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

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Reviews

Editorial

Sleep, sex and psychosocial health: Expanding the horizons of behavioral sleep medicine

Miguel Meira e Cruz[1,2,A–F], Monica Levy Andersen[3,4,B,D–F]

[1] Sleep Unit, Cardiovascular Center of the University of Lisbon, Lisbon School of Medicine, Portugal
[2] Centro Europeu do Sono, Lisbon, Portugal
[3] Federal University of São Paulo, São Paulo, Brazil
[4] Sleep Institute, Associação Fundo de Incentivo à Pesquisa (AFIP), São Paulo, Brazil

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Address for correspondence
Miguel Meira e Cruz
E-mail: mcruz@medicina.ulisboa.pt

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Intimacy, including partnered sex or masturbation, may modestly improve sleep continuity via neuroendocrine and circadian pathways, supporting cautious, ethical and patient-centred integration into behavioral sleep medicine.

In an era marked by the rising prevalence of sleep disturbances and the escalating dependence on pharmacological treatment, the search for effective, low-risk and accessible behavioral interventions is both urgent and timely. Within this context, the study by Lastella et al., "Sleep on it: A pilot study exploring the impact of sexual activity on sleep outcomes in cohabiting couples,"[1] offers a scientifically stimulating, albeit preliminary, contribution. By assessing sexual behavior, including both partnered activity and masturbation, as a pre-sleep activity through ecological polysomnography and subjective outcomes, the authors uncover suggestive evidence that intimacy may offer relevant insights for individual and potentially public health strategies, although further validation is clearly warranted.

The findings, highlighting modest improvement in wake after sleep onset (WASO) and increased sleep efficiency, invite cautious consideration of intimacy as a potential behavioral factor in sleep health. These effects are likely mediated through the neuroendocrine mechanisms involving oxytocin, prolactin and dopamine, and the downregulation of stress hormones, such as cortisol, pathways well-known for influencing both sleep and emotional regulation. However, it is crucial to interpret such benefits within the scope and limitations of the original sample (7 couples), and not to overstate their generalizability.

Drawing from neurochronobiological insights,[2] sexual and romantic behaviors, although distinct in function, are both modulated by circadian rhythms, affective neurobiology and sleep architecture. The neurochemical correlates of intimacy and sexual engagement may influence the regulation of rapid eye movement (REM) sleep, emotional processing and the stability of relational bonding. The circadian timing of these behaviors seems to be critical[3] and may potentiate their effects, opening the door to chrono-sexual interventions, tailored approaches that align intimacy with individual circadian typologies for optimized outcomes.

Importantly, the possible role of these behavioral pathways extends beyond sleep outcomes. The exploration of sexual activity (whether partnered or solitary) as a coping mechanism for psychosocial stress,[4] anxiety, depressive symptoms, and obsessive-compulsive tendencies[5] opens a line of investigation that is both clinically relevant and ethically delicate. While pharmacological interventions remain essential for many, they are not without side effects, including REM sleep suppression, cognitive impairment and the risk of dependency. In contrast, sexually intimate behavior, when voluntary and contextually appropriate, may provide a socially reinforcing alternative with fewer adverse consequences. Yet, such behavioral suggestions must be personalized and consensual, with attention to the risk of coercion or the disruption of spontaneity within relationships.

The relational aspect further deserves careful interpretation. The observed synchronization in REM sleep among cosleeping partners suggests that intimacy may enhance sleep through both physiological and interpersonal pathways. Still, such phenomena are contingent upon the quality and dynamics of the relationship. Masturbation, also included in the Lastella et al.'s study, showed similar outcomes,[1] which implies that the sleep-promoting effects of sexual behavior are not solely dependent on relational engagement, but may stem from neurobiological mechanisms inherent to sexual arousal and release.

We recognize the controversy surrounding the concept of framing sexual behavior as a 'therapy.' While we do not suggest replacing validated clinical interventions, we advocate for further empirical exploration of intimacy-related behaviors as part of a broader behavioral health framework, especially in cases where pharmacological options are contraindicated or poorly tolerated.

To that end, we support a more nuanced approach that integrates sexual activity into the therapeutic dialogue not as a universal recommendation, but as an optional behavioral strategy, applicable in select, consensual and informed contexts. This complements, rather than replaces, current therapeutic modalities and supports a more person-centered model of care.

Recent literature exploring sex differences and sleep-related vulnerabilities also provides a relevant backdrop to these discussions. For instance, emerging evidence indicates that women are more likely to suffer from insomnia and subjective sleep complaints, partly due to hormonal fluctuations, caregiving roles and circadian misalignment. Men, conversely, may be underdiagnosed for disorders like obstructive sleep apnea, which presents differently across sexes and is often correlated with different behavioral triggers.[6] Furthermore, the influence of hormones on sleep architecture (e.g., the role of estrogen in REM sleep regulation) suggests the need to tailor behavioral interventions, including those involving intimacy, according to sex and the hormonal status. These insights underscore the importance of stratifying sleep interventions not only by age and relationship context, but also by sex-related physiological and behavioral traits.

Toward strategic integration: Implementing sexual behavior principles in sleep medicine

In light of these insights, a strategic framework is warranted to explore the integration of sexual behavior principles into the broader field of behavioral sleep medicine. This integration must be cautious, respectful of ethical boundaries and rooted in scientific evidence. The approach can unfold across 4 domains: clinical practice; research; education and training; and public health policy.

1. Clinical practice

Clinicians may begin by sensitively incorporating sexual health discussions into sleep consultations, recognizing both partnered intimacy and solitary sexual activity as potentially relevant factors. These conversations must be framed with care to avoid coercion or discomfort. Couple-based behavioral therapies may consider the relational context of sleep disruption and intimacy, and interventions could be personalized based on the chronotype, the attachment style and relational dynamics (Fig. 1).

2. Research

Future studies should focus on randomized controlled trials to examine the short- and long-term effects of sexual activity, partnered or solo, on sleep architecture, emotional regulation and circadian physiology.[7] Mechanistic studies exploring neuroendocrine responses, sex-specific outcomes and chronobiological alignment are needed to establish evidence-based recommendations.

3. Education and training

Healthcare providers in sleep medicine should receive interdisciplinary training, including sexual health literacy, psychological theory and cultural competence. Programs must prepare clinicians to discuss sensitive topics respectfully and ethically, equipping them to address the diverse needs of patients.

4. Public health policy

Public health messaging can help destigmatize discussions around sexual behavior and sleep health, while policy development can promote holistic models of care that value intimacy, psychosocial wellness and non-pharmacological strategies. Importantly, these efforts must ensure inclusivity and avoid prescriptive norms.

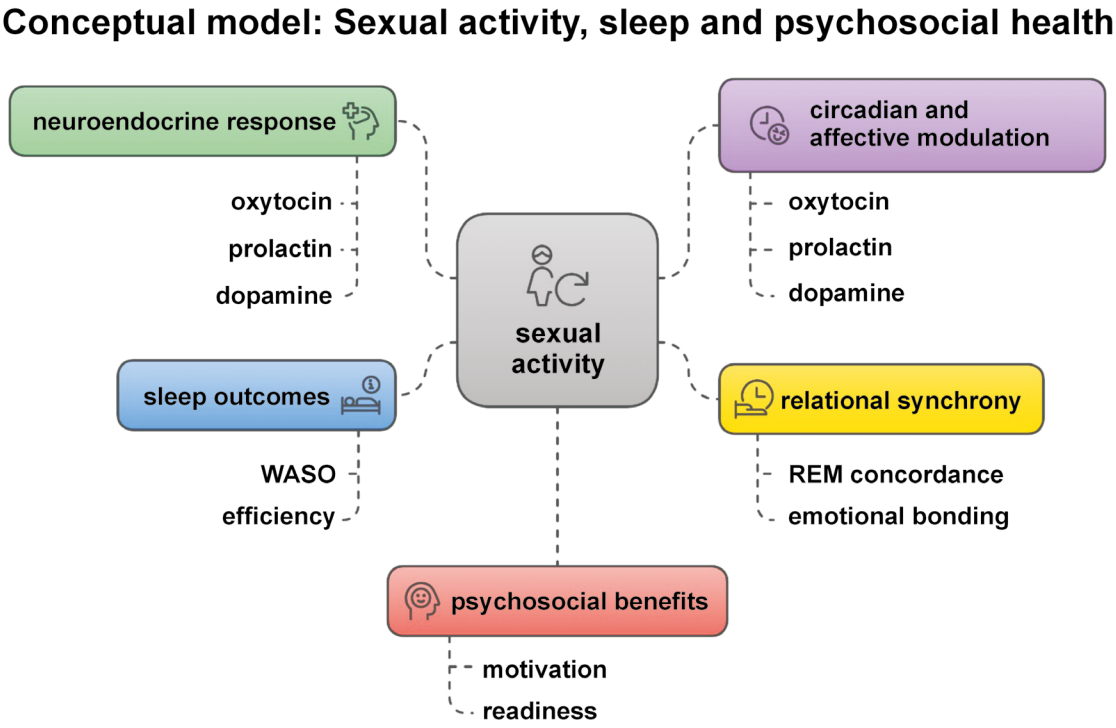

Fig. 1. Conceptual model illustrating the interplay between sexual activity, sleep regulation and psychosocial health

The diagram represents the integrative pathways through which sexual activity, whether solo or partnered, may exert therapeutic effects on sleep and psychological wellbeing. Sexual activity stimulates neuroendocrine responses, including the release of oxytocin, prolactin and dopamine, which in turn promote improved sleep architecture (e.g., reduced wake after sleep onset (WASO), increased sleep efficiency) and enhanced emotional regulation. Concurrently, sexual behavior interacts with circadian and affective systems, influenced by timing, hormonal rhythms and emotional states, which modulate both sleep and mood. These processes contribute to psychosocial benefits, such as increased motivation and readiness the following day, and to relational synchrony, particularly in cohabiting couples, reflected in sleep stage (i.a., rapid eye movement (REM)) concordance and emotional bonding. Together, these pathways support the inclusion of intimacy and sexual behavior as potential non-pharmacological strategies within the therapeutic framework of sleep medicine and behavioral health.

In conclusion, the integration of sexual behavior principles into behavioral sleep medicine offers a promising avenue for non-invasive and patient-centered care. However, this potential must be approached with scientific humility, ethical vigilance and cultural sensitivity.

ORCID iDs

Miguel Meira e Cruz https://orcid.org/0000-0001-6076-0878
Monica Levy Andersen https://orcid.org/0000-0002-1894-6748

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Editorial

The grand issue of mandibular condyle fractures: Development of treatment approaches

Marcin Kozakiewicz[A–F]

Department of Maxillofacial Surgery, Medical University of Lodz, Poland

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation;
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Address for correspondence
Marcin Kozakiewicz
E-mail: marcin.kozakiewicz@umed.lodz.pl

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The following description outlines how the current standards for treating mandibular condyle fractures hatched, so that they have grown into mature techniques for guiding patients to recovery.

The aim of the present article is to highlight the long way of mandibular condylar process fracture treatment leading to a satisfactory and low-complication-rate outcome.

Treatment modalities for condylar process fractures remain the most controversial issue in maxillofacial traumatology. This is strange, since a fracture of the mandible is the most common fracture of the facial skeleton, and the condylar process is the most common location of mandibular fractures.[1] This epidemiology is due to the rather prominent anatomical position of the mandible and some difficulties in protecting it.

The earliest records on the treatment of mandibular fractures date back to the Bronze Age.[2] In the "Edwin Smith Papyrus" there is a case report written in 1700 B.C. The author of this military medical guide advises not treating an open fracture of the mandible, since the patient will die anyway.

This state of medical knowledge persisted until the Napoleonic Wars, when Desault pointed out that in the treatment of mandibular condylar process fractures, it was very important to achieve good contact between two bone fragments.[3] The impetus for the development of maxillofacial traumatology appeared to be armed conflicts, i.e., World Wars I and II, the Korean War, and the Vietnam War, due to the involvement of large, rich countries and a rapidly increasing number of patients. Lambotte introduced the term 'osteosynthesis' (1907), Kazanjian used the splinting of the teeth, tooth ligation and bone sutures (1914–1918), Ivy introduced a ligature with a loop (1922), and Ginestet introduced Kirshner wires and external immobilization (1936). Later, onlay individual silver splints were used for the immobilization of reduced bone fragments. Then, the era of closed treatment in all mandibular fractures, including condylar fractures, set in.[4] However, in 1886, the young and relatively unknown surgeon Carl Hansmann presented his experience with the plate osteosynthesis system at the annual meeting of the German Surgical Society.[5] He described 2 patients with mandibular fractures he treated with his method.

Today, Hansmann is undisputedly considered the inventor of plate osteosynthesis. His subcutaneous plate, fixed with percutaneous screws, bears little resemblance to the plate systems of today. It was open reduction and rigid fixation (ORIF), so called today. Halsted refined this system, introduced it to the USA and used head screws for open internal osteosynthesis in 1893.[6,7] Open reduction and rigid fixation stayed in the shadow of closed treatment for many consecutive decades because of its high complication rates, especially the risk of osteomyelitis. Except for a few isolated reports, plate osteosynthesis was therefore unable to gain widespread acceptance. This was due to the use of inadequate implant materials (corrosion); plates and screws often caused inflammatory lesions, and in the worst cases, osteomyelitis with its catastrophic consequences. This, in turn, led to the general view of "not too much metal for bone." As a result, the chosen implants were too small, and therefore too weak, which, in addition to corrosion problems, caused instability in the fracture area, which is fatal in osteosynthesis. Non-physiological handling of the bone, i.e., the extensive elevation of the periosteum and too rapid rotations during pre-drilling with insufficient cooling led to premature loosening of the screws. The reduction of susceptibility to corrosion occurred with the invention of chromium-cobalt-molybdenum (Cr-Co-Mo) alloy Vitallium, used by Bigelow in 1943.[8] However, external fixation was still used as during WWI.

To overcome this situation and advance toward modern maxillofacial treatment, scientific progress was necessary, i.e., new materials (stainless steel patented in 1915; stainless steel with the addition of Mo to increase resistance to chloride-induced rusting in 1930; the miniaturization of osteosynthetic materials; the development of materials dedicated to maxillofacial surgery, not just the adaptation of orthopedic solutions), the improvement of anesthesiology (first general anesthesia by Hanaoka using tsūsensan in 1804; the creation of specialization in 1912; the introduction of the Macintosh laryngoscope curved blades in 1943; general anesthesia with intubation and relaxation by Pokrzywnicki in Poland in 1947; the replacement of flammable gases with Halothane in 1956, which was then gradually replaced with halogenated ethers, introduced in 1970s), the development of radiological techniques (pantomography: 1970s; computed tomography (CT) scanning: 1980s), as well as the introduction of effective bactericidal medications (penicillin: after World War II; chlorhexidine: 1950s; metronidasol: 1960; lincomycin: 1962; clindamycin: 1966) and specially designed surgical instruments.

The truly modern approach to ORIF is the post-WWII period. First, Robinson should be recalled.[9] His L-shape plate is made of stainless steel, which gives it a thinness not met with other plates described in his time, with regard to using the casting method. Robinson used periangular skin access and reported no facial nerve weakness in his patients. He originally recommended ORIF when a patient could not be immobilized with maxillomandibular ligation, but he noticed ORIF was beneficial for most patients with mandibular condylar process fractures. He recommended ORIF for multiple fractures of the mandible, when more stability is needed than is obtained with wire sutures, which were widely used up to 1980s. He mentioned the use of only 2 screws per fracture as an advantage (he used self-tapping screws, 2.7 mm in diameter, with bicortical anchorage).

In those times, the most important question was: "close or open treatment?"[10] Surprisingly, most maxillofacial surgeons believed that most fractures of the condylar process of the mandible should be treated non-surgically, and certainly most fractures of the mandibular head. Alternatively, in terms of surgical treatment, it was speculated whether in the case of a mandibular head fracture, the entire lower fragment should be removed, which prevents ankylosis, but irreversibly degrades the stomatognathic system. Korzon summed up his research in 1971: "Assessing the long-term results of closed and conservative treatment of the fractures of the condylar process of the mandible, it was found that only conservative treatment does not always give good results. It fails in fractures with significant displacements, with dislocation, and especially in old fractures of the condylar process of the mandible. Incorrect positioning of bone fragments causes a number of disorders in the masticatory system."[10] Nowadays we know the proper answer, but then it was the hottest issue, or even the voice of the pioneers of the upcoming revolution.

At that time there was a resurgence of interest in the Vitallium alloy. Luhr studied the rigid fixation of the facial skeleton with Vitallium in 1960s and proposed compression osteosynthesis in 1967.[6] Luhr and Spiessl reintroduced the idea of using miniature plates in osteosynthesis in 1968 and 1972. Luhr recalled the idea of using self-tapping screws (1968) and used extraoral approaches.[11] Spiessl adapted the plates to the dimensions of the mandible and popularized the compression osteosynthesis of the mandible in the USA, beginning in 1971. He also used compression fixations without plates, employing lag screws bicortically (1974) and eliminating intermaxillary postoperative immobilization.[12] And finally, the era of intraoral approaches opened (Luhr, 1985).[13] Michelet and Moll described mandibular fractures treated with Vitallium miniplates and monocortical immobilization without intermaxillary ligatures in 1971.[14] Plate sizes were reduced,[6] and based on the abovementioned study, Champy developed monocortical miniplate osteosynthesis in 1976.[15] It is still used today as the primary ORIF technique.

Although the possibility of the anatomical reduction of fragments and the restoration of temporomandibular joint (TMJ) function enforced the superiority of open treatment, the breakthrough came 30 years ago, when

an Austrian team developed a technique for the osteosynthesis of mandibular head fractures.[16] Surprisingly, and invariably since those days, US centers have not been interested in developing the treatment of condylar process fractures. This seems to be due to the fear of complications and unstable treatment results (and related patient claims). And yet, safe and effective protocols for the management of even severely comminuted fractures of the condylar process are known.[17]

In 1990s, surgical steel was displaced by titanium (Ti) alloys as more biocompatible.[18] Another boost came from Strasbourg – extending Kessler's 1980 study describing ideal osteosynthesis lines in the mandibular body, Meyer et al. described them in the mandible condyle.[19] Since then, miniaturized materials and the knowledge of how to apply them effectively have been available.

The still asked question from the 1960s was finally answered by Neff.[20] More predictable treatment results can be obtained with ORIF vs. closed treatment. It seems today that by proposing closed treatment to most patients, the clinician assumes responsibility for potentially subjecting many patients to severe dysfunction of TMJ and the entire stomatognathic system. Anyway, it is clear that applying ORIF for the fractures of the mandibular head will be the responsibility of a small number of specialized centers, to which patients from a wider area of the country will be referred, while patients with simpler types of condylar fractures will be managed in any department of maxillofacial surgery.

Currently, a range of effective techniques and materials is available for ORIF, enabling the successful treatment of mandibular condyle fractures. The following types of fractures are distinguished: base; neck; and mandibular head. The complexity of surgical treatment increases as these fractures are listed. Many osteosyntheses still use bicortical fixation and there is a multitude of available fixation materials. The current state of the art in managing mandibular condylar process fractures can be summarized as follows: system 2.0; self-tapping screws; Ti alloys; antibiotic prophylaxis; nasal/submental intubation; oral disinfection; intraoral approaches; limited periosteal detachment; monocortical anchorage; the consideration of bone stress areas; water cooling; no intermaxillary immobilization after surgery; and the removal of the fixation material after the period of bone union formation (Fig. 1). The ORIF modalities are continually evolving to enhance efficacy, and the field offers substantial scope for further innovation.

Concluding, it must be stated that over the past decades, the approach to fracture treatment has rapidly evolved from closed treatment to ORIF. The current scientific evidence indicates that most patients will benefit from open treatment and osteosynthesis. This reflects the progress in diagnostic techniques, fixation materials and surgical skill.

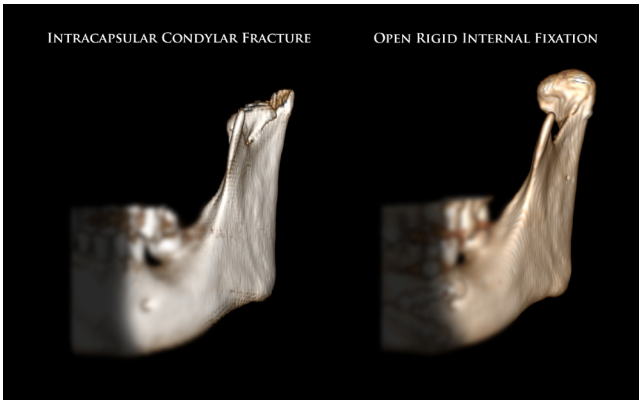

Fig. 1. State of the art in mandible condyle osteosynthesis – fixation of the mandible head with a headless compression screw. Open rigid internal fixation (ORIF) provides in such a case the restoration of bone anatomy, dental occlusion and the temporomandibular joint (TMJ) function

ORCID iDs

Marcin Kozakiewicz https://orcid.org/0000-0001-9200-2828

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Perspective

Laminate veneers phenomena: Esthetics above biology and function?

Pedro Santos Diamantino[1,A,D,F], Guilherme Schmitt De Andrade[1,A,D,F], Mariana Gadelho Gimenez Diamantino[1,A,D,F], Carlos Eduardo Francci[2,A,D,F], Guilherme de Siqueira Ferreira Anzaloni Saavedra[1,A,D,E], João Paulo Mendes Tribst[3,A,D–F]

[1] Institute of Science and Technology, São Paulo State University (Unesp), São José dos Campos, Brazil
[2] Department of Biomaterials and Oral Biology, University of São Paulo School of Dentistry (FO-USP), Brazil
[3] Department of Reconstructive Oral Care, Academic Centre for Dentistry Amsterdam (ACTA), University of Amsterdam and Vrije Universiteit Amsterdam, Amsterdam, the Netherlands

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Address for correspondence
João Paulo Mendes Tribst
E-mail: joao.tribst@gmail.com

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Keywords: minimally invasive dentistry, esthetic dentistry, adhesive dentistry, evidence-based dentistry, ethical dental practice

Indirect laminate veneers can deliver excellent esthetic results, but their use must remain secondary to biological and functional principles, requiring an ethical and evidence-based approach during treatment planning.

Porcelain laminate veneers, first introduced in the early 20th century, have gained popularity due to advancement in ceramic materials, adhesive systems and computer-aided design/computer-aided manufacturing (CAD/CAM) technologies.[1] These innovations have enabled more conservative and predictable preparations while meeting the rising demand for esthetic dental treatment – a phenomenon influenced by modern beauty standards and the concept of "emotional dentistry."

It is well known that the veneers bonded to enamel have greater fracture resistance than those bonded to dentin, making minimally invasive dentistry critical not only for biological reasons, but also for the durability of restorations. When properly planned and executed, porcelain veneers offer an effective solution with a success rate exceeding 90% in long-term studies.[2,3]

Key success factors include detailed case planning, enamel preservation, tooth vitality, appropriate material selection, and adherence to the technique. Failure often results from ignoring limitations – especially in young patients with large pulp chambers and minimal wear – or from overtreatment, driven by profit rather than clinical need. Problems such as microleakage, fractures and detachment are more common when the technique is poorly executed.[3–7]

Indications for veneers include minor color corrections, abnormal tooth contours, diastemas, recession exposing dentin, malformations, and wear.[3–8] They do not include patients seeking cosmetic enhancement due to societal pressure. In comparison with full crowns, veneer preparations are about 50% more conservative.[7–9] They are typically used to treat resistant tooth discoloration, morphological changes, diastemas, incisal wear, fractures, or non-esthetic conditions.[10–13]

A growing concern is that untrained or underqualified practitioners, driven by high demand, may overlook conservative alternatives, leading to overtreatment and irreversible damage. Ethical practice requires full disclosure of all restorative options, prioritizing preservation over esthetics when appropriate.[7] When performed correctly, veneer treatment is highly durable, with studies showing survival rates of 92% at 6 years and 86% at 12 years.[1] Success is also related to the dentist's and technician's expertise.

Ultimately, 3 pillars underpin successful outcomes: understanding the treatment concept; technical accuracy; and long-term maintenance. These steps focus on minimizing complications and extending the longevity of the restoration. As the number of prosthetic procedures increase, this raises critical questions about how they align with the principles of preventive dentistry and the importance of dental tissue preservation in modern practice.[3]

Furthermore, patient age must be considered, especially for the young. Younger individuals have larger pulp chambers; thus, even minimal tooth reduction (0.5 mm) risks postoperative sensitivity and potential pulp damage. Additionally, the longevity of veneers – while high (85–95% over 5–20 years) – still necessitates replacement eventually, leading to further tooth reduction.[8–10]

Enamel wear from aging also affects veneer viability. Enamel thickness decreases with age, especially after 50, increasing the risk of exposing dentin during preparation. Adhesion to enamel is more predictable than in the case of dentin, where moisture control is difficult and structural properties are inferior, raising the risk of fractures. Adhesive cementation can mitigate some issues, but not all.[11]

A critical question arises: Are we overtreating? Despite being termed "conservative," veneer preparation may remove up to 30% of healthy enamel.[8] The goal of restorative dentistry should be to preserve natural tooth structures while addressing disease and function. When esthetic concerns are the only issue, less invasive options like whitening or orthodontics should be prioritized. Veneers should not substitute appropriate orthodontic treatment, especially in younger patients or those with crowded teeth, as this may require excessive tooth reduction and increase complications.[10]

Informed consent is essential. Patients must understand biological costs, the need for enamel preservation and the long-term implications of dentin exposure. Gurel et al. found failure rates of 89.3% when veneer margins extended into dentin vs. significantly higher survival when the margins remained in enamel.[2]

Another option would be treatment with direct composite veneers, which can offer good esthetics and preserve healthy tooth structures. Though more prone to degradation and staining than ceramics, their reversibility and repairability make them ideal for borderline cases. Studies show survival rates of ~80–100% over 3–6 years for composite restorations.[3]

The veneer philosophy has evolved. Initially, no-prep veneers were preferred to avoid invasiveness. However, they often lead to overcontoured restorations and soft tissue irritation. A standard reduction of 0.3–1 mm is now recommended to allow proper material thickness and esthetics. Standardized diamond burs and mock-ups help guide tooth reduction while preserving enamel.[12] Yet, aggressive preparation is still common, particularly in esthetic-driven practices. Preparation should be individualized, especially in additive cases, such as diastemas or worn teeth, to avoid unnecessary enamel removal. No-prep veneers are only suitable when natural tooth contours allow a 0.3-millimeter material thickness without overbuilding.[13,14]

Veneer materials have also advanced.[15] Feldspathic ceramics, though esthetically superior, are brittle and difficult to fabricate in thin layers.[16] Newer materials for indirect restorations like lithium disilicate offer greater fracture resistance and are suitable for ultra-thin veneers (as thin as 0.3 mm).[14–17]

From a laboratory perspective, a chamfer finish line remains important for predictable fabrication. Diagnostic tools like mock-ups, temporary composite veneers and wax-ups enable clinicians to visualize final outcomes and determine necessary reduction more accurately.[12]

Digital dentistry has emerged as a key ally. Approaches like the anatomical shell technique aim to replicate natural tooth morphology, improving predictability and reducing reliance on manual skills.[12–17] Digital systems facilitate obtaining high-quality esthetic results and enable the reproduction of various kinds of tooth morphology.[18] However, limitations remain, especially in replicating the optical dynamics of single anterior teeth, something 3D-printing technologies may overcome when associated with intraoral scanning.[19]

In the context of material selection, lithium disilicate ceramics – particularly those fabricated through milling – can show superior wear resistance as compared to feldspathic ceramics, which demonstrate lower wear performance, but behave similarly to heat-pressed lithium disilicate ceramics.[20]

In summary, veneers should not be a default esthetic solution. Treatment decisions must be grounded in ethical practice, clinical need and respect for biological preservation. Dentists must guide patients responsibly, proposing conservative options first, and ensuring long-term function and esthetics are achieved with minimal harm.

After shared decision making on an indirect veneer, one possible clinical protocol can be summarized as follows (Fig. 1 and 2):

1. **Initial Assessment.** Comprehensive photographic documentation of the patient's smile should be performed, including images with lips at rest, during maximum smile, intraoral views, profile views, and detailed photographs of the maxillary anterior region. This step should be conducted in accordance with ethical treatment

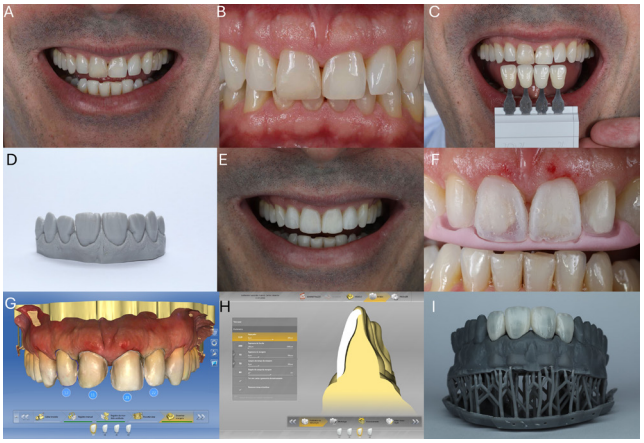

Fig. 1. A – maximum smile photograph; B – intraoral smile photograph; C – color selection; D – morphological model of a natural tooth; E – composite resin veneers fabricated using a matrix obtained from the molded model; F – intraoral mock-up with composite resin veneers; G – minimally invasive preparations for ceramic veneers; H – selection of teeth and type of restoration to be scanned in the CEREC software (Dentsply Sirona, Charlotte, USA); I – printed model (Geller type) used for the verification of adaptation, adjustment, finishing, and placement of the ceramic laminates

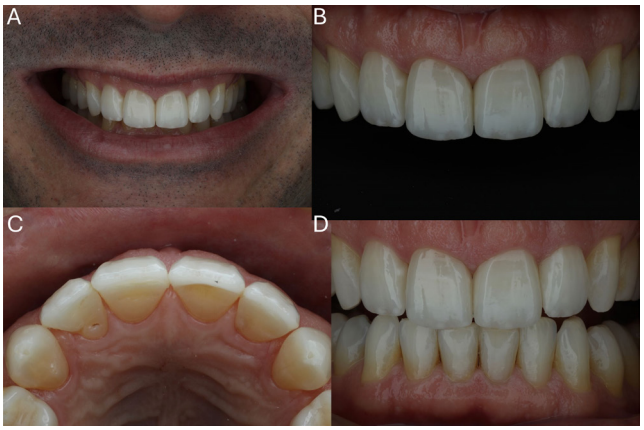

Fig. 2. A – adapted laminates with extrinsic stain and glaze at maximum smile; B – view of maxillary anterior teeth; C – occlusal view; D – intermaxillary relationship during anterior guidance

planning and shared decision making, ensuring that both esthetic and functional needs are met.

2. **Shade and Proportion Analysis.** Select the appropriate tooth shade under standardized lighting conditions. Evaluate the height-to-width ratio of central incisors to assist in determining the ideal tooth shape. Measure the gingival sulcus depth to assess the potential need for soft tissue modification.
3. **Tooth Morphology Selection and Mock-up.** Choose a morphological tooth model that harmonizes with the patient's facial and dental proportions. Fabricate provisional resin veneers, using a matrix derived from the selected model. Place the mock-up intraorally to evaluate esthetics and function. Scan the intraoral mock-up to generate a digital model with corrected proportions.
4. **Gingival Correction.** If indicated, perform a gingivectomy guided by the mock-up and the previously obtained sulcus measurements to refine the gingival zeniths and improve symmetry.
5. **Tooth Preparation.** After soft tissue healing, prepare the teeth for veneers, using the mock-up or a silicone index as a guide. This approach ensures minimally invasive preparation, prioritizing enamel preservation.
6. **Digital Workflow and Design.** Capture intraoral scans of the prepared teeth. Utilize CAD software to design restorations based on the scanned mock-up, adjusting design parameters and finalizing the morphology as needed.
7. **Model Printing and Verification.** Digitally edit and print physical models to verify the fit and adaptation of the restorations. Perform adjustments, finishing and extrinsic staining on the ceramic veneers before final placement.
8. **Cementation.** Bond the milled ceramic laminate veneers, using adhesive cementation techniques under proper isolation.
9. **Final Evaluation.** Acquire postoperative photographs from multiple angles, including frontal views with lips at rest and smiling, as well as intraoral, occlusal and profile views. These images can be later used to assess esthetic outcomes and verify incisal edge-to-lip harmony.

Conclusions

Indirect veneers are a reliable solution for esthetic dental issues, offering long-term success with significantly less tooth reduction than full crowns. However, ethical and effective treatment requires individualized diagnosis, patient-centered planning and the consideration of all available alternatives. Clinicians must be properly trained and inform patients of the benefits, limitations and potential risks, especially regarding dentin exposure. To preserve healthy tooth structures, veneer preparations should be carefully planned, using tools like diagnostic wax-ups, mock-ups and/or silicone guides.

ORCID iDs

Pedro Santos Diamantino https://orcid.org/0000-0001-9951-9045
Guilherme Schmitt De Andrade https://orcid.org/0000-0002-5697-2947
Mariana Gadelho Gimenez Diamantino https://orcid.org/0000-0003-2299-9751
Carlos Eduardo Francci https://orcid.org/0000-0003-3638-5005
Guilherme de Siqueira Ferreira Anzaloni Saavedra https://orcid.org/0000-0001-7108-0544
João Paulo Mendes Tribst https://orcid.org/0000-0002-5412-3546

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Original paper

Global consensus report of the World Federation for Laser Dentistry (WFLD) on laser-assisted caries treatment and prevention

Samir Nammour[1,A–F], Aldo Brugnera Junior[2,A–F], Toni Zeinoun[3,A–F], Jacek Matys[4,B–D,F], Sonia Bordin-Aykroyd[5,B–D,F], Paul Nahas[6,C,D,F], Marwan El Mobadder[1,B–D,F], Sajee Sattayut[7,A–F], Darinca Carmen Todea[8,A,B,D–F], Akira Aoki[9,A,D–F], Kinga Grzech-Leśniak[10,11,A–F]

[1] Department of Dental Sciences, Faculty of Medicine, University of Liège, Belgium
[2] São Carlos Institute of Physics (IFSC), University of São Paulo (USP), Brazil
[3] Department of Periodontology, Faculty of Dental Medicine, Lebanese University, Beirut, Lebanon
[4] Department of Dental Surgery, Faculty of Dentistry, Wroclaw Medical University, Poland
[5] School of Pharmacy, De Montfort University, Leicester, UK
[6] Department of Restorative and Esthetic Dentistry, Faculty of Dental Medicine, Lebanese University, Rafic Hariri Campus, Hadath, Beirut, Lebanon
[7] Lasers in Dentistry Research Group, Khon Kaen University, Thailand
[8] Department of Oral Rehabilitation and Dental Emergencies, School of Dentistry, Victor Babeș University of Medicine and Pharmacy, Timișoara, Romania
[9] Department of Periodontology, Graduate School of Medical and Dental Sciences, Institute of Science Tokyo, Tokyo, Japan
[10] Department of Integrated Dentistry, Faculty of Dentistry, Wroclaw Medical University, Poland
[11] Department of Periodontics, School of Dentistry, Virginia Commonwealth University, Richmond, 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

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Address for correspondence
Kinga Grzech-Leśniak
E-mail: kinga.grzech-lesniak@umw.edu.pl

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Abstract

Background. This article presents the global consensus report of the World Federation for Laser Dentistry (WFLD) on laser-assisted caries prevention and management. Laser-assisted caries removal is a minimally invasive approach based on selective ablation, targeting demineralized tissues while preserving the adjacent healthy enamel and dentin. This approach aligns with the principles of modern conservative dentistry. Erbium-doped yttrium aluminum garnet (Er:YAG) and erbium, chromium-doped yttrium scandium gallium garnet (Er,Cr:YSGG) lasers are highly absorbed by water and hydroxyapatite (HAP), enabling precise ablation with minimal thermal diffusion and collateral damage. Laser wavelengths can also exhibit bactericidal effects through thermal and photomechanical mechanisms, reducing the microbial load in carious lesions.

Objectives. The aim was to review, evaluate and consolidate the current evidence on the use of laser technologies in caries prevention and treatment, in light of the emerging scientific knowledge and clinical advancements.

Material and methods. This summary is based on the current evidence from in vitro, ex vivo and clinical studies evaluating the interaction of erbium-based lasers (Er:YAG, Er,Cr:YSGG) with enamel and dentin, their effect on the microbial load, fluoride uptake and resin adhesion, and their use in photobiomodulation (PBM) therapy.

Results. Laser irradiation induces physicochemical changes in enamel, such as superficial melting and recrystallization, reducing porosity and increasing resistance to acid attacks. Fluoride uptake is enhanced through microstructural modifications, especially when combined with topical fluoride. Subablative laser settings synergize with fluoride to enhance retention without damaging enamel. Laser conditioning before fissure sealing improves surface energy and resin adhesion, reducing microleakage. Photobiomodulation promotes remineralization in early lesions via cellular stimulation.

Conclusions. Laser-assisted caries treatment offers precise, minimally invasive management of dental caries with added benefits, such as microbial reduction, structural enhancement and improved adhesion. Careful control of parameters is essential to balance efficacy and safety. Further studies are needed to standardize protocols and confirm long-term outcomes in clinical practice.

Keywords: dental caries, lasers, dental caries prevention, evidence-based dentistry

Highlights

- This consensus report provides evidence-based guidance on the role of laser technologies in caries prevention and minimally invasive management.
- Laser-assisted approaches enhance enamel resistance, facilitate fluoride uptake, improve restorative outcomes, and support the treatment of dentin hypersensitivity.
- Standardized protocols and synergistic use with conventional preventive methods are essential for safe and effective clinical application.

Introduction

Dental caries remains one of the most widespread and burdensome oral diseases globally, affecting both children and adults, and contributing significantly to impaired oral health and reduced quality of life.[1,2] Despite advances in preventive strategies and restorative techniques, including fluoride use, fissure sealants and minimally invasive dentistry, the prevalence of caries continues to be high.[3,4] Traditional approaches are limited by issues such as patient compliance, variable long-term effectiveness and the recurrent need for retreatment. Consequently, there is growing interest in adjunctive methods that can strengthen hard tissues, reduce microbial challenges and support biological repair while preserving tooth structure.

Over the past few decades, lasers have emerged as a promising technology in dentistry. Continuous improvements in laser devices have led to greater safety, precision and versatility, opening new avenues for their use in both the prevention and treatment of caries. A variety of laser systems, including CO_2, neodymium-doped yttrium aluminum garnet (Nd:YAG), erbium-doped yttrium aluminum garnet (Er:YAG), erbium, chromium-doped yttrium scandium gallium garnet (Er,Cr:YSGG), argon, and diode lasers, have been investigated for dental applications.[5] Their distinct wavelengths and tissue interactions allow them to induce morphological and chemical changes in enamel and dentin, such as melting, recrystallization and the occlusion of dentinal tubules. These modifications can reduce enamel solubility, increase acid resistance and improve patient comfort by reducing hypersensitivity.[4]

In preventive dentistry, laser irradiation has demonstrated the ability to enhance enamel resistance to demineralization, particularly when combined with fluoride or other remineralizing agents.[4] Subablative protocols can promote fluoride uptake without causing structural damage, offering synergistic protection against acid attacks. Certain wavelengths also possess bactericidal effects, reducing the microbial load on tooth surfaces and within incipient lesions. Collectively, these attributes suggest that lasers can play an important role in reducing the risk of caries initiation and progression.[5,6]

From a therapeutic perspective, erbium-based lasers allow the selective ablation of carious dentin, facilitating minimally invasive caries removal while preserving healthy tissue. This aligns with the philosophy of modern conservative dentistry. Additionally, photobiomodulation (PBM) therapy, formerly referred to as low-level laser therapy (LLLT), has been explored as a complementary strategy to stimulate pulp and dentin healing, and promote lesion reversal when combined with agents such as casein phosphopeptide–amorphous calcium phosphate (CPP–ACP).[6] Moreover, a microscopic and crystallographic analysis of dental enamel melted using a 445-nm diode laser showed a significant increase in acid resistance.[7] These innovative applications expand the potential of lasers beyond traditional cutting instruments, positioning them as multipurpose tools in caries management.

Nevertheless, several challenges remain before widespread clinical adoption can be recommended. Outcomes vary considerably depending on the laser type, wavelength, energy settings, and irradiation protocols used. While in vitro and short-term clinical studies are promising, robust evidence on long-term efficacy, cost-effectiveness and biomechanical safety is still limited. The lack of standardized guidelines further complicates clinical decision making and may hinder optimal integration of lasers into daily practice.

In this context, the World Federation for Laser Dentistry (WFLD) has developed the present consensus report on laser-assisted caries treatment and prevention. Drawing on the expertise of an international panel of specialists, this document aims to critically evaluate the available evidence, provide practical recommendations for preventive and therapeutic use of lasers in caries management, and identify key areas for future research. By establishing a unified framework, the WFLD seeks to guide clinicians toward evidence-based adoption of laser technologies, ultimately contributing to improved patient outcomes and advancing minimally invasive dentistry.

Methods

A modified Delphi approach was used to develop the consensus report. The panel consisted of 11 specialists from 8 countries (Belgium, Brazil, Lebanon, the United Kingdom, Thailand, Romania, Japan, and Poland), all of whom are active members of the WFLD. Eight experts

were assigned to prepare a section of the manuscript according to their specific field of expertise. The drafted sections were then compiled and redistributed anonymously to all panel members for critical review and comments. One designated coordinator integrated the suggested corrections and produced a revised draft of the manuscript. This version was subsequently reviewed in detail by 2 independent experts, followed by a group discussion to resolve any remaining disagreement. The final approval of the consensus document was obtained once all experts agreed with the revised version. This structured process ensured transparency, minimized individual bias, and guaranteed that the final consensus reflected the collective expertise and international agreement of the panel.

Background on laser applications in dentistry

The use of lasers in dentistry spans several decades, but recent technological advances have significantly improved their safety, precision and versatility. A variety of lasers, including CO_2,[8,9] Nd:YAG,[10,11] Er:YAG,[12–15] Er,Cr:YSGG,[16] argon,[17] and diode lasers,[18] have been utilized for caries-related applications. These lasers differ in terms of wavelength, interaction with the tissue and depth of energy penetration, leading to various biological and chemical effects on enamel and dentin substrates. When applied under appropriate parameters, laser irradiation has been shown to induce desirable morphological and chemical changes, including the surface melting, fusion and recrystallization of dental hard tissues.[8] These effects are believed to reduce enamel porosity, decrease acid solubility and increase the acid resistance of hydroxyapatite (HAP) crystallites, considered as key factors in caries prevention.

In the preventive context, laser irradiation has been associated with enhanced enamel resistance to demineralization.[19] Studies report that laser treatment can either directly alter the crystalline structure of enamel or synergize with topical agents like fluoride to enhance its uptake and incorporation into HAP.[20,21] This dual action not only provides mechanical resistance to acid attacks, but may also alter diffusion pathways for acid, thereby slowing down the progression of incipient lesions. Additionally, certain laser wavelengths exhibit bactericidal properties, making lasers capable of reducing the microbial load on tooth surfaces and within early carious lesions.[22] This antimicrobial effect further contributes to the caries-preventive potential of laser applications.

From a therapeutic perspective, lasers offer the possibility of selective caries removal, targeting demineralized tissues while preserving the adjacent healthy enamel and dentin.[23] This minimally invasive approach aligns with the principles of modern conservative dentistry. In cases involving dentin, some laser systems have been reported to occlude dentinal tubules, which can reduce hypersensitivity and improve patient comfort. For example, CO_2 laser irradiation has demonstrated the ability to increase dentin resistance to artificial caries-like lesions.[24] Another emerging area in the context of caries treatment is the use of PBM therapy, formerly known as LLLT. Photobiomodulation does not aim to alter hard tissues directly, but instead stimulates cellular responses that may aid in remineralization or pulp–dentin complex healing. When combined with remineralizing agents such as CPP–ACP or bioactive glass, PBM may accelerate lesion reversal and promote biological repair mechanisms.[25]

Despite the promising clinical and in vitro results, the underlying mechanisms governing laser-induced beneficial changes in enamel and dentin are not yet fully elucidated. While some studies describe chemical transformations, such as carbonate loss, changes or modifications in the HAP crystallite lattice, spectroscopic and ultrastructural evidence remains incomplete. Effects vary not only with the laser type, but also with specific parameters, such as pulse duration, energy density, repetition rate, and the presence of adjunctive agents like fluoride.

Moreover, the long-term clinical efficacy and cost-effectiveness of laser-assisted caries management remain topics of ongoing research. Standardization of protocols and deeper understanding of laser–tissue interactions at the molecular level are essential before widespread clinical adoption can be recommended.

Laser-assisted caries prevention

Tooth decay remains one of the most common oral diseases worldwide.[26] In response to the limitations of traditional preventive methods, dental research has turned to innovative approaches, including the use of lasers. This technology, originally reserved for restorative treatment, is increasingly being recognized as a promising preventive tool. Several types of lasers are now being studied and applied in dental practices, often in combination with other methods like fluoride treatment, fissure sealing and enhanced oral hygiene. This integrated approach aims to strengthen the natural protection of the teeth, thus reducing the risk of demineralization and cavity formation.

Several procedures are cited in the literature to increase the enamel resistance to caries. Below we summarize the main methods playing a key role in preventing tooth decay.

Enamel modification for acid resistance

Laser-assisted caries prevention strategies increasingly focus on enhancing the acid resistance of enamel – a critical barrier against demineralization and caries formation.[27] By altering both the physical and chemical

properties of enamel, laser irradiation can strengthen its structure and improve its protective capabilities.[7] Depending on the energy level, wavelength and type of laser used, these effects range from superficial melting and prism disorganization to improved uptake of topical fluoride. While high-power lasers, such as Er:YAG and Er,Cr:YSGG, enable enamel fusion and surface glazing, lower-energy systems, such as argon or diode lasers, are employed to enhance fluoride retention without damaging the tissue. Recent clinical studies suggest that combining subablative laser protocols with fluoride application offers a synergistic approach, reinforcing enamel resistance to acid attacks in a minimally invasive manner.

On the other hand, recent in vitro studies indicate that CO_2 laser irradiation (commonly at ~10.6 µm) can improve the acid resistance of tooth enamel, particularly when combined with fluoride treatment. For example, human premolar enamel surfaces irradiated with a pulsed CO_2 laser showed significantly lower calcium (Ca) release and shallower erosion depths as compared to controls and those treated with Nd:YAG lasers. However, these benefit effects diminished over extended acid exposure times and did not affect subsurface enamel layers.[28] Moreover, the application of CO_2 laser through a topical amine fluoride solution enhanced fluoride uptake and increased surface acid resistance in both sound and demineralized enamel, with fewer surface alterations observed via scanning electron microscopy (SEM) than after laser alone.[24,29,30] Similarly, combining CO_2 laser irradiation with acidulated phosphate fluoride (APF) demonstrated a synergistic effect, further reducing demineralization as compared to either treatment alone.[30,31] One study revealed that while CO_2 laser alone slightly prevented enamel erosion, it provided the most effective protection when paired with a stannous fluoride (AmF/NaF/$SnCl_2$) solution, particularly in human enamel models.[32] Together, these findings suggest that CO_2 laser treatment, especially when used with fluoride agents, can enhance enamel resistance to acid challenges, though effects may be limited to superficial layers and are most pronounced when complemented by fluoride therapy.

Structural fusion and laser surface effects on enamel

Mature dental enamel is composed of a predominantly Ca-based material, primarily in the form of HAP, which constitutes approximately 96% of its composition. During laser irradiation, the generated heat can induce the dehydration of enamel, potentially leading to the formation of surface microfissures. The literature indicates that laser-induced melting and glazing of dental enamel can result in the development of these microcracks on the irradiated surface. Such fissures may, over time, contribute to the structural failure of enamel within the oral cavity, culminating in the physical destruction of the tooth. Considering these detrimental effects, recent research has focused on the application of low-power lasers that do not cause significant heating or fusion of enamel, thus minimizing the risk of damage.[27,30,33]

When interacting with the structure of enamel, lasers induce its fusion through heat, followed by rapid re-solidification. This fusion process alters the structural integrity of enamel by disrupting the prism (rod) structure, which results in the loss of the characteristic orientation of HAP crystallites. Additionally, the organic interrod matrix undergoes disintegration. These physical alterations hinder and slow down the progression of acid attacks within enamel by disrupting the preferred pathways (inter-prismatic and under-crystallite spaces) for acid infiltration. Furthermore, heating and subsequent melting of enamel trigger chemical changes in its constituent phases, which can further enhance enamel resistance to acidic degradation.

By modifying the structure of enamel through melting it superficially, lasers can make it more resistant to acid attacks responsible for cavities.

Physicochemical alterations of enamel through laser irradiation

The effects of various types of lasers on dental hard tissues are being thoroughly investigated. These effects are highly dependent on laser-specific parameters, including wavelength, energy output and the technique of irradiation.[34] Among laser devices, erbium lasers (Er:YAG and Er,Cr:YSGG) are especially relevant in dentistry due to their high absorption by water. This property enables their safe application with water spray, effectively reducing thermal damage during the ablation of hard tissues.[34–37]

In enamel, which has a lower water content than dentin, the action of erbium lasers at low energy densities primarily removes organic materials, such as proteins and lipids, located between enamel rods. This results in the creation of a micro-roughened surface, which can enhance adhesion in restorative procedures.[38] However, it is important to note that enamel cutting generally requires significantly higher power settings as compared to dentin. For instance, the effective ablation of enamel may necessitate power levels up to 2 W and energy densities around 31.44 J/cm^2 at a frequency of 10 Hz.[39]

For the preparation of dentin, which is more sensitive to thermal effects due to its organic matrix and proximity to pulp, Nahas et al. recommend lower energy settings – specifically, 60 mJ at 10 Hz in the micro-short pulse (MSP) mode – in combination with water irrigation and air cooling to minimize the risk of pulpal damage.[40]

Cracks on the surface of enamel can occur as a result of laser irradiation due to the rapid thermal changes induced by laser energy. When enamel is exposed to high-intensity laser beams, as in the case of Er:YAG lasers, the abrupt rise and fall in temperature may generate thermal stress within the structure of enamel, exceeding the

tensile strength of the material, and resulting in microcracks or fissures on the surface. Early experimental investigations had already documented such morphological changes: Hibst and Keller demonstrated that Er:YAG laser irradiation could cause microexplosions and crack formation in enamel and dentin,[41] while Fried et al.[42] and McCormack et al.[43] confirmed that rapid thermal expansion, under insufficient cooling conditions, promoted surface fissures and irregularities visible under SEM.[42–44] Similarly, White et al. reported microcracks in the enamel irradiated by CO_2 lasers, emphasizing that energy density and the pulse mode are the critical determinants of surface safety.[45] Subsequent investigations on CO_2 lasers confirmed that even at subablative fluences, enamel exhibited subsurface cracking and carbonization, especially in the absence of water cooling.[46]

Comparable structural alterations have been described with other wavelengths. Nd:YAG irradiation has been associated with crater formation, localized melting and subsurface cracks in enamel due to its deeper penetration and high thermal load.[47,48] In a comparative study, Nd:YAG-treated enamel exhibited a higher frequency of fissures than Er:YAG-irradiated samples, particularly under dry conditions.[49] Argon lasers, although primarily used for curing and caries prevention, have also been shown to induce localized thermal stress and fissures in enamel prisms when applied at high energy densities.[50] These cross-wavelength findings consistently point to the risk of thermomechanical stress concentration whenever energy delivery exceeds the capacity of the tissue for rapid heat dissipation.

More recent studies have refined these observations with advanced imaging. For instance, using atomic force microscopy (AFM), SEM and energy-dispersive X-ray spectroscopy (EDS), Rodríguez Vilchis et al. observed triangular cracks in enamel, with depths ranging from approx. 250 nm to 750 nm and widths between 2.58 µm and 5.37 µm, along with the accompanying craters and irregular surfaces.[51] Likewise, nanoscale X-ray computed tomography (nano-CT) confirmed that, while glazing and surface roughness occurred on the irradiated enamel, subsurface structural damage was minimal and prismatic enamel architecture largely remained intact, suggesting that surface alterations may not always translate into deeper compromise.[52]

These thermally induced modifications could nevertheless act as stress concentrators, weakening enamel and potentially jeopardizing adhesive performance or mechanical integrity. Despite the expanding clinical adoption of lasers in dentistry, there remains a pronounced gap in research regarding the long-term mechanical quality of laser-treated enamel, particularly concerning the risk of tooth fracture. Most mechanical investigations have focused on dentin rather than enamel. For example, the dentin beams irradiated without water cooling, using Er:YAG or Q-switched Er:YSGG lasers, exhibited visible surface cracks and a significant reduction in bending strength – from approx. 142 MPa in controls to 91 MPa in the dry-irradiated group.[53] By contrast, comparable tests on enamel, such as the assessments of fracture toughness, load-bearing capacity or crack propagation under functional conditions, are notably scarce. Earlier descriptive studies largely focused on identifying surface fissures,[42,45–47,50] but they did not extend to quantitative biomechanical evaluations.

This lacuna in the literature underscores a critical oversight: the risk of tooth fracture due to laser-induced structural alterations in enamel remains insufficiently studied, with scant mechanical testing performed thus far. Rigorous biomechanical evaluations of laser-treated enamel are urgently needed to ensure both safety and efficacy in clinical applications.

To summarize, erbium lasers provide a versatile tool for the modifications of hard tissues. High energy levels are recommended for efficient enamel cutting, while dentin benefits from lower energy parameters to preserve its structural and biological integrity. This differentiation is essential when preparing enamel and dentin surfaces for bonded restorations (Table 1).[34,38–40] However, the irradiation conditions must be reduced for dentin conditioning before bonded fillings (Table 2).

Combined use of laser and fluoride in restorative dentistry

The combination of laser irradiation and topical fluoride application has emerged as a promising strategy to enhance enamel resistance against acid demineralization.

Table 1. Recommended parameters for erbium-doped yttrium aluminum garnet (Er:YAG) and erbium, chromium-doped yttrium scandium gallium garnet (Er,Cr:YSGG) lasers for cutting enamel and dentin

Parameters for ablation	Enamel	Dentin
Energy per pulse [mJ]	200–500	100–300
Frequency [Hz]	10–20	10–20
Power [W]	2–6	1–3
Water spray	high	high
Fluency [J/cm^2]	15.72 or 31.44	–
Pulse duration	–	the shortest pulse duration

Table 2. Recommended parameters for Er:YAG and Er,Cr:YSGG lasers for dentin pre-bonding conditioning

Parameters for dentin conditioning before bonding	Dentin
Energy per pulse [mJ]	30–60
Frequency [Hz]	10–20
Power [W]	0.6–2.0
Fluency [J/cm^2]	20.769
Air/water ratio [mL per min]	6/4
Pulse duration [µs]	50–100

Fluoridation, a traditional yet highly effective method, involves applying fluoride in the form of varnish or gel to strengthen enamel and increase its acid resistance by promoting remineralization.[54–57] Low-energy laser treatment can further improve fluoride uptake and retention by modifying enamel chemistry and microstructure without causing thermal damage. This synergistic interaction is being investigated both in preventive and restorative dentistry, with reported benefits including enhanced enamel protection, as well as improved adhesion and sealing performance of restorative materials.

One of promising adjunctive strategies in laser-assisted caries prevention involves the combination of low-energy laser irradiation with topical fluoride application.[58] The argon laser, operating at low power – typically in the milliwatt range – and emitting in the blue visible spectrum, has been widely studied in this context.[17,58] Due to its interaction at the electronic level of atoms, visible light from such lasers is hypothesized to induce chemical changes in the HAP structure of enamel. Specifically, this interaction may facilitate the substitution of hydroxyl ions (OH^-) with fluoride ions (F^-), which are more electronegative. This chemical alteration enhances fluoride uptake and contributes to greater acid resistance of the enamel surface.[17,19,20,58–65]

Supporting this hypothesis, an in vivo clinical study assessed fluoride retention following laser irradiation under subablative settings, i.e., low energy levels insufficient to cause morphological changes or damage to enamel.[66] The results confirmed that laser application after fluoride treatment significantly improved fluoride retention in dental enamel without compromising structural integrity.[66] These findings underscore the clinical potential of low-energy lasers as safe and effective adjuncts to conventional fluoride-based preventive strategies.

Laser preconditioning for improved adhesion and sealing

Laser surface conditioning for fissure sealants

Pit and fissure areas – narrow grooves typically found on the occlusal surfaces of molars and premolars – are highly susceptible to dental caries.[67] These regions tend to trap plaque and debris, and are difficult to clean effectively with a toothbrush, particularly in children and adolescents. To counter this risk, fissure sealants are widely used as a preventive measure. The application of fluid resin into pits and fissures creates a physical barrier, which blocks the entry of food particles and bacteria, thereby reducing caries incidence in these vulnerable zones.[67] The procedure is simple, minimally invasive, and provides long-term protection.

Recent advances in preventive dentistry have introduced laser irradiation as a surface preconditioning tool to improve the adhesion of sealants.[68] By modifying the surface of enamel – without the need for acid etching – lasers can enhance resin retention and sealant longevity. In particular, laser preparation has shown potential for increasing micromechanical interlocking and reducing microleakage. Furthermore, laser use extends beyond the application of sealants. In modern caries prevention strategies, lasers play a growing role in enhancing enamel resistance by promoting fluoride incorporation into the HAP structure. This synergistic effect contributes to increased acid resistance and long-term protection against demineralization. Nonetheless, these technological approaches should always be integrated with fundamental oral hygiene practices and a balanced diet. Laser-based preventive strategies are most effective when used as part of a comprehensive caries management protocol.[67,68]

Laser-assisted adhesion strategies in restorative dentistry

Bonded restorations in dentistry rely essentially on resin micromechanical interlock with the bonded surfaces.

Among the lasers commonly used in dentistry are erbium lasers, such Er:YAG and Er,Cr:YSSG, which are particularly effective for cutting and preparing dental hard tissues. The effects of various laser types on hard tissues can be summarized as follows:

The CO_2 laser enhances dentin resistance to caries by melting dentin and obliterating dentinal tubules.[24] However, overheating of the surface in a localized area may modify the chemical composition of dentin due to melting and vaporization of certain components.[69] Moreover, such irradiation negatively affects the shear bond strength (SBS) of the composite materials bonded to dentin.[69]

Diode lasers are not typically used for the ablation of dental hard tissues. However, when used to irradiate bonding systems at low energy levels prior to photopolymerization, they can increase SBS to enamel. Furthermore, this enhancement in bond strength is not affected by thermal aging.[70] In addition, the diode laser pre-treatment of non-carious cervical lesions acts better against bacterial microinfiltration as compared to chemical surface treatment.[71] Moreover, diode laser irradiation does not affect the microleakage of the restored materials, whether it is resin composite, glass ionomer or resin-modified glass ionomer.[72]

The Nd:YAG laser is commonly used in dentinal treatment. Controversial results can be found in the literature. Studies by Sun et al.[73] and Landmayer et al.[74] showed that bonding to dentin after being irradiated with Nd:YAG at 124.4 mJ/cm^2 enhanced the shear and microtensile bond strength of the composite, even after thermocycling. Although the composition of dentin was modified and some of the collagen fibers were removed, the hardness and roughness of dentin surface were equivalent to those of the unirradiated dentin.[73,74] On the other hand, Hamidi et al. were totally against the use of Nd:YAG before bonding to dentin.[10] The reported microtensile bond

strength to the dentin irradiated with 50 mJ was lower as compared to the unirradiated dentin.[10] Even though, using Nd:YAG at 60 mJ does not affect the collagen layer needed for the hybrid layer.[75] Low SBS to Nd:YAG-irradiated dentin was also reported by Al Ahdal et al.[22]

Erbium laser (Er:YAG and Er,Cr:YSGG) beams are highly absorbed by water. They can be employed under water spray, thus reducing thermal damage during the ablation of hard tissues.[34] The ablation effect of erbium lasers is more pronounced on dentin than enamel due to the difference in water content. At low energy densities, they act on enamel through removing organic particles between enamel rods, thus creating a rough surface.[38] However, the conventional etching of enamel is still giving better surface preparation for composite bonding than laser alone. Moreover, combining acid etching with laser increases bond strength when compared to the conventional etching of enamel.[76]

Latest studies have shown successful bond strength of the composite to the dentin irradiated with erbium lasers, and laser use was recommended as an alternative technique to the conventional one.[16,77] It has been demonstrated that the irradiation of dentin modifies its surface by eliminating the smear layer and exposing dentinal tubules for bonding infiltration.[23] Caries removal and cavity preparation with the use of erbium lasers are less invasive procedures as compared to those performed with rotary burs or chemical agents.[23] Unfortunately, the results published by Karatas et al. prove the contrary, but only for the Er,Cr:YSGG laser, which negatively affected SBS to the irradiated dentin by altering the structure of the hybrid layer.[78] Cutting enamel and dentin requires high power, especially for enamel (up to 2 W), and energy density of 31.44 J/cm^2 at a frequency of 10 Hz.[39] Nahas et al. recommended conditioning for dentin preparation to receive a bonded restoration with a decrease in the level of the delivered energy to 60 mJ, with a frequency of 10 Hz, the MSP mode under water irrigation and air cooling.[40] Another study by Demir et al. used 200 mJ, 20 Hz and pulse duration of 50 μs; that way, the laser beam eliminated the smear layer, opened dentinal tubules and prepared the surface of dentin for the bonding composite.[79]

Laser-assisted caries removal and treatment

Laser-assisted caries removal is a minimally invasive approach that exploits selective ablation mechanisms, where laser energy specifically targets carious tissues due to the differences in water and mineral content, allowing the preservation of healthy enamel and dentin. Er:YAG and Er,Cr:YSGG lasers are highly absorbed by water and HAP, efficiently ablating carious dentin through microexplosive effects and rapid vaporization. The water/air spray assures minimal thermal diffusion and prevents collateral pulp damage.[47] Certain laser wavelengths exhibit bactericidal properties, making lasers capable of reducing the microbial load in carious lesions via thermal and photomechanical disruption, contributing to improved clinical outcomes.[9,80,81] Laser irradiation induces physicochemical modifications in enamel, such as superficial melting and recrystallization, thus increasing resistance to acid attacks responsible for caries initiation.[82,83] Prior to the placement of fissure sealants, laser conditioning modifies enamel morphology, creating microroughness and improving surface energy, which significantly enhances resin adhesion and sealant durability, reducing microleakage.[84–86]

Photobiomodulation facilitates remineralization in combination with remineralizing agents, promoting mineral deposition and accelerating repair mechanisms in early carious lesions.[87,88]

Optimal laser treatment outcomes depend on precise control of parameters, such as wavelength, energy density, pulse duration, and cooling mechanisms to balance effective ablation with the preservation of structural integrity and biological function. Continuous investigation into the mechanisms and optimization of laser-based caries treatment is critical for standardizing protocols, and ensuring their effectiveness and safety for widespread clinical adoption.

Enhancing enamel resistance and remineralization: The role of Er,Cr:YSGG and Er:YAG lasers

Effect on sound enamel

Multiple in vitro studies have demonstrated that Er,Cr:YSGG laser irradiation enhances the acid resistance of sound enamel. Ulusoy et al. used SEM and EDS to show decreased solubility of both primary and permanent enamel after irradiation.[89] Serdar-Eymirli et al. confirmed that combining laser with fluoride or CPP–ACP significantly increased enamel microhardness.[90] Mahdi and Hussein validated this synergistic effect in a randomized clinical trial on primary teeth, using APF gel.[91]

Effect on enamel erosion

Studies confirm that the Er,Cr:YSGG laser helps protect enamel against acid erosion. Hadi and Ali found increased enamel microhardness and reduced mineral loss after combining laser with APF.[92] AlShamrani et al. showed superior protection against acid challenges with the same combination.[93] Fornaini et al. emphasized that subablative parameters create structural changes that enhance acid resistance without damaging enamel.[94]

As for the Er:YAG laser, several studies have evaluated its preventive effect with regard to enamel acid demineralization, yielding mixed results. A systematic review and

meta-analysis by Lombardo et al. summarized findings from 6 such studies comparing laser to no treatment.[8] Four of these studies found that the Er:YAG laser did not significantly improve enamel resistance, as measured by parameters such as microhardness, lesion depth and Ca dissolution. However, 2 studies reported a positive effect: one showed a 41% reduction in mineral loss, and another found a significant decrease in the lesion depth when the laser was applied at a 4-millimeter distance with water cooling at 2 mL/s. When combined with other interventions, the Er:YAG laser demonstrated enhanced efficacy. It significantly increased enamel microhardness when used with 5% fluoride varnish and it reduced Ca dissolution when combined with 1.23% APF gel. Furthermore, the combination of the laser with 2% NaF gel resulted in a 54% reduction in acid-induced mineral loss as compared to only 24% with the gel alone.[8]

Remineralization of initial carious lesions

The Er,Cr:YSGG laser enhances the efficacy of remineralizing agents in treating initial enamel lesions. Cheng et al. concluded that laser with CPP–ACP significantly improved enamel microhardness and reduced the lesion depth.[95] Damar et al. observed improved outcomes with theobromine-containing products.[96] Yilmaz et al. confirmed similar effects in primary enamel.[97]

One in vitro study assessed the effect of the Er:YAG laser irradiation combined with APF therapy on the remineralization of white spot lesions (WSLs).[98] The study found that the combination treatment significantly enhanced enamel resistance to acid attacks as compared to either treatment alone. Laser irradiation was shown to increase the size of HAP crystallites by melting, causing the recrystallization of enamel, and subsequently decreasing the permeability of enamel and enhancing its resistance to acid attacks.[98]

Laser parameters and protocols

The most common parameters used in caries-related studies include: Er,Cr:YSGG laser at 2,780 nm; power 0.25–0.50 W; energy density 4.5–9.0 J/cm^2; frequency 20 Hz; and pulse duration 140 µs. The water/air spray (20/20% or modified) is used to limit thermal effects. Subablative settings are the key to ensuring enamel integrity while promoting mineral uptake.

Pulse energies between 100 and 200 mJ and fluence values ranging from 12.7 to 44.4 J/cm^2 are effective in reducing enamel demineralization. A frequency of 10 Hz and pulse duration between 250 and 400 µs are commonly used settings in the protocols aimed at improving enamel acid resistance.

Carefully selected parameters, such as moderate pulse energies, appropriate fluence, and controlled frequency and pulse duration, are critical for achieving optimal results without compromising enamel integrity. As research progresses, laser-based treatment is poised to become a valuable adjunct in minimally invasive, preventive dental care protocols (Table 3).

Marginal integrity and microleakage in Class V restorations

Class V restorations, typically found at the cervical third of the tooth, present challenges due to the mixed substrates of enamel, dentin and cementum, as well as their susceptibility to microleakage. Laser-based cavity preparation, particularly using Er:YAG and Er:Cr:YSGG lasers, has been explored as an alternative to traditional burs to improve surface quality and bond strength at the enamel and cementum margins. However, several studies have demonstrated that microleakage at the cementum margin of the restoration, in particular, with composite resin, was greater than the cervical margin at some laser parameters.[104–107] Consequently, the parameter settings for laser devices for the cervical margin of Class V preparation comprising cementum need to be thoroughly investigated and should differ from the standard recommendations typically applied for enamel or dentin. No clinical trials were identified; only in vitro studies were found, as microleakage must be examined in the extracted teeth.

The Er:YAG laser (2,940 nm) was used under variable energy and frequency settings. Low-energy settings ranging from 75 to 200 mJ at 2–20 Hz proved to be effective in comparison with traditional bur preparation methods; the Er,Cr:YSGG laser (2,780 nm), set at 175 mJ and 20 Hz, also demonstrated effective sealing of restorations when acid etching was used.[108] The comparison of total-etching and self-etching adhesives and selective etching of enamel (with no etching for cementum) showed no significant differences in microleakage when using the Er:YAG laser at 200 mJ and 20 Hz.[109] Other studies demonstrated comparable or less microleakage when using the Er:YAG and Er,Cr:YSGG lasers vs. bur preparation in the groups that utilized a primer with glass ionomer cement or a self-etching adhesive with a composite resin restoration.[106,107,110] Based on a scoping review, the following recommendations for minimizing microleakage are made for the cementum margin of Class V restorations, which can also be applied to the enamel margins (Table 4).

Recommended laser parameters

Laser type: Er:YAG (2,940 nm) or Er,Cr:YSGG (2,780 nm).
Energy per pulse: 100–200 mJ.
Frequency: 10–20 Hz.
Pulse duration: 100–200 µs.
Water spray: ≥5 mL/min.

Table 3. Selected in vitro studies on enamel microhardness

Study	Sample size	Laser settings	Chemical agent	Outcomes
Mahdi and Hussein[91] 2024	80 primary posterior teeth	power of 0.25 W, 0.50 W and 0.75 W, with a pulse width of 140 µs and a repetition rate of 20 Hz, the irrigation system provided 40% air and 60% water spray, energy transmission was facilitated through a fiber optic system equipped with a 600-µm beam diameter, the MZ6 sapphire gold tip; the laser was applied from a distance of 1–2 mm in the non-contact (H) mode	1.23% APF gel	the combined application of Er;Cr:YSGG laser under subablative parameters and APF gel demonstrated a superior potential for remineralizing the enamel of primary teeth affected by WSLs
Yilmaz et al.[97] 2020	89 primary anterior maxilla and mandible teeth	laser type: Er,Cr:YSGG wavelength: not explicitly stated in the text, but known to be 2,780 nm for Er,Cr:YSGG power: 0.5 W frequency: 20 Hz water/air spray ratio: 60/40% pulse energy: 25 mJ fluence: 8.84 J/cm^2 pulse duration: 60 µs tip diameter: 600 µm irradiation distance: approx. 1.0–1.5 mm from the target	CPP–ACP	combining subablative Er:YSGG laser irradiation with CPP–ACP application provided superior protection against enamel erosion as compared to either method used alone
Babu Kl et al.[99] 2025	380 primary molars	laser type: Er,Cr:YSGG wavelength: 2,780 nm energy density (fluence): 4.64 J/cm^2 pulse energy: 0.25 W repetition rate (frequency): 20 Hz pulse duration: 140 µs water/air spray ratio: 55/65% irradiation mode: subablative, scanning motion tip-to-tissue distance: 1 mm tip type: MZ6 sapphire	fluoride	a synergistic effect in increasing enamel surface microhardness with remineralizing agents
Subramaniam and Pandey[100] 2014	30 anterior teeth	laser type: Er,Cr:YSGG mode: non-contact distance: 15 mm pulse duration: 140 µs power: 4 W water/air spray ratio: 60/40% frequency: 50 Hz irradiation time: 20 s	CPP–ACP (GC Tooth Mousse)	the combined action of laser and CPP–ACP resulted in significantly higher surface microhardness as compared to the application of CPP–ACP alone
Ghelejkhani et al.[101] 2021	35 third molars	100 mJ energy, 10 Hz frequency and energy density 8 J/cm^2, with 35–40% water and 50% air at a distance of 1 mm for 30 s (Waterlase; BIOLASE Technology, San Clemente, USA)	CPP–ACP (GC Tooth Mousse)	fluoride varnish increased enamel surface microhardness, while GC Tooth Mousse had no such effect; laser therapy before the application of remineralizing agents did not significantly enhance enamel resistance to demineralization
Molaasadollah et al.[102] 2017	20 primary teeth	0.5 W power, 20 Hz frequency, 60% water, 40% air, pulse duration of 5 ±1 s	1.23% APF gel for 4 min	Er,Cr:YSGG laser irradiation + 1.23% APF gel was not significantly different from the application of fluoride gel alone in enhancing the remineralization of WSLs
Mendes da Silva VR et al.[103] 2019	150 bovine enamel slabs	the laser operated with a pulse width of 140 µs, a repetition rate of 20 Hz and variable output power (0–6 W), delivered via a 750 µm × 6 mm sapphire tip (MS75) in focused mode at a distance of 1 mm from the surface; 3 parameter settings were used: – P1: 0.25 W, 2.8 J/cm^2 energy density, 56 W/cm^2 power density – P2: 0.50 W, 5.7 J/cm^2 energy density, 1,136 W/cm^2 power density – P3: 0.75 W, 8.5 J/cm^2 energy density, 17,004 W/cm^2 power density	1.23% APF gel (pH: 3.6–3.9)	Er,Cr:YSGG laser (0.50 W, 20Hz, 5.7 J/cm^2, 1,136 W/cm^2) with fluoride was the only treatment capable of controlling the progression of enamel erosion

APF – acidulated phosphate fluoride; CPP–ACP – casein phosphopeptide–amorphous calcium phosphate; Er,Cr:YSGG – erbium, chromium-doped yttrium scandium gallium garnet; WSL – white spot lesion.

Table 4. Laser parameters and microleakage outcomes for Class V restorations of the cementum margin (in vitro studies)

Study	Laser type	Wavelength [nm]	Pulse duration [μs]	Energy [mJ]	Frequency [Hz]	Conditioning for the cementum	Microleakage at the cervical margin
Arami et al.[12] 2014	Er:YAG	2,940	not mentioned	300	10	acid etching	significantly more microleakage than in bur preparation
Ozel et al.[104] 2009	Er:YAG	2,940	not mentioned	150–700	5–20	self-etching adhesive	only 200 mJ/20 Hz showed comparable microleakage to the bur
Korkmaz et al.[106] 2010	Er:YAG	2,940	not mentioned	200	20	self-etching adhesive	no significant difference between Er:YAG and the bur
Phanombualert et al.[107] 2015	Er:YAG	2,940	200	50, 75 and 100	15	self-etching adhesive	less microleakage than in the case of the bur, and other settings of energy of 75 and 100 mJ
Fattah et al.[108] 2013	Er,Cr:YSGG	2,780	140	175	20	laser only acid etching	the best sealing at the cervical margin with acid etching
Delme et al.[110] 2005	Er:YAG	2,940	100	200	10	laser only acid etching	significantly more microleakage than in bur preparation, but acid etching did not seem to show less microleakage
Baygin et al.[111] 2012	Er,Cr:YSGG	2,780	140–200	not mentioned	20	acid etching self-etching adhesive	the least microleakage in the group with acid etching

Er:YAG – erbium-doped yttrium aluminum garnet; QSP – quantum square pulse (laser mode).

Conditioning recommendations by margin type

Enamel margins

Preparation: laser or a bur

Conditioning: 3 steps of acid etching, primer and bonding, or a self-etching adhesive

Material: a resin composite with good enamel adhesion

Cementum (cervical) margins

Preparation: laser or a bur.

Conditioning: 3 steps of acid etching, primer and bonding, or a self-etching adhesive.

Avoid: laser-only surface conditioning.

Material: a resin composite or glass ionomer.

Adjunctive benefits of laser use in preventive dentistry: Management of tooth hypersensitivity

Although dentin hypersensitivity (DH) is not a direct outcome of carious lesions, it is frequently encountered in clinical practice as a comorbidity of non-carious cervical lesions, enamel erosion or preventive interventions, such as scaling and polishing, orthodontic movement, or even conservative laser treatment. Therefore, its effective management is essential to ensure patient comfort and the long-term success of preventive care strategies.

Laser-assisted therapies, especially using high- and low-level laser systems, have emerged as valuable tools for managing DH. These approaches complement preventive and restorative procedures by reducing pain, sealing the exposed dentinal tubules and improving patient compliance with minimally invasive dental protocols.

Dentin hypersensitivity is defined as a short, sharp pain arising from the exposed dentin in response to thermal, tactile, osmotic, or chemical stimuli, and is not attributable to any other form of dental pathology.[112,113] It significantly affects patient quality of life, and is commonly associated with gingival recession and enamel or cementum loss, which expose dentinal tubules to external stimuli.[21,114]

Role of photobiomodulation

Photobiomodulation has emerged as a non-invasive, effective adjunct in the management of DH, owing to its dual mechanism – the occlusion of dentinal tubules and the modulation of nerve activity.[113,115] The lasers used in DH treatment are broadly categorized by power and mechanism:

– high-power lasers (e.g., Nd:YAG, Er:YAG, Er,Cr:YSGG) deliver photothermal energy, melting and recrystallizing dentin to physically seal dentinal tubules (Table 5)[116–118];
– low-level lasers (e.g., diode 660–830 nm) induce PBM, altering pain perception via anti-inflammatory and neural mechanisms (Table 6).[119–121]

Scanning electron microscopy has confirmed that laser-treated surfaces show occluded or narrowed dentinal tubules, reducing fluid flow and the transmission of stimuli.[113]

Clinical performance

High-power lasers like Nd:YAG and neodymium-doped yttrium aluminum perovskite (Nd:YAP) have shown rapid desensitization with protocols such as 0.5 W at 10 Hz for

Table 5. High-energy lasers (a photothermal effect) – evidence from clinical (in vivo) and laboratory (in vitro) studies

Laser type	Wavelength	Parameters	Sessions	Outcomes
Nd:YAG	1,064 nm (e.g., Nammour et al., 2022[124])	0.5 W 10 Hz ~8,400 J/cm^2 2 passages	1 session	the effect of dentinal tubule sealing lasted 12 months
Er:YAG	2,940 nm (e.g., Sajith et al., 2024[125])	~20 J/cm^2 continuous contact	6 sessions, biweekly	VAS dropped from 7.8 to 0.6 at 12 months
Er,Cr:YSGG	2,780 nm (e.g., Yilmaz et al., 2011[126])	0.5 W 30 s non-contact	1 session	comparable to GaAlAs diode laser, a significant DH drop
CO_2	10,600 nm (e.g., Belal and Yassin, 2014[127])	2 W pulsed	1 session	up to 30% tubule occlusion (SEM), a safe temperature profile

Nd:YAG – neodymium-doped yttrium aluminum garnet; VAS – visual analog scale; GaAlAs – gallium aluminum arsenide; DH – dentin hypersensitivity; SEM – scanning electron microscopy.

Table 6. Selection of in vivo treatment procedures with low-level lasers (photobiomodulation (PBM))

Laser type	Wavelength	Parameters	Sessions	Outcomes
Diode (660–810 nm)	810 nm (e.g., Naghsh et al., 2020[121])	30–100 mW 120 s fluence ≤ 4 J/cm^2	4 sessions, weekly	a significant VAS reduction sustained over 1–2 months
Diode (904–980 nm)	808–904 nm (e.g., Moura et al., 2019[122])	100 mW 10 s per point 4 J/cm^2	4 sessions, 48 h apart	reduced DH for up to 24 weeks
GaAlAs (805–830 nm)	830 nm (e.g., Praveen et al., 2018[123])	~40 mW 60 s <10 J/cm^2	1 session	a greater DH reduction than with a topical desensitizer alone

Nd:YAG, producing a significant and sustained reduction in DH.[114,118] The Er,Cr:YSGG laser has similarly demonstrated safe and effective outcomes when using subablative protocols.[115]

Photobiomodulation, particularly with diode lasers (660–1,064 nm), has proven effective in neuromodulation and dentin repair. Studies report reduced VAS scores sustained over months after 6 sessions using 50–100 mW for 50–120 s per point.[121,123,128]

Combination therapies

Synergistic effects are observed when laser therapy is combined with desensitizing agents, such as KNO_3, fluoride gels or CPP–ACP pastes.

For example, Guanipa-Ortiz et al. demonstrated a 77% reduction in DH with diode laser and casein phosphopeptide–amorphous calcium phosphate fluoride (CPP–ACPF) paste,[129] while Jomaa et al. reported 9-month relief when combining diode laser with 1.23% NaF gel (Table 7).[130]

Comparative efficacy

Several systematic reviews affirm the superiority or at least equivalence of laser therapy in comparison with conventional agents.[132,133] However, the Cochrane review by Mahdian et al. emphasized the need for high-quality, standardized trials due to heterogeneous methodologies and short follow-ups.[113] Laser therapy, particularly when

Table 7. Combination therapy (laser + chemical agent) – evidence from clinical (in vivo) and laboratory (in vitro) studies

Study	Therapy type	Protocol	Agent	Outcomes
Nammour et al.[124] 2022	Nd:YAG + KNO_3	1,064 nm 1 W	5% KNO_3	up to 74% DH reduction in fluorotic dentition
Guanipa-Ortiz et al.[129] 2019	diode + CPP–ACPF paste	808 nm 4 J/cm^2	GC MI Paste Plus	a 77% reduction in DH, improved oral health scores
Jomaa et al.[130] 2023	diode (810 nm) + fluoride gel	200–500 mW 30–60 s	1.23% NaF	long-lasting DH suppression over 9 months
Medhat et al.[131] 2024	Er:YAG + N-CAP adhesive	2,940 nm 628 J/cm^2	N-CAP	increased penetration and occlusion (SEM)

CPP–ACPF – casein phosphopeptide–amorphous calcium phosphate fluoride; N-CAP – nanocarbonate apatite.

individualized by wavelength, energy and treatment protocol, offers a valuable tool in the management of DH. The promising outcomes are observed in combination strategies that integrate lasers with topical agents, simultaneously targeting neural modulation and dentinal tubule sealing.[116,117,128]

Photodynamic therapy in carious lesions

Scientific publications support the idea that photodynamic therapy (PDT) can play a beneficial role in caries treatment, predominantly by reducing the microbial load in deep lesions and supporting conservative, minimally invasive dentistry approaches (Table 8).[134–142]

Table 8. Summary of different goals and outcomes of photodynamic therapy (PDT) in the literature

PDT in caries treatment	Evidence highlights
Antimicrobial potential	shown consistently in lab and early clinical use[134,135,138,140,142]
Temporary microbial reduction	effective reduction of CFU, but DNA presence may persist[135,140,142]
Additional benefits in pediatric cases	reduced tissue removal, improved margins, short-term microbial decline[134,138,139]
Protocol variability	wide variability – photosensitizer, light type, energy, timing[140–142]
Not standalone treatment	best used alongside caries management (e.g., selective excavation, restoration)[134–142]
Lack of standardization	no universal protocol exists yet[141,142]

CFU – colony-forming units.

Literature reports

Photodynamic therapy is gaining attention as an adjunctive method for managing carious lesions, mainly because of its antimicrobial activity and its compatibility with minimally invasive treatment strategies. A growing number of laboratory and clinical studies demonstrate that antimicrobial photodynamic therapy (aPDT) can reduce cariogenic microorganisms, such as *Streptococcus mutans* and *Lactobacillus* spp., in infected dentin.[134–136] Although the degree of this reduction varies across studies, aPDT generally enhances disinfection when combined with the selective removal of carious tissue.[135]

In pediatric dentistry, aPDT has shown particularly encouraging results. Randomized clinical trials on primary molars indicate that aPDT used alongside selective caries removal improves microbial control and may support better restoration performance during follow-up.[134,137–139] One 12-month clinical study reported favorable marginal integrity of the composite restorations placed after aPDT, suggesting a potential clinical benefit beyond microbial reduction.[139] Other investigations similarly show reduced bacterial load in deep lesions and good short-term clinical outcomes, including maintained pulp vitality.[138] In vitro research confirms that aPDT can be effective against cariogenic biofilms, although the outcome is strongly dependent on the photosensitizer concentration, wavelength and irradiation time.[140,141] Systematic reviews emphasize the promise of PDT, while highlighting that inconsistent protocols and relatively few high-quality trials still limit firm clinical recommendations.[141,142] Given the current evidence, PDT should be considered an adjunctive approach rather than a replacement for conventional caries management. When used together with selective excavation and standard restorative procedures, PDT may enhance dentin disinfection and support improved treatment outcomes.[134–142]

Conclusion

There is scientific evidence that PDT can play a beneficial role in caries treatment, primarily by reducing the microbial load in deep lesions and supporting conservative, minimally invasive dentistry approaches. Additionally, PDT has been associated with clinical improvement in restoration margins over time. However, the effectiveness of the therapy remains highly dependent on protocol variables (photosensitizer choice, concentration, light wavelength/intensity, and exposure duration), and long-term outcomes and standardization are lacking.

Currently, PDT should be used as an adjunct to conventional caries treatment, rather than as a standalone approach.

Economic aspect of integrating lasers into routine dental care

Laser treatment, like many other dental procedures, requires special equipment and trained practitioners.[143] Depending on the specificity of the treatment, different types of laser might be recommended. Unfortunately, the effective cost of a laser machine ranges from a low price, as in the case of diode lasers,[144,145] and increases with the complexity of manufacturing.[146] Not only is the cost of a laser machine important, but also the accessories used could affect the final price of the treatment. An example of these accessories are delivery tips of different diameters, where a simple intervention at different areas might require more than one tip.[143] Although some tips can last for more than one procedure, these accessories, as well as handpieces with the embedded mirrors that degrade with time, will eventually need maintenance or replacement.[143] Therefore, the cost of laser treatment could be high, and thus may create a burden in some economically affected countries, since increasing the fee for

dental treatment to reflect the cost of the machine, the tips, or any other accessories is difficult to justify. Consequently, in these countries, laser educational programs in universities should be the first step toward the integration of laser concepts, preparing practitioners to acquire laser practice in their dental cabinet, starting from low-cost diode lasers.[144,145] Moreover, manufacturers should increase their efforts and assure special prices for newly interested societies in laser treatment or through government subsidies, and couple their machines with long-term warranty, as well as enlarge their infrastructure for proper maintenance and repair to support dentists continuing laser treatment within their dental procedures.

Discussion with a global conclusion

This consensus report provides comprehensive and evidence-based guidance on the use of laser technologies in caries prevention and management. The integration of lasers, ranging from erbium and diode systems to argon and CO_2 devices, demonstrates a significant promise in enhancing enamel acid resistance, improving fluoride uptake, facilitating minimally invasive caries removal, and optimizing restorative outcomes. Laser-assisted strategies also offer adjunctive benefits in treating DH, and preparing tooth surfaces for sealants and bonding agents.

While diverse protocols and device types are currently in use, their clinical success hinges on precise parameter control and proper technique selection. Synergistic approaches combining lasers with conventional agents such as fluoride varnishes, sealants or desensitizers show the greatest potential for durable, patient-centered outcomes. Despite the encouraging data, gaps remain in standardization and long-term validation.

The WFLD endorses continuous clinical education, research and collaboration to refine laser protocols, promote safety and expand global accessibility. As part of an integrated preventive model, laser-assisted dentistry represents a valuable complement to traditional practices, reinforcing the goal of preserving healthy tooth structure and improving overall oral health.

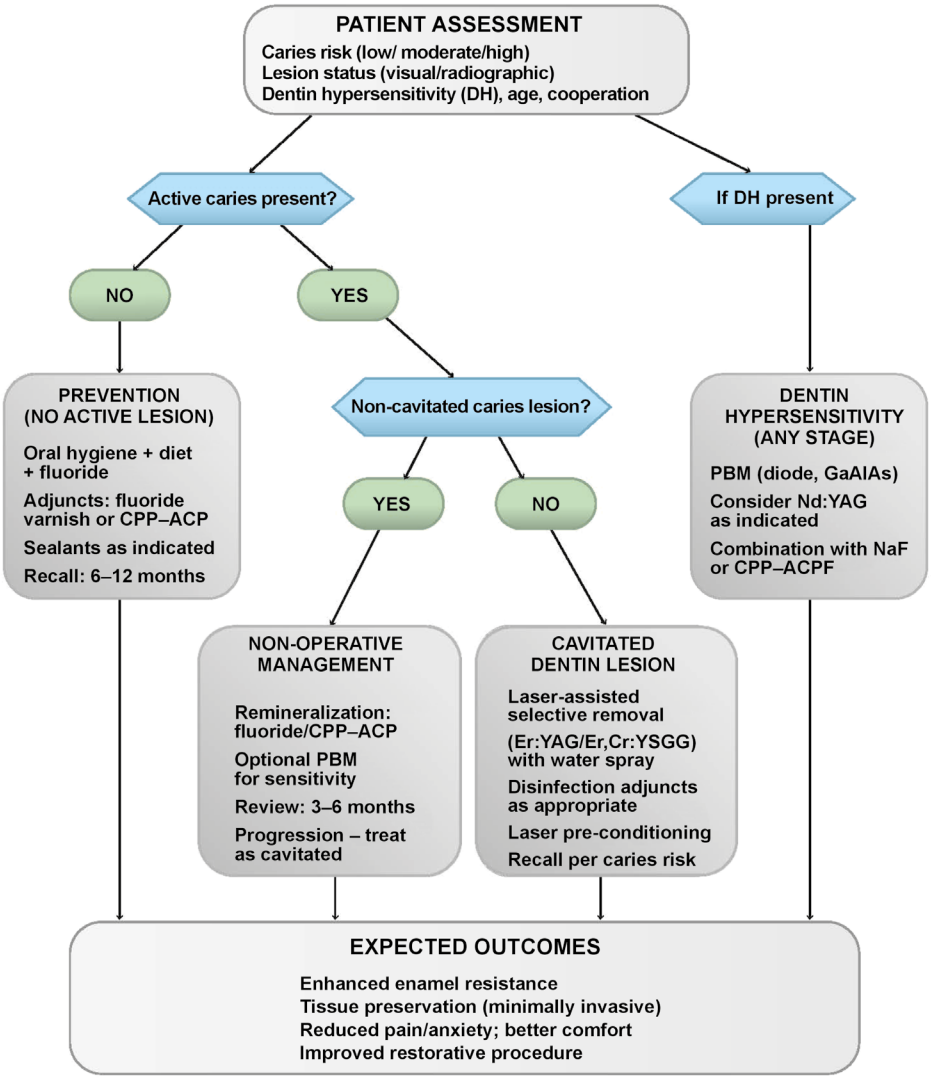

Fig. 1. Flow diagram: Laser-assisted caries treatment and prevention

Although numerous in vitro and laboratory studies demonstrate promising effects of laser-assisted protocols in enhancing enamel resistance, facilitating remineralization and improving restorative outcomes, the number of well-designed clinical trials remains limited. Consequently, the current evidence base is stronger in experimental settings than in real-world clinical applications, which represents a significant limitation of this consensus report.

The adoption of laser-assisted dentistry is limited by the high cost of devices, variability between systems, and the operator learning curve that requires specific training. The consensus was developed using a modified Delphi process, which ensured transparency, but did not include multiple structured voting rounds. Furthermore, much of the supporting evidence is derived from laboratory studies, while high-quality randomized controlled clinical trials remain scarce. Despite these limitations, this report provides the most comprehensive synthesis to date, and offers a valuable framework for future research and clinical guidelines.

While laboratory data provides encouraging support for the potential of laser-assisted caries prevention and management, robust clinical trials and long-term follow-up studies are still required to confirm these benefits and to establish standardized, evidence-based protocols for daily practice.

Figure 1 presents a flow diagram of the clinical decision-making process for laser-assisted caries treatment and prevention.

Ethics approval and consent to participate

Not applicable.

Data availability

Not applicable.

Consent for publication

Not applicable.

Use of AI and AI-assisted technologies

Not applicable.

ORCID iDs

Samir Nammour https://orcid.org/0000-0003-0321-9764
Aldo Brugnera Junior https://orcid.org/0000-0002-8634-743X
Toni Zeinoun https://orcid.org/0000-0002-8715-5392
Jacek Matys https://orcid.org/0000-0002-3801-0218
Sonia Bordin-Aykroyd https://orcid.org/0000-0003-2989-3431
Paul Nahas https://orcid.org/0000-0002-8303-5107
Marwan El Mobadder https://orcid.org/0000-0003-1813-2688
Sajee Sattayut https://orcid.org/0000-0001-7111-9381
Darinca Carmen Todea https://orcid.org/0000-0002-1654-4067
Akira Aoki https://orcid.org/0009-0000-4468-5118
Kinga Grzech-Leśniak https://orcid.org/0000-0002-5700-4577

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Original paper

New algorithm/tool used to achieve the periodontal risk assessment, diagnosis and prognosis (GF-PeDRA©): A clinical study with 221 patients

Gustavo Vicentis de Oliveira Fernandes[1,A–F], Juliana Campos Hasse Fernandes[2,A–F]

[1] Missouri School of Dentistry and Oral Health, A.T. Still University, St. Louis, USA
[2] Private researcher, St. Louis, USA

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation;
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Address for correspondence
Gustavo Vicentis de Oliveira Fernandes
E-mail: gustfernandes@gmail.com

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Abstract

Background. Periodontal diagnosis and risk assessment are extremely important to assess the individual likelihood of developing periodontal disease or experiencing its progression.

Objectives. The goal was to introduce and validate a new algorithm through providing the periodontal/peri-implant diagnosis (comparing the one by the professionals vs. the automated tool), risk assessment and prognosis, and to establish cut-off limits with a new scoring system.

Material and methods. GF-PeDRA© has 18 parameters to be assessed, achieving an octadecagon picture. The parameters are as follows: the probing depth (PD); the number of interproximal sites with bone loss; clinical attachment loss (CAL); radiographic bone loss (RBL); bleeding on probing (BoP); the bone loss pattern; tooth loss; the evidence of progression; the need for complex rehabilitation; the patient's age; biofilm accumulation; smoking; diabetes; extension and distribution; peri-implant disease; other systemic conditions; furcation involvement; and necrotizing lesions. The new scoring system, GF-PeDRA©, is based on the percentage of the octadecagon area obtained: for areas ≥0% and ≤9%, the prognosis is good; ≥10% and ≤24%, fair; ≥25% and ≤37%, poor; ≥38% and ≤49%, questionable; and ≥50%, hopeless.

Results. A total of 221 patients were included, with 34 (15.38%) smokers and 28 (12.67%) diabetics. The evaluators individually achieved the diagnosis ($\kappa = 0.83$); therefore, 37 out of 221 cases were revised, and the final clinical diagnosis was established. Afterward, all information was inserted into GF-PeDRA© to obtain an automated diagnosis. Comparing them (the professionals vs. GF-PeDRA©), the total agreement level was achieved ($\kappa = 1.0$). The average GF-PeDRA© score was 28.64%, with a median (*Me*) of 32.2%. Forty-eight (21.72%) patients were classified as having a good prognosis for periodontal treatment, 43 (19.46%) had a fair prognosis, 43 (19.46%) had a poor prognosis, 68 (30.77%) had a questionable prognosis, and 19 (8.60%) had a hopeless prognosis.

Conclusions. GF-PeDRA© proved to be a helpful tool in diagnosing, and providing risk assessment and prognosis. New clinical studies must be conducted to validate the presented GF-PeDRA© scoring system.

Keywords: diagnosis, prognosis, risk assessment, algorithm, periodontics

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Highlights

- GF-PeDRA© is a reliable diagnostic tool: The new algorithm showed perfect agreement (κ = 1.0) with the professional periodontal and peri-implant diagnoses, confirming its accuracy and potential as a digital aid for clinicians.
- Comprehensive, parameter-based assessment: By integrating 18 clinical and systemic parameters into an octadecagon model, GF-PeDRA© allows the simultaneous evaluation of diagnosis, risk and prognosis, offering a holistic view of a patient's periodontal status.
- Innovative prognostic scoring system: The percentage-based scoring approach effectively stratifies prognosis from good to hopeless, yet further clinical studies are needed to validate and refine its predictive value in diverse patient populations.

Introduction

The new classification system for periodontal and peri-implant diseases and conditions was introduced in 2018, following an international workshop's deliberations and consensus reports.[1] It is the most evidence-based and clinically relevant system ever proposed. It is considered the first major update to the classification since 1999.[2] Since then, educational institutions and dentists have been utilizing this new classification, following the stipulated principles. It comprises the reclassification of disease modalities into novel schemes, including staging and grading for periodontitis, indicating the severity and extent of the disease, and considering the patient's overall health status.[3] As with all new system implementations, a learning curve is inevitably necessary through the experiences and correct interpretations of the guidelines.

Predictive, preventive, personalized, and participatory periodontology ('5Ps')[4] represents the future of periodontics. A predictive approach using high-tech tools for diagnosis permits a better detection of patients at risk and the early diagnosis of periodontitis/peri-implantitis, when it is easier to treat it successfully. It is organized as personalized prevention based on a single patient's genetic and microbiological status,[5,6] and customized therapy tailored to the medical reality of the specific patient. Finally, the patient's active role can be emphasized through participatory collaboration.

Risk assessment for periodontal/peri-implant treatment has become essential in determining predictability. Periodontal risk assessment is a systematic approach to evaluating the individual likelihood of developing periodontal disease or experiencing its progression. This process is essential for identifying at-risk individuals and implementing preventive or therapeutic interventions tailored to their needs. Several periodontal risk assessment tools have already been developed and validated.[7,8] A systematic review from 2015 addressed 5 risk assessment tools.[8] The most often used and widely accepted one is the periodontal risk assessment (PRA) tool.[9] It is considered as a valid system, enabling the identification of patients at high risk for periodontal re-infection and progression after treatment with the use of only 6 criteria.[9]

However, facing all advances in the periodontal/peri-implant classification, employing only several parameters or analyzing only some factors to predict a periodontal risk can be insufficient to reflect a "total" reality about the patient's periodontal/peri-implant condition. Without considering other parameters, such as non-chronic or necrotizing forms of periodontitis, additional complex clinical information that is often difficult to obtain, or other potential risk factors (e.g., environmental exposure and genetic predispositions), the assessment of the patient's condition may be incomplete. Addressing these requirements is essential for developing an unbiased prognostic system.

Therefore, the goal of the present study was to introduce and validate a new algorithm/tool through providing (1) the periodontal/peri-implant diagnosis (comparing the professional (specialist) one vs. the automated tool), (2) risk assessment and prognosis, as well as (3) to establish cut-off limits for a clinically significant disease with a new scoring system (GF – Periodontal Diagnosis and Risk Assessment (GF-PeDRA©)). To our knowledge, this is the first automated tool described in the literature for periodontal and peri-implant diagnosis and risk assessment, integrating 18 parameters into a dynamic spider chart that automatically updates, thereby enhancing clinical interpretation, education, and monitoring of disease progression and risk factors.

Material and methods

The study was approved by the local research ethics committee at A.T. Still University, St. Louis, USA (No. of approval: ATSU – IRB GF20240929-001), and was conducted in compliance with Good Clinical Practice, the Declaration of Helsinki and the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines.[10] Prior to the commencement of the study, all patients signed the informed consent to participate, permitting their inclusion. The recruitment period and study duration ranged from April 2022 to July 2023. All participants were evaluated by an expert/specialist in periodontics (G.V.O.F., over 16 years of experience) and

individually revised by a general dentist (J.C.H.F., over 14 years of clinical experience); in case of any divergence, the case was revisited and discussed until clarification and definition were achieved. The validation sample comprised 221 patients.

Eligibility criteria

The included patients were ≥18 years old, and were periodontally evaluated in the university clinic during the period of recruitment and assessment, without any restrictions for a systemic condition or language. Patients who did not wish to participate in the study and refused to sign the informed consent were excluded.

GF-PeDRA© – presentation and variables

In line with the multifactorial nature of periodontal and peri-implant diseases, no single factor can be solely responsible for their development. A literature-based analysis identified 18 systemic and local predictors with suggested options, which were subsequently incorporated into this tool, producing an octadecagon (an eighteen-sided polygon) representation(Fig. 1):

1. Highest probing depth (PD) value – A. 0–3 mm; B. 4 mm; C. 5 mm; D. 6 mm; and E. >6 mm;

2. Number of interproximal sites with bone loss – A. 0; B. 1; C. 2; D. >2 and <8; E. ≥8 and <12; and F. ≥12;

3. Highest clinical attachment loss (CAL) value – A. 0; B. 1–2 mm; C. 3–4 mm; and D. >4 mm;

4. Maximum radiographic bone loss (RBL), represented by the percentage (%) of bone loss (it was calculated following the original recommendations of the classification system) – A. 0%; B. 5%; C. 10%; D. 12%; E. 14%; F. 15%; G. 21%; H. 28%; I. 34%; J. 40%; K. 46%; L. 51%; M. 56%; N. 61%; O. 66%; P. 71%; Q. 76%; R. 81%; S. 86%; T. 91%; and U. 100%;

5. Percentage of sites with bleeding on probing (BoP) – A. 0–3%; B. 4–7%; C. 8–9%; D. ≥10% and ≤30%; and E. >30% up to 100%;

6. Bone loss pattern (observe the overall pattern in the arches) – A. none; B. horizontal bone loss; and C. vertical bone loss;

7. Tooth loss, including periodontally hopeless teeth planned for extraction – A. none; B. none due to periodontitis; C. loss of up to 4 teeth due to periodontitis; and D. loss of 5 or more teeth due to periodontitis;

8. Evidence of progression over 5 years (progression must be observed by comparing the sites in the initial and periodontal charts after 5 years) – A. no loss; B. <2 mm; C. 2 mm; and D. >2 mm;

9. Need for complex rehabilitation – A. no need; B. <20 remaining teeth; C. masticatory dysfunction; D. bite collapse, drifting or flaring; and E. secondary occlusal trauma (mobility >II);

10. Patient's age, varying from 13 to 120 years;

11. CAL and biofilm accumulation – A. no CAL and no/low level of biofilm; B. lower CAL despite heavy biofilm deposits; C. CAL proportionate to the biofilm level; and D. higher CAL, disproportionate to the biofilm level;

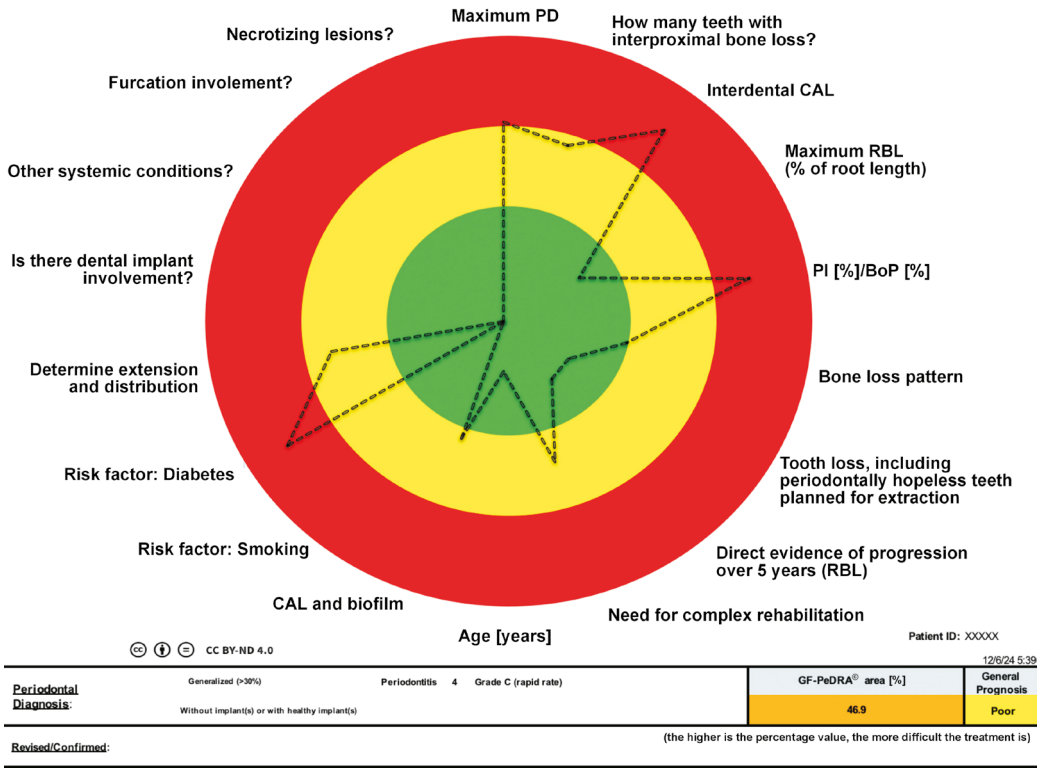

Fig. 1. GF-PeDRA© tool for periodontal diagnosis, prognosis and risk assessment with a new scoring system

PD – probing depth; CAL – clinical attachment loss; RBL – radiographic bone loss; PI – plaque index; BoP – bleeding on probing.

12. Smoking – A. non-smoker; B. <10 cigarettes/day; and C. ≥10 cigarettes/day;

13. Diabetes – A. non-diabetic (HbA1c up to 5.6%); B. HbA1c >5.6% and <7.0%; and C. HbA1c ≥ 7.0%;

14. Extension and distribution of the disease – A. healthy periodontium; B. localized (≤30%); C. generalized (>30%); D. molar–incisor (localized, ≤30%); and E. molar–incisor (generalized, >30%);

15. Peri-implant disease – A. without implant(s) or with healthy implant(s) in the mouth; B. peri-implant mucositis in up to 2 implants; C. peri-implant mucositis in 3 implants or more; D. peri-implantitis in 1 implant; E. peri-implantitis in 2 implants; F. peri-implantitis in 3 implants; and G. peri-implantitis in 4 implants or more;

16. Other systemic conditions (other than diabetes; stress, chronic obstructive pulmonary disease (COPD), cardiac disease, hyper/hypothyroidism, arthritis, atherosclerosis, respiratory disorders, gastrointestinal disorders, renal disorders, cancer, Alzheimer's disease, adverse pregnancy, immunopathies and hematologic disorders, hereditary disorders relevant to the formation and maintenance of connective tissue and bone, granulomatous disease, osteoporosis, rheumatism, inflammatory vascular disease, and Sjögren's syndrome) – A. no; B. yes, 1 or 2 (controlled); C. yes, 3 or more (controlled); D. yes, 1 or 2 (non-controlled); and E. yes, 3 or more (non-controlled);

17. Furcation involvement – A. no; B. Class I furcation (<3 mm of horizontal attachment loss); C. Class II furcation (≥3 mm of horizontal attachment loss); D. Class III furcation ('through and through' furcation involvement without direct clinical visualization); E. Class IV furcation ('through and through' furcation involvement with direct clinical visualization); and

18. Necrotizing lesions – A. no; B. gingival necrosis, gingival pain, spontaneous bleeding, the ulceration of the gingival margin, and halitosis; C. gingival necrosis, severe deep pain, spontaneous bleeding, halitosis, punched-out gingival papilla (inverted architecture), the loss of the alveolar bone, pseudo-membrane formation, lymph gland enlargement, low-grade fever.

Following the 2017 World Workshop on the Classification of Periodontal and Peri-implant Diseases (Tonetti, Greenwell & Kornman, 2018), these 18 parameters have been combined in an octadecagon that permits to provide an automated diagnosis and visualizes the risk for disease development. Each vector/factor has its own scale for risk profiles, as detailed above. For some parameters, the response is dichotomic: yes or no; and for others, there is a gradual increase according to the presentation. A comprehensive evaluation using this functional diagram provides an individual total risk profile and prognosis for periodontal treatment. The new scoring system, GF-PeDRA©, is based on the percentage of the octadecagon area obtained: for areas ≥0% and ≤9%, the prognosis is good; ≥10% and ≤24%, fair; ≥25% and ≤37%, poor; ≥38% and ≤49%, questionable; and ≥50%, hopeless. According to the data inserted for each item, a new design is presented in the form of octadecagon, suggesting a different GF-PeDRA© score. Each variable can achieve 100% in the tool's weight, totaling 18 times 100%, which is used to calculate the GF-PeDRA© score.

Table 1 presents in detail the weight applied for the calculation of the GF-PeDRA© score. (Note: All the numbers can be adjusted after a greater sample size is evaluated in future studies).

Table 1. Weight per variable used to obtain the GF-PeDRA© score

Variable analyzed	Options	Weight within the tool [%]
1. Highest PD value	A. 0–3 mm	0
	B. 4 mm	15
	C. 5 mm	50
	D. 6 mm	80
	E. >6 mm	100
2. Number of interproximal sites with bone loss	A. 0	0
	B. 1	10
	C. 2	20
	D. >2 and <8	50
	E. ≥8 and <12	75
	F. ≥12	100
3. Highest CAL value	A. 0	0
	B. 1–2 mm	35
	C. 3–4 mm	70
	D. >4 mm	100
4. Maximum RBL	A. 0%	0
	B. 5%	5
	C. 10%	8
	D. 12%	12
	E. 14%	15
	F. 15%	20
	G. 21%	25
	H. 28%	30
	I. 34%	35
	J. 40%	40
	K. 46%	45
	L. 51%	50
	M. 56%	55
	N. 61%	60
	O. 66%	65
	P. 71%	70
	Q. 76%	75
	R. 81%	80
	S. 86%	90
	T. 91%	95
	U. 100%	100
5. Percentage of sites with BoP	A. 0–3%	0
	B. 4–7%	10
	C. 8–9%	40
	D. ≥10% and ≤30%	80
	E. >30% up to 100%	100
6. Bone loss pattern	A. none	0
	B. horizontal bone loss	50
	C. vertical bone loss	100
7. Tooth loss, including periodontally hopeless teeth planned for extraction	A. none	0
	B. none due to periodontitis	30
	C. loss of up to 4 teeth due to periodontitis	70
	D. loss of 5 or more teeth due to periodontitis	100
8. Evidence of progression over 5 years	A. no loss	0
	B. <2 mm	30
	C. 2 mm	70
	D. >2 mm	100

Table 1. - continuation

Variable analyzed	Options	Weight within the tool [%]
9. Need for complex rehabilitation	A. no need	0
	B. <20 remaining teeth	60
	C. masticatory dysfunction	80
	D. bite collapse, drifting or flaring	100
	E. secondary occlusal trauma (mobility >II)	100
10. Patient's age (between 13 and 120 years)	≥13 and ≤52	20
	≥53 and ≤82	40
	≥83 and ≤102	60
	≥103 and ≤114	85
	≥115 and ≤120	100
11. CAL and biofilm accumulation	A. no CAL and no/low level of biofilm	0
	B. lower CAL despite heavy biofilm deposits	25
	C. CAL proportionate to the biofilm level	50
	D. higher CAL, disproportionate to the biofilm level	100
12. Smoking	A. non-smoker	0
	B. <10 cigarettes/day	50
	C. ≥10 cigarettes/day	100
13. Diabetes	A. non-diabetic (HbA1c up to 5.6%)	0
	B. HbA1c >5.6% and <7.0%	50
	C. HbA1c ≥ 7.0%	100
14. Extension and distribution of the disease	A. healthy periodontium	0
	B. localized (≤30%)	30
	C. generalized (>30%)	70
	D. molar–incisor (localized, ≤30%)	70
	E. molar–incisor (generalized, >30%)	100
15. Peri-implant disease	A. without implant(s) or with healthy implant(s) in the mouth	0
	B. peri-implant mucositis in up to 2 implants	10
	C. peri-implant mucositis in 3 implants or more	30
	D. peri-implantitis in 1 implant	40
	E. peri-implantitis in 2 implants	60
	F. peri-implantitis in 3 implants	80
	G. peri-implantitis in 4 implants or more	100
16. Other systemic conditions (other than diabetes)	A. no	0
	B. yes, 1 or 2 (controlled)	30
	C. yes, 3 or more (controlled)	60
	D. yes, 1 or 2 (non-controlled)	80
	E. yes, 3 or more (non-controlled)	100
17. Furcation involvement	A. no	0
	B. Class I furcation (<3 mm of horizontal attachment loss)	20
	C. Class II furcation (≥3 mm of horizontal attachment loss)	50
	D. Class III furcation ('through and through' furcation involvement without direct clinical visualization)	85
	E. Class IV furcation ('through and through' furcation involvement with direct clinical visualization)	100
18. Necrotizing lesions	A. no	0
	B. gingival necrosis, gingival pain, spontaneous bleeding, the ulceration of the gingival margin, and halitosis	70
	C. gingival necrosis, severe deep pain, spontaneous bleeding, halitosis, punched-out gingival papilla (inverted architecture), the loss of alveolar bone, pseudo-membrane formation, lymph gland enlargement, low-grade fever	100

Statistical analysis

A descriptive analysis was performed. The data retrieved was uploaded into the Excel software (v. 16.91, Microsoft Office; Microsoft Corporation, Redmond, USA). Inter-rater agreement among the professionals was assessed using Cohen's kappa test, followed by the comparison of the results obtained by the professionals and those generated by the automated GF-PeDRA© tool.

Results

Demographic data

A total of 221 patients were enrolled (age – median (*Me*): 46 years; mode: 30;58), 42.4% male and 57.6% female. All demographic data is included in Table 2.

Table 2. Demographic data of the participants

Parameters		Data
Gender	M	42.4%
	F	57.6%
Mean age [years]		46.73 (range: 18–93)
No. of teeth assessed		5,301
Subgroups (No. of patients by age)	≥18 and ≤20	18
	≥21 and ≤30	35
	≥31 and ≤40	35
	≥41 and ≤50	37
	≥51 and ≤60	34
	>60	62
Smoker?	non-smokers	187 (84.62%)
	smokers	34 (15.38%) 21: <10 cigarettes/day 13: ≥10 cigarettes/day
Diabetic?	non-diabetics	193 (87.33%)
	diabetics	28 (12.67%) 20: HbA1c >5.6% and <7.0% 8: HbA1c ≥ 7.0%

M – male; F – female.

Clinical data

A total of 28 patients were diagnosed as periodontally healthy, 55 with plaque-induced gingivitis, and 138 with periodontitis. When stratifying the periodontitis cases, 33 patients were diagnosed with Periodontitis I (A = 10; B = 19; and C = 4); 18 had Periodontitis II (A = 1; B = 11; and C = 6); 35 had Periodontitis III (A = 3; B = 20; and C = 12); and 52 had Periodontitis IV (A = 0; B = 35; and C = 17). Only one case of molar/incisor pattern was observed. No peri-implant disease or necrotizing condition was found.

The mean CAL value found was 3.19 mm (min: 0 mm; max: 14 mm; *Me:* 2 mm; mode: 0 mm); the mean number of non-adjacent interdental surfaces with interproximal bone loss was 6 per patient. The bone loss pattern was horizontal in 135 cases (61.09%), and in 3 cases (1.36%), vertical. Twenty-five (11.31%) patients had furcation involvement. The mean percentage of BoP was 28.67% (min: 0%; max: 100%; *Me*: 19%; mode = 15%). The mean PD was 5.31 mm (min: 2 mm; max: 14 mm; *Me*: 5 mm; mode: 5 mm) (supplementary material, available from the corresponding author upon reasonable request).

GF-PeDRA© score and inter-agreement level

Comparing the diagnoses individually achieved by the evaluators, there was a good level of agreement (κ = 0.83). Therefore, 37 out of 221 patients were revised (Table 3, red letters), and the cases were discussed to reach a tie-break and establish the final clinical periodontal diagnosis. Afterward, all information was inserted into the algorithm/tool (GF-PeDRA©) and a diagnosis for each patient was automatically obtained; the GF-PeDRA© diagnosis was compared to the final clinical periodontal diagnosis made by the professionals, resulting in a perfect agreement level (100%, κ = 1.0) (Table 3).

Table 3. Diagnosis provided by the evaluators and the GF-PeDRA© tool, along with the GF-PeDRA© score for prognosis.

Patient number	Diagnosis (GVdOF)	Diagnosis (JCHF)	Final diagnosis (after discussion)	Diagnosis (GF-PeDRA©)	GF-PeDRA© score (0–100%)	Prognosis
1	Periodontitis III-C	Periodontitis IV-C	Periodontitis III-C	Periodontitis III-C	41.4	Questionable
2	Periodontitis IV-B	Periodontitis IV-B	Periodontitis IV-B	Periodontitis IV-B	47.9	Questionable
3	Periodontitis IV-B	Periodontitis IV-B	Periodontitis IV-B	Periodontitis IV-B	41.0	Questionable
4	Gingivitis	Gingivitis	Gingivitis	Gingivitis	16.8	Fair
5	Gingivitis	Gingivitis	Gingivitis	Gingivitis	15.4	Fair
6	Gingivitis	Healthy periodontium	Gingivitis	Gingivitis	9.4	Good
7	Gingivitis	Gingivitis	Gingivitis	Gingivitis	15.4	Fair
8	Periodontitis III-C	Periodontitis IV-C	Periodontitis IV-C	Periodontitis IV-C	48.8	Questionable
9	Periodontitis I-B	Periodontitis I-B	Periodontitis I-B	Periodontitis I-B	25.4	Poor
10	Gingivitis	Gingivitis	Gingivitis	Gingivitis	12.7	Fair
11	Periodontitis IV-C	Periodontitis IV-C	Periodontitis IV-C	Periodontitis IV-C	51.5	Hopeless
12	Gingivitis	Gingivitis	Gingivitis	Gingivitis	17.4	Fair
13	Periodontitis I-B	Periodontitis II-B	Periodontitis I-B	Periodontitis I-B	36.7	Poor
14	Gingivitis	Gingivitis	Gingivitis	Gingivitis	11.2	Fair
15	Periodontitis III-C	Periodontitis III-C	Periodontitis III-C	Periodontitis III-C	39.8	Questionable
16	Periodontitis III-B	Periodontitis III-B	Periodontitis III-B	Periodontitis III-B	32.4	Poor
17	Gingivitis	Gingivitis	Gingivitis	Gingivitis	7.4	Good
18	Gingivitis	Gingivitis	Gingivitis	Gingivitis	7.4	Good
19	Healthy periodontium	Healthy periodontium	Healthy periodontium	Healthy periodontium	1.7	Good
20	Periodontitis IV-B	Periodontitis IV-B	Periodontitis IV-B	Periodontitis IV-B	46.6	Questionable
21	Periodontitis IV-B	Periodontitis IV-B	Periodontitis IV-B	Periodontitis IV-B	48.2	Questionable
22	Gingivitis	Gingivitis	Gingivitis	Gingivitis	11.4	Fair
23	Periodontitis III-B	Periodontitis III-B	Periodontitis III-B	Periodontitis III-B	40.2	Questionable
24	Gingivitis	Gingivitis	Gingivitis	Gingivitis	17.1	Fair
25	Periodontitis IV-B	Periodontitis IV-B	Periodontitis IV-B	Periodontitis IV-B	44.8	Questionable
26	Periodontitis IV-C	Periodontitis IV-C	Periodontitis IV-C	Periodontitis IV-C	59.8	Hopeless
27	Gingivitis	Gingivitis	Gingivitis	Gingivitis	7.4	Good
28	Periodontitis I-B	Periodontitis I-B	Periodontitis I-B	Periodontitis I-B	24.4	Fair
29	Gingivitis	Gingivitis	Gingivitis	Gingivitis	9.7	Good
30	Periodontitis II-B	Periodontitis II-B	Periodontitis II-B	Periodontitis II-B	39.1	Questionable
31	Periodontitis I-B	Periodontitis I-B	Periodontitis I-B	Periodontitis I-B	27.1	Poor
32	Periodontitis II-B	Periodontitis II-B	Periodontitis II-B	Periodontitis II-B	26.8	Poor
33	Healthy periodontium	Healthy periodontium	Healthy periodontium	Healthy periodontium	7.4	Good

Patient number	Diagnosis (GVdOF)	Diagnosis (JCHF)	Final diagnosis (after discussion)	Diagnosis (GF-PeDRA©)	GF-PeDRA© score (0–100%)	Prognosis
34	Gingivitis	Gingivitis	Gingivitis	Gingivitis	11.7	Fair
35	Periodontitis III-B	Periodontitis IV-B	Periodontitis IV-B	Periodontitis IV-B	45.7	Questionable
36	Gingivitis	Gingivitis	Gingivitis	Gingivitis	11.2	Fair
37	Gingivitis	Gingivitis	Gingivitis	Gingivitis	13.2	Fair
38	Periodontitis I-C	Periodontitis I-C	Periodontitis I-C	Periodontitis I-C	37.7	Poor
39	Healthy periodontium	Healthy periodontium	Healthy periodontium	Healthy periodontium	2.9	Good
40	Periodontitis IV-C	Periodontitis IV-C	Periodontitis IV-C	Periodontitis IV-C	64.1	Hopeless
41	Healthy periodontium	Gingivitis	Healthy periodontium	Healthy periodontium	1.5	Good
42	Healthy periodontium	Healthy periodontium	Healthy periodontium	Healthy periodontium	7.4	Good
43	Gingivitis	Gingivitis	Gingivitis	Gingivitis	13.5	Fair
44	Healthy periodontium	Gingivitis	Healthy periodontium	Healthy periodontium	3.2	Good
45	Gingivitis	Gingivitis	Gingivitis	Gingivitis	11.2	Fair
46	Healthy periodontium	Healthy periodontium	Healthy periodontium	Healthy periodontium	0.6	Good
47	Gingivitis	Gingivitis	Gingivitis	Gingivitis	13.2	Fair
48	Gingivitis	Gingivitis	Gingivitis	Gingivitis	6.4	Good
49	Gingivitis	Gingivitis	Gingivitis	Gingivitis	9.7	Good
50	Periodontitis IV-B	Periodontitis IV-C	Periodontitis IV-C	Periodontitis IV-C	43.4	Questionable
51	Gingivitis	Gingivitis	Gingivitis	Gingivitis	11.2	Fair
52	Periodontitis II-B	Periodontitis III-B	Periodontitis II-B	Periodontitis II-B	32.1	Poor
53	Periodontitis IV-B	Periodontitis IV-B	Periodontitis IV-B	Periodontitis IV-B	45.6	Questionable
54	Gingivitis	Healthy periodontium	Gingivitis	Gingivitis	9.7	Good
55	Healthy periodontium	Healthy periodontium	Healthy periodontium	Healthy periodontium	1.0	Good
56	molar/incisor Periodontitis III-C	molar/incisor Periodontitis III-C	molar/incisor Periodontitis III-C	molar/incisor Periodontitis III-C	52.6	Hopeless
57	Gingivitis	Gingivitis	Gingivitis	Gingivitis	7.4	Good
58	Periodontitis III-C	Periodontitis IV-C	Periodontitis III-C	Periodontitis III-C	40.2	Questionable
59	Gingivitis	Gingivitis	Gingivitis	Gingivitis	13.5	Fair
60	Gingivitis	Gingivitis	Gingivitis	Gingivitis	10.2	Fair
61	Periodontitis II-C	Periodontitis II-C	Periodontitis II-C	Periodontitis II-C	41.3	Questionable
62	Periodontitis III-B	Periodontitis III-B	Periodontitis III-B	Periodontitis III-B	36.2	Poor
63	Periodontitis III-C	Periodontitis III-C	Periodontitis III-C	Periodontitis III-C	41.6	Questionable
64	Gingivitis	Gingivitis	Gingivitis	Gingivitis	9.7	Good
65	Gingivitis	Gingivitis	Gingivitis	Gingivitis	11.2	Fair
66	Periodontitis I-B	Periodontitis I-B	Periodontitis I-B	Periodontitis I-B	31.2	Poor
67	Periodontitis I-C	Periodontitis I-B	Periodontitis I-C	Periodontitis I-C	36.1	Poor
68	Gingivitis	Gingivitis	Gingivitis	Gingivitis	12.3	Fair
69	Periodontitis I-A	Periodontitis I-A	Periodontitis I-A	Periodontitis I-A	31.5	Poor
70	Periodontitis III-C	Periodontitis III-C	Periodontitis III-C	Periodontitis III-C	45.0	Questionable
71	Periodontitis II-B	Periodontitis II-B	Periodontitis II-B	Periodontitis II-B	39.0	Questionable
72	Periodontitis IV-C	Periodontitis IV-C	Periodontitis IV-C	Periodontitis IV-C	46.8	Questionable
73	Periodontitis III-C	Periodontitis III-C	Periodontitis III-C	Periodontitis III-C	45.0	Questionable
74	Gingivitis	Gingivitis	Gingivitis	Gingivitis	9.4	Good
75	Periodontitis I-B	Periodontitis IV-B	Periodontitis I-B	Periodontitis I-B	38.5	Questionable
76	Periodontitis II-C	Periodontitis II-C	Periodontitis II-C	Periodontitis II-C	35.2	Poor
77	Periodontitis I-C	Periodontitis I-C	Periodontitis I-C	Periodontitis I-C	34.1	Poor
78	Periodontitis IV-B	Periodontitis IV-B	Periodontitis IV-B	Periodontitis IV-B	49.6	Questionable
79	Healthy periodontium	Healthy periodontium	Healthy periodontium	Healthy periodontium	3.5	Good
80	Healthy periodontium	Gingivitis	Healthy periodontium	Healthy periodontium	12.4	Fair

Patient number	Diagnosis (GVdOF)	Diagnosis (JCHF)	Final diagnosis (after discussion)	Diagnosis (GF-PeDRA©)	GF-PeDRA© score (0–100%)	Prognosis
81	Periodontitis IV-C	Periodontitis IV-B	Periodontitis IV-B	Periodontitis IV-B	39.0	Questionable
82	Periodontitis III-B	Periodontitis IV-B	Periodontitis III-B	Periodontitis III-B	43.3	Questionable
83	Periodontitis IV-C	Periodontitis IV-C	Periodontitis IV-C	Periodontitis IV-C	55.5	Hopeless
84	Periodontitis IV-B	Periodontitis IV-B	Periodontitis IV-B	Periodontitis IV-B	41.0	Questionable
85	Periodontitis II-A	Periodontitis II-A	Periodontitis II-A	Periodontitis II-A	42.3	Questionable
86	Periodontitis I-A	Periodontitis I-A	Periodontitis I-A	Periodontitis I-A	35.9	Poor
87	Periodontitis II-B	Periodontitis II-B	Periodontitis II-B	Periodontitis II-B	41.5	Questionable
88	Periodontitis II-B	Periodontitis II-B	Periodontitis II-B	Periodontitis II-B	33.5	Poor
89	Periodontitis III-B	Periodontitis III-B	Periodontitis III-B	Periodontitis III-B	40.6	Questionable
90	Periodontitis IV-C	Periodontitis IV-C	Periodontitis IV-C	Periodontitis IV-C	63.3	Hopeless
91	Periodontitis II-B	Periodontitis II-B	Periodontitis II-B	Periodontitis II-B	40.3	Questionable
92	Gingivitis	Gingivitis	Gingivitis	Gingivitis	17.4	Fair
93	Gingivitis	Gingivitis	Gingivitis	Gingivitis	15.4	Fair
94	Periodontitis I-A	Periodontitis II-A	Periodontitis I-A	Periodontitis I-A	26.8	Poor
95	Periodontitis III-B	Periodontitis III-B	Periodontitis III-B	Periodontitis III-B	29.4	Poor
96	Healthy periodontium	Healthy periodontium	Healthy periodontium	Healthy periodontium	3.5	Good
97	Periodontitis I-B	Periodontitis I-B	Periodontitis I-B	Periodontitis I-B	26.3	Poor
98	Gingivitis	Gingivitis	Gingivitis	Gingivitis	7.4	Good
99	Periodontitis I-B	Periodontitis I-B	Periodontitis I-B	Periodontitis I-B	27.7	Poor
100	Gingivitis	Healthy periodontium	Gingivitis	Gingivitis	7.4	Good
101	Periodontitis IV-B	Periodontitis IV-B	Periodontitis IV-B	Periodontitis IV-B	53.1	Hopeless
102	Periodontitis III-B	Periodontitis III-B	Periodontitis III-B	Periodontitis III-B	48.4	Questionable
103	Periodontitis I-A	Periodontitis I-A	Periodontitis I-A	Periodontitis I-A	32.2	Poor
104	Periodontitis III-C	Periodontitis IV-C	Periodontitis III-C	Periodontitis III-C	42.2	Questionable
105	Periodontitis IV-B	Periodontitis IV-B	Periodontitis IV-B	Periodontitis IV-B	42.9	Questionable
106	Healthy periodontium	Healthy periodontium	Healthy periodontium	Healthy periodontium	2.9	Good
107	Periodontitis III-B	Periodontitis IV-B	Periodontitis III-B	Periodontitis III-B	39.3	Questionable
108	Periodontitis IV-B	Periodontitis IV-B	Periodontitis IV-B	Periodontitis IV-B	37.3	Poor
109	Periodontitis IV-B	Periodontitis IV-B	Periodontitis IV-B	Periodontitis IV-B	52.6	Hopeless
110	Periodontitis III-B	Periodontitis III-B	Periodontitis III-B	Periodontitis III-B	43.3	Questionable
111	Periodontitis IV-C	Periodontitis IV-C	Periodontitis IV-C	Periodontitis IV-C	58.2	Hopeless
112	Healthy periodontium	Healthy periodontium	Healthy periodontium	Healthy periodontium	5.8	Good
113	Periodontitis IV-B	Periodontitis IV-B	Periodontitis IV-B	Periodontitis IV-B	46.3	Questionable
114	Periodontitis II-B	Periodontitis II-B	Periodontitis II-B	Periodontitis II-B	37.6	Poor
115	Periodontitis I-B	Periodontitis I-B	Periodontitis I-B	Periodontitis I-B	30.0	Poor
116	Gingivitis	Gingivitis	Gingivitis	Gingivitis	17.4	Fair
117	Gingivitis	Gingivitis	Gingivitis	Gingivitis	9.4	Good
118	Periodontitis IV-B	Periodontitis IV-B	Periodontitis IV-B	Periodontitis IV-B	47.1	Questionable
119	Gingivitis	Gingivitis	Gingivitis	Gingivitis	8.4	Good
120	Periodontitis IV-B	Periodontitis III-B	Periodontitis IV-B	Periodontitis IV-B	50.0	Hopeless
121	Periodontitis IV-B	Periodontitis IV-B	Periodontitis IV-B	Periodontitis IV-B	50.8	Hopeless
122	Periodontitis III-C	Periodontitis III-C	Periodontitis III-C	Periodontitis III-C	40.2	Questionable
123	Periodontitis IV-C	Periodontitis IV-C	Periodontitis IV-C	Periodontitis IV-C	46.6	Questionable
124	Periodontitis IV-B	Periodontitis IV-B	Periodontitis IV-B	Periodontitis IV-B	52.7	Hopeless
125	Periodontitis I-B	Periodontitis I-B	Periodontitis I-B	Periodontitis I-B	30.2	Poor
126	Healthy periodontium	Healthy periodontium	Healthy periodontium	Healthy periodontium	5.2	Good
127	Gingivitis	Healthy periodontium	Gingivitis	Gingivitis	9.7	Good

Patient number	Diagnosis (GVdOF)	Diagnosis (JCHF)	Final diagnosis (after discussion)	Diagnosis (GF-PeDRA©)	GF-PeDRA© score (0–100%)	Prognosis
128	Periodontitis II-C	Periodontitis II-C	Periodontitis II-C	Periodontitis II-C	40.8	Questionable
129	Healthy periodontium	Healthy periodontium	Healthy periodontium	Healthy periodontium	5.8	Good
130	Periodontitis IV-B	Periodontitis IV-B	Periodontitis IV-B	Periodontitis IV-B	43.2	Questionable
131	Periodontitis III-B	Periodontitis III-B	Periodontitis III-B	Periodontitis III-B	41.9	Questionable
132	Periodontitis II-B	Periodontitis I-B	Periodontitis I-B	Periodontitis I-B	26.6	Poor
133	Periodontitis II-C	Periodontitis II-C	Periodontitis II-C	Periodontitis II-C	39.0	Questionable
134	Healthy periodontium	Healthy periodontium	Healthy periodontium	Healthy periodontium	7.5	Good
135	Periodontitis III-B	Periodontitis IV-B	Periodontitis III-B	Periodontitis III-B	39.6	Questionable
136	Periodontitis I-A	Periodontitis I-A	Periodontitis I-A	Periodontitis I-A	23.1	Fair
137	Periodontitis IV-B	Periodontitis IV-B	Periodontitis IV-B	Periodontitis IV-B	40.0	Questionable
138	Periodontitis IV-B	Periodontitis IV-B	Periodontitis IV-B	Periodontitis IV-B	37.3	Poor
139	Periodontitis III-B	Periodontitis III-B	Periodontitis III-B	Periodontitis III-B	41.6	Questionable
140	Healthy periodontium	Healthy periodontium	Healthy periodontium	Healthy periodontium	1.0	Good
141	Periodontitis II-B	Periodontitis II-B	Periodontitis II-B	Periodontitis II-B	24.9	Fair
142	Periodontitis III-B	Periodontitis III-B	Periodontitis III-B	Periodontitis III-B	34.8	Poor
143	Periodontitis I-A	Periodontitis II-A	Periodontitis I-A	Periodontitis I-A	30.1	Poor
144	Periodontitis III-B	Periodontitis III-B	Periodontitis III-B	Periodontitis III-B	39.2	Questionable
145	Periodontitis I-B	Periodontitis I-B	Periodontitis I-B	Periodontitis I-B	24.9	Fair
146	Gingivitis	Gingivitis	Gingivitis	Gingivitis	13.3	Fair
147	Healthy periodontium	Healthy periodontium	Healthy periodontium	Healthy periodontium	3.3	Good
148	Periodontitis II-C	Periodontitis II-C	Periodontitis II-C	Periodontitis II-C	35.7	Poor
149	Gingivitis	Gingivitis	Gingivitis	Gingivitis	9.4	Good
150	Gingivitis	Gingivitis	Gingivitis	Gingivitis	6.4	Good
151	Periodontitis I-B	Periodontitis I-B	Periodontitis I-B	Periodontitis I-B	29.8	Poor
152	Periodontitis I-B	Periodontitis I-B	Periodontitis I-B	Periodontitis I-B	21.6	Fair
153	Gingivitis	Gingivitis	Gingivitis	Gingivitis	9.7	Good
154	Periodontitis I-B	Periodontitis I-B	Periodontitis I-B	Periodontitis I-B	35.6	Poor
155	Periodontitis IV-B	Periodontitis IV-B	Periodontitis IV-B	Periodontitis IV-B	55.0	Hopeless
156	Gingivitis	Healthy periodontium	Gingivitis	Gingivitis	7.4	Good
157	Periodontitis IV-B	Periodontitis IV-B	Periodontitis IV-B	Periodontitis IV-B	51.7	Hopeless
158	Periodontitis IV-B	Periodontitis III-B	Periodontitis IV-B	Periodontitis IV-B	41.8	Questionable
159	Periodontitis II-C	Periodontitis II-C	Periodontitis II-C	Periodontitis II-C	35.1	Poor
160	Periodontitis IV-C	Periodontitis IV-C	Periodontitis IV-C	Periodontitis IV-C	45.5	Questionable
161	Periodontitis IV-B	Periodontitis IV-B	Periodontitis IV-B	Periodontitis IV-B	40.9	Questionable
162	Gingivitis	Gingivitis	Gingivitis	Gingivitis	7.4	Good
163	Gingivitis	Gingivitis	Gingivitis	Gingivitis	13.2	Fair
164	Gingivitis	Gingivitis	Gingivitis	Gingivitis	14.3	Fair
165	Periodontitis I-C	Periodontitis I-C	Periodontitis I-C	Periodontitis I-C	37.1	Poor
166	Healthy periodontium	Healthy periodontium	Healthy periodontium	Healthy periodontium	3.5	Good
167	Healthy periodontium	Healthy periodontium	Healthy periodontium	Healthy periodontium	5.2	Good
168	Periodontitis IV-B	Periodontitis IV-B	Periodontitis IV-B	Periodontitis IV-B	54.9	Hopeless
169	Periodontitis IV-C	Periodontitis IV-C	Periodontitis IV-C	Periodontitis IV-C	40.4	Questionable
170	Periodontitis IV-B	Periodontitis IV-B	Periodontitis IV-B	Periodontitis IV-B	44.6	Questionable
171	Periodontitis IV-C	Periodontitis IV-C	Periodontitis IV-C	Periodontitis IV-C	54.5	Hopeless
172	Periodontitis I-B	Periodontitis I-B	Periodontitis I-B	Periodontitis I-B	34.5	Poor
173	Gingivitis	Gingivitis	Gingivitis	Gingivitis	17.1	Fair
174	Periodontitis IV-B	Periodontitis IV-B	Periodontitis IV-B	Periodontitis IV-B	47.3	Questionable

Patient number	Diagnosis (GVdOF)	Diagnosis (JCHF)	Final diagnosis (after discussion)	Diagnosis (GF-PeDRA©)	GF-PeDRA© score (0–100%)	Prognosis
175	Gingivitis	Gingivitis	Gingivitis	Gingivitis	17.1	Fair
176	Periodontitis IV-B	Periodontitis IV-B	Periodontitis IV-B	Periodontitis IV-B	35.7	Poor
177	Periodontitis I-A	Periodontitis I-A	Periodontitis I-A	Periodontitis I-A	22.4	Fair
178	Periodontitis III-B	Periodontitis III-B	Periodontitis III-B	Periodontitis III-B	47.0	Questionable
179	Periodontitis III-B	Periodontitis IV-B	Periodontitis III-B	Periodontitis III-B	37.0	Poor
180	Periodontitis IV-C	Periodontitis IV-B	Periodontitis IV-B	Periodontitis IV-B	44.1	Questionable
181	Gingivitis	Healthy periodontium	Gingivitis	Gingivitis	10.0	Fair
182	Gingivitis	Gingivitis	Gingivitis	Gingivitis	15.1	Fair
183	Healthy periodontium	Healthy periodontium	Healthy periodontium	Healthy periodontium	4.9	Good
184	Periodontitis II-B	Periodontitis II-B	Periodontitis II-B	Periodontitis II-B	29.5	Poor
185			Gingivitis	Gingivitis	9.7	Good
186	Periodontitis III-A	Periodontitis III-A	Periodontitis III-A	Periodontitis III-A	38.0	Questionable
187	Periodontitis III-A	Periodontitis III-A	Periodontitis III-A	Periodontitis III-A	42.6	Questionable
188	Healthy periodontium	Healthy periodontium	Healthy periodontium	Healthy periodontium	3.3	Good
189	Periodontitis I-B	Periodontitis I-B	Periodontitis I-B	Periodontitis I-B	31.8	Poor
190	Healthy periodontium	Healthy periodontium	Healthy periodontium	Healthy periodontium	10.7	Fair
191	Periodontitis III-B	Periodontitis III-A	Periodontitis III-A	Periodontitis III-A	36.3	Poor
192	Periodontitis III-B	Periodontitis III-B	Periodontitis III-B	Periodontitis III-B	44.0	Questionable
193	Healthy periodontium	Gingivitis	Healthy periodontium	Healthy periodontium	3.5	Good
194	Gingivitis	Gingivitis	Gingivitis	Gingivitis	22.0	Fair
195	Periodontitis I-A	Periodontitis I-A	Periodontitis I-A	Periodontitis I-A	25.5	Poor
196	Periodontitis III-B	Periodontitis IV-B	Periodontitis III-B	Periodontitis III-B	41.7	Questionable
197	Healthy periodontium	Healthy periodontium	Healthy periodontium	Healthy periodontium	7.5	Good
198	Periodontitis III-B	Periodontitis III-B	Periodontitis III-B	Periodontitis III-B	42.4	Questionable
199	Periodontitis IV-C	Periodontitis IV-C	Periodontitis IV-C	Periodontitis IV-C	45.3	Questionable
200	Periodontitis III-C	Periodontitis III-C	Periodontitis III-C	Periodontitis III-C	41.2	Questionable
201	Periodontitis I-B	Periodontitis I-B	Periodontitis I-B	Periodontitis I-B	33.4	Poor
202	Healthy periodontium	Healthy periodontium	Healthy periodontium	Healthy periodontium	14.3	Fair
203	Periodontitis IV-B	Periodontitis IV-B	Periodontitis IV-B	Periodontitis IV-B	43.3	Questionable
204	Periodontitis III-C	Periodontitis III-C	Periodontitis III-C	Periodontitis III-C	47.2	Questionable
205	Periodontitis III-B	Periodontitis III-B	Periodontitis III-B	Periodontitis III-B	37.1	Poor
206	Periodontitis IV-B	Periodontitis IV-B	Periodontitis IV-B	Periodontitis IV-B	42.2	Questionable
207	Gingivitis	Gingivitis	Gingivitis	Gingivitis	9.7	Good
208	Periodontitis I-B	Periodontitis I-B	Periodontitis I-B	Periodontitis I-B	28.9	Poor
209	Periodontitis II-B	Periodontitis IV-B	Periodontitis II-B	Periodontitis II-B	39.7	Questionable
210	Gingivitis	Gingivitis	Gingivitis	Gingivitis	11.2	Fair
211	Healthy periodontium	Healthy periodontium	Healthy periodontium	Healthy periodontium	11.6	Fair
212	Healthy periodontium	Gingivitis	Healthy periodontium	Healthy periodontium	5.5	Good
213	Periodontitis IV-B	Periodontitis IV-B	Periodontitis IV-B	Periodontitis IV-B	47.1	Questionable
214	Periodontitis IV-B	Periodontitis IV-B	Periodontitis IV-B	Periodontitis IV-B	47.7	Questionable
215	Periodontitis IV-C	Periodontitis IV-C	Periodontitis IV-C	Periodontitis IV-C	51.3	Hopeless
216	Gingivitis	Gingivitis	Gingivitis	Gingivitis	9.7	Good
217	Periodontitis III-C	Periodontitis III-C	Periodontitis III-C	Periodontitis III-C	48.0	Questionable
218	Periodontitis I-A	Periodontitis II-A	Periodontitis I-A	Periodontitis I-A	19.4	Fair
219	Periodontitis I-A	Periodontitis I-A	Periodontitis I-A	Periodontitis I-A	18.2	Fair
220	Periodontitis IV-C	Periodontitis IV-C	Periodontitis IV-C	Periodontitis IV-C	51.4	Hopeless
221	Periodontitis IV-C	Periodontitis III-C	Periodontitis IV-C	Periodontitis IV-C	50.7	Hopeless

Furthermore, the new GF-PeDRA© score was achieved for each patient (a range from 0% to 100%). The mean GF-PeDRA© score was 28.64% (min: 0.6%; max: 64.1%; *Me*: 32.2%). Then, analyzing the GF-PeDRA© scores of the 221 patients enrolled, 48 (21.73%) were classified as having a good prognosis for periodontal treatment, 43 (19.46%) had a fair prognosis, 43 (19.46%) had a poor prognosis, 68 (30.77%) had a questionable prognosis, and 19 (8.60%) had a hopeless prognosis. Table 3 presents detailed results per patient.

Discussion

The concept of periodontal risk assessment was introduced as a systematic approach to evaluating the patient's risk for developing periodontal disease.[6,7] It is worth remembering that periodontal disease is a prevalent condition that affects millions of people worldwide,[11] posing significant risks to oral and systemic health. Thereby, accurate risk assessment is critical for effectively managing and preventing periodontal disease, guiding clinicians in tailoring preventive and therapeutic strategies to individual patients. Over the years, various tools have been developed and modified to enhance their predictive accuracy and clinical utility. However, given the changes in the classification system – since many existing tools were developed and reported based on older versions, and are therefore outdated despite their usefulness – and the various shortcomings observed, this study aimed to introduce, test and validate a new algorithm/tool for periodontal diagnosis, risk assessment and treatment prognosis (GF-PeDRA©).

The proposed tool provides a practical and visually intuitive chart, with one version tailored for professionals and another for patients, to facilitate the demonstration and explanation of clinical findings. To the best of our knowledge, this is the first report in the literature describing a periodontal/peri-implant diagnostic and automated tool that integrates the evaluation of an extensive set of parameters (n = 18) based on the new classification system, thereby enhancing diagnostic accuracy, educational utility, and patient comprehension of periodontal and peri-implant conditions.

Among the available tools, one of the most important is PRA,[9] which intends to help generate data and information for the clinician based on 6 parameters, whereas GF-PeDRA© has 3 times the number of parameters assessed. The criteria used in PRA are summarized in a hexagonal functional diagram, identifying patients as low-, moderate- or high-risk. All the factors evaluated in PRA were also taken into consideration in the new algorithm/tool (GF-PeDRA©): (1) the probing pocket depth (PPD); (2) tooth loss (the number of missing teeth from 1 to 28 (wisdom teeth are not included)); (3) BoP; (4) bone loss over age (bone loss/age, % alveolar bone loss) – reporting the amount of alveolar bone loss at the most advanced site in increments of 10% (in the case of periapical radiographs, the % alveolar bone loss is compared with the distance measured at 1 mm apical from the cemento-enamel junction (CEJ) to the root apex, and for bitewing radiographs, the % alveolar bone loss is calculated with 10% per 1 mm); (5) the environment considering smoking only (non-smoker; former smoker – if tobacco use cessation occurred 5 years ago or earlier; occasional smoker – up to 10 cigarettes per day; smoker – up to 20 cigarettes per day; and heavy smoker – more than 20 cigarettes per day); and (6) the systemic condition of the patient (diabetes type I or II, interleukin 1 (IL-1) gene polymorphism, or stress).

Despite its validation and widespread international use, the PRA system, when compared to more recently developed tools,[12–16] and in light of advances in research and updates to the classification system, may transmit imprecise or incomplete information to clinicians and patients, as it is based on a relatively limited set of factors. The PRA system presents several limitations. (1) It allows the selection of 2, 4 or 6 sites per tooth or implant, which may lead to inconsistencies in data collection. (2) It accounts for tooth loss without considering the underlying reason for extraction. (3) It lacks parameters necessary to accurately assess disease severity (e.g., interdental CAL), complexity (e.g., furcation involvement, masticatory dysfunction, secondary occlusal trauma, severe ridge defects, bite collapse drifting or flaring, and fewer than 20 remaining teeth) and extent (localized, generalized or the molar–incisor pattern). (4) It incorporates highly subjective personal parameters, such as stress and socioeconomic factors, that are difficult to define and compare objectively. (5) Finally, all parameters within the PRA system are weighted equally, which may lead to unrealistic results, although adjusting this limitation is not straightforward.

Genetic factors are correlated to predisposition and play a crucial role in periodontal disease susceptibility. Polymorphisms in specific genes, such as those encoding interleukins and other inflammatory mediators, have been linked to an increased risk of periodontitis.[17,18] Although it is highly important, it is not a simple factor to be observed; it was indirectly approached in GF-PeDRA©. Similarly, considering systemic conditions beyond diabetes enables a better understanding of the bidirectional relationship between periodontal disease and other systemic disorders (e.g., cardiovascular disease, osteoporosis and obesity). These conditions are closely associated with systemic inflammation, which can exacerbate periodontal disease.[19] In recognition of the importance of these systemic factors, GF-PeDRA© also incorporated them into its assessment framework.

Some parameters have been standard for periodontal assessment tools, such as smoking and diabetes, 2 of the most significant risk factors or truly acknowledged modifying factors. Smoking has been consistently linked with

an increased risk of periodontitis due to its adverse effects on the immune response and tissue healing.[20] Similarly, diabetes is associated with an elevated risk of periodontal disease, primarily due to the impact of hyperglycemia on immune function and tissue integrity.[21] Other recognized parameters are PPD and BoP, which are direct indicators of periodontal health; PPD measures the pseudo-pocket or the severity of tissue destruction, while BoP reflects the inflammation level and disease activity.[17]

Comparing risk assessment models/tools, it is possible to observe significant differences in various approaches; some focus more on clinical parameters, whereas others emphasize systemic and genetic factors. Studies have shown that some tools offer higher predictive accuracy than traditional PRA, particularly in identifying patients at risk for rapid disease progression.[22] Incorporating genetic and systemic factors into a modified version of PRA improved its capacity to identify high-risk individuals who may not yet present with severe clinical manifestations, and thus enhanced its reliability. However, this enhancement also made the assessment less practical for routine clinical use due to the limited accessibility of such data. Therefore, the clinical utility of these models varies depending on their complexity and the resources available to the practitioner. For example, some models are more accessible to general practitioners due to their simplicity, while others require specialized knowledge and equipment.[23] Thereby, although GF-PeDRA© has more questions and parameters/factors to be addressed, it can be considered a simple tool with high accuracy.

Some authors have invited readers to rethink diseases such as peri-implantitis, which is not approached by many available tools.[24] GF-PeDRA© refers to this aspect among its numerous reliable items: (1) highest PD value; (2) number of interproximal sites with bone loss; (3) highest CAL value; (4) maximum RBL; (5) percentage of sites with BoP; (6) bone loss pattern; (7) tooth loss, including periodontally hopeless teeth planned for extraction; (8) evidence of progression over 5 years; (9) need for complex rehabilitation; (10) patient's age; (11) CAL and biofilm accumulation; (12) smoking; (13) diabetes; (14) extension and distribution of the disease; (15) peri-implant disease; (16) other systemic conditions (other than diabetes); (17) furcation involvement; and (18) necrotizing lesions. Some of the information is not easy to reach. It should be emphasized that clinical experience remains indispensable for the accurate interpretation of individual cases; for instance, bone loss around prosthetic crowns[25] should not be misclassified as periodontitis.

Limitations

It is necessary to consider a more extended period to obtain all data, making the result more precise and reliable. Even though the inclusion of 221 patients occurred by chance, more than half of the participants were diagnosed with periodontitis, and around 25% had severe periodontitis – a prevalence higher than that reported in the general population, where approx. 42% of individuals over 30 years old have periodontitis, and 7.8% present with severe forms, according to the National Institutes of Health (NIH).[11] As this was an initial observational study of the proposed tool, future research should perform appropriate sample size calculations to ensure representative results. Increasing the sample size could yield a distribution of periodontitis cases more consistent with population estimates.

Conclusions

The new algorithm/tool (GF-PeDRA©) proved to help diagnose periodontal/peri-implant conditions. It provides a new and feasible scoring system for risk assessment (the GF-PeDRA© score) and the prognosis of periodontal treatment, which must be validated in future clinical studies with more patients. Then, the longitudinal evaluation of patients is recommended to confirm the proposed prognosis and improve the reliability of this new system.

Ethics approval and consent to participate

The study was approved by the local research ethics committee at A.T. Still University, St. Louis, USA (No. of approval: ATSU – IRB GF20240929-001). All participants signed an informed consent form.

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.

Use of AI and AI-assisted technologies

Not applicable.

ORCID iDs

Gustavo Vicentis de Oliveira Fernandes
https://orcid.org/0000-0003-3022-4390
Juliana Campos Hasse Fernandes
https://orcid.org/0000-0001-7603-3544

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Original paper

Effect of various beverages on the surface roughness and color stability of different denture base resins: An in vitro study

Mai Salah El-Din[1,A,D,F], Passent Ellakany[2,D,E,F], Shaimaa Mohamed Fouda[3,A,B,F], Mohammed Moustafa Gad[3,C,E,F]

[1] Department of Prosthodontics, Alexandria University Main Hospital, Egypt
[2] Division of Prosthodontics, Department of Restorative Sciences, School of Dentistry, University of Alabama at Birmingham, USA
[3] Department of Substitutive Dental Sciences, College of Dentistry, Imam Abdulrahman Bin Faisal University, Dammam, 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

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Address for correspondence
Mai Salah El-Din
E-mail: mai.ebrahim.dent@alexu.edu.eg

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Abstract

Background. Denture base materials can be highly sensitive to the effects of daily beverage consumption, manifesting in alterations to their surface texture or color.

Objectives. The study aimed to evaluate the effect of different beverages (Pepsi, coffee and tea) on the surface roughness (Ra) and color stability of 3 types of denture base materials.

Material and methods. A total of 120 specimens ($n = 10$/group) were fabricated from 3 different denture base materials, namely heat-polymerized polymethyl methacrylate (HP), thermoformed polyamide (PA) and acetal (AC). The surface roughness and color stability of the specimens were evaluated 3 times: before immersion in beverages; after 30 days of immersion; and after 90 days of immersion in artificial saliva (a control group) and Pepsi, coffee and tea (test groups). The data analysis was performed using two-way analysis of variance (ANOVA) to compare the results of Ra and color change (ΔE) between denture base resins and beverages.

Results. The differences between the materials, beverages and time were significant for Ra values, as well as the interaction between materials and beverages, and between beverages and time. The findings indicated significant differences in ΔE between denture base materials. In comparison to PA and AC, HP exhibited lower ΔE values. A significant change in color was observed over time for all of the tested materials.

Conclusions. The tested beverages increased Ra and caused change in the color stability for all materials. The observed color change was correlated with the duration of the immersion, and was more evident in thermoformed resins.

Keywords: polymethyl methacrylate, denture base, thermoformed resin, surface properties, chromogen solution

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Highlights

- Thermoformed denture base resins are most affected by beverages such as coffee, tea and Pepsi.
- Patient education on limiting the intake of these beverages is recommended to prevent increased surface roughness, discoloration, and premature denture replacement.

Introduction

Removable dental prostheses remain an essential prosthetic treatment in many conditions of oral rehabilitation.[1] Although polymethyl methacrylate (PMMA) resin is the material of choice used in the fabrication of denture bases, it possesses certain drawbacks, such as the presence of residual monomer, surface porosity, difficult processing, as well as weak flexural and tensile strength.[2–4]

Thermoformed denture base materials are used in the fabrication of dental prostheses, particularly removable partial or complete dentures, preformed clasps, flexible partial denture frameworks, temporary crowns and bridges, orthodontic appliances, antisnoring devices, different types of mouth guards, and splints.[4,5] These materials offer numerous advantages, including light weight, flexibility, good stability, and retention. The most significant advantage of thermoformed resins is the minimal content or absence of free monomers or metal alloys in the material, which reduces the risk of allergic reactions.[4,6] In addition, thermoformed materials possess non-metal clasps and exhibit a natural aesthetic color, which blends with the color of gingival tissues.[7] This enables clinicians to use undercut areas for enhanced retention, a property that is unattainable with conventional denture base resins.[6]

The acetal resin has several advantages, including flexibility and resistance to occlusal wear and fracture. Therefore, it is considered an optimal material for the fabrication of frameworks and clasps for removable partial dentures,[8] provisional bridges, artificial teeth for removable dentures, and orthodontic appliances. The main disadvantage of acetal is its lack of translucency, which hinders its ability to match the aesthetic appearance of thermoplastic acrylic and polycarbonate.[9]

Polyamide (nylon) is a monomer-free substance that is considered suitable for patients allergic to methyl methacrylate. In addition, it is light in weight and impervious to oral fluids.[10] However, its stiffness makes it ill-suited for the application in occlusal rests or denture elements that necessitate rigidity.[11,12]

Denture discoloration may be attributed to intrinsic and/or extrinsic factors including material composition, wear and exposure to stains.[13] Discoloration of denture base resin has been observed following the use of beverages, oral fluids and denture cleansers.[14] Accordingly, denture base materials must possess adequate color stability to achieve optimal aesthetics and serviceability.[14,15]

Several denture base materials are available on the market, and numerous beverages are consumed by different populations. Therefore, it is important to assess the effect of commonly consumed beverages on the surface roughness (Ra) and color stability of denture base materials.[16] Although there have been several studies on Ra and color stability of acrylic resin denture bases, the comparison of these resins with polyamides and acetal after immersion in beverages remains limited.[14] Therefore, the current study evaluated the effect of different beverages, specifically Pepsi, coffee and tea, on Ra and color stability of 3 types of denture base materials: heat-polymerized PMMA (HP); thermoformed polyamide (PA); and acetal (AC). The null hypothesis stated that Ra and color stability of the 3 denture base resins would not be affected by immersion in the tested beverages.

Material and methods

A total of 120 specimens made of 3 denture base resins (HP, PA, AC) were used in the study. The specimens were fabricated by pouring melted baseplate wax (Cavex Set Up; Cavex Holland BV, Haarlem, Netherlands) inside silicon molds measuring 20 mm × 20 mm × 3 mm.[17] Four wax specimens were invested at a time in dental stone (Denston Turkish Dental Stone Type 3; DentaCarts, Cairo, Egypt) within a metal flask (61B Two Flask Compress; Handler Manufacturing, Westfield, USA) following the conventional method for HP. A special dental flask (SABILEX dental flask; Sabilex, Buenos Aires, Argentina) was designed for the injection molding technique in thermoformed resins. A sprue measuring 4 mm in diameter was attached to each specimen at 1 corner, and the 4 sprues were then integrated into a single sprue, which emerged through the flask orifice at one side. Wax was melted away following the investment in the dental stone. The stone surfaces with mold spaces were coated with a separating medium (Acrostone Separating Medium; Acrostone Dental & Medical Supplies, Cairo, Egypt) and then left to dry. Heat-polymerized PMMA (Acrostone, Cairo, Egypt) powder and liquid were mixed according to the manufacturer's instructions. Once the dough stage was reached, HP was packed into the mold spaces following the conventional method. Subsequently, it was transferred to a thermostatically controlled water bath, where it underwent a short curing cycle at 70°C for 1.5 h.

Thereafter, it was brought to boil at 100°C for 1 h. Thermoformed polyamide (Sabilex FlexiUltra, shade: pink 78; Sabilex) and AC (Bio Dentaplast; bredent GmbH & Co. KG, Senden, Germany) are available in the form of granules, which are contained in cartridges of different sizes. During the injection process, the cartridge was aligned with the opening of the flask within the electric furnace (BIOSTRONG 400; Sabilex). In accordance with the manufacturer's guidelines, PA and AC were plasticized for 15 min at 280°C under a pressure of 7.5 bars. The specialized dental flask was bench-cooled for 15–20 min before opening.[18]

The specimens were then subjected to a finishing process that employed the same technique and was carried out by the same operator in order to standardize the pressure exerted. It was achieved by using a tungsten carbide bur (HM79GX-040-HP; Meisinger, Centennial, USA) at a low speed for 2 min, followed by polishing with the use of a new set of a polishing kit for each group material. The polishing procedure was performed using a pre-polishing brown rubber disc at 1,500 rpm for 1 min, followed by a fine pumice for 2 min, and finally using a Tripoli compound for 2 min. The specimens were stored in distilled water at room temperature for 48 h to reduce the residual monomer before testing.

The specimens of each material (n = 40/material) were immersed in artificial saliva (control group), Pepsi, coffee, or tea (test groups) (n = 10/group) (Fig. 1). All the steps were accomplished by a single researcher (MMG) to ensure standardization. The composition of the beverage solutions and preparation procedures are delineated in Table 1. The specimens were stored in different containers holding 50 mL of the tested beverages for 15 min (average time for which a beverage is consumed during the day), followed by storage in distilled water until the next day. This procedure was repeated daily for 30 days (T_1) and 90 days (T_2).[19] To prevent fungal growth, the solutions were refreshed and changed daily. Artificial saliva and Pepsi were used at room temperature,[20] while coffee and tea were utilized at 50°C[21] to mimic the temperature of actual use. The surface roughness values were measured at baseline (T_0), T_1 and T_2 using a non-contact optical interferometric profilometer (Contour GT-K; Bruker Nano GmbH, Berlin, Germany) at a resolution of 0.01 mm. Each specimen was scanned at 5 sites, and the average Ra value of each specimen was calculated.

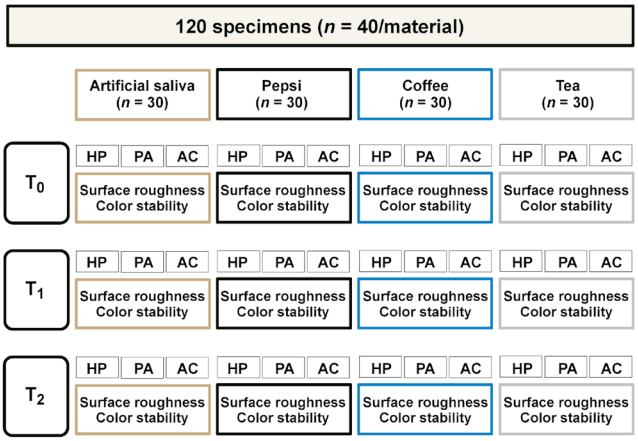

Fig. 1. Flowchart of specimen immersion and testing

HP – heat-polymerized polymethyl methacrylate; PA – thermoformed polyamide; AC – acetal; T_0 – before immersion in beverages; T_1 – immersion for 30 days; T_2 – immersion for 90 days.

Color change (ΔE) was evaluated using a spectrophotometer (Color-Eye® 7000A; X-Rite, Grand Rapids, USA). The color variation was calculated according to the International Commission on Illumination (CIE) at T_0, T_1 and T_2. The CIE L*a*b system measures color variation between 2 points, based on the following formula[20] (Equation 1):

$$\Delta E = [(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2]^{½} \quad (1)$$

where:

ΔL – difference in lightness/darkness value;

Δa – difference in the red/green axis;

Δb – difference in the yellow/blue axis.

Before testing, the spectrophotometer was calibrated according to the manufacturer's instructions. Each specimen was placed against the port, after which the support arm was locked. Four measurements were recorded for each specimen, and the mean ΔL, Δa and Δb values were calculated.[20] The color change (ΔE) was quantified between T_1 and T_0, as well as between T_2 and T_0.[20] Subsequently, the ΔE values were converted

Table 1. Immersion solutions used in the current study

Material	Name and manufacturer	Composition/preparation
Artificial saliva	Orthana; Biofac A/S, Kastrup, Denmark	mucin, methyl-4-hydroxybenzoate, benzalkonium chloride, EDTA, H_2O_2, xylitol, peppermint oil, spearmint oil, mineral salts
Coffee	Nescafé® Classic; Nestlé, São Paulo, Brazil	15 g of Nescafé® Classic was poured into 500 mL of boiling distilled water and stirred.
Tea	Yellow Lipton; Unilever Gulf, Dubai, United Arab Emirates	The tea solution was prepared by immersing 5 tea bags into 500 mL of boiling distilled water for 10 min. Both tea and coffee solutions were used at 50°C[19] to mimic the temperature of actual use.
Pepsi	Pepsi; Al Jomaih Beverages Company, Dammam, Saudi Arabia	The beverage was obtained from containers with capacities of 355 mL, 591 mL and 710 mL, stored at room temperature. There was no special preparation for the Pepsi group.

EDTA – ethylenediaminetetraacetic acid.

to the National Bureau of Standards (NBS) units (trace = 0.0–0.5, slight = 0.5–1.5, noticeable = 1.5–3.0, appreciable = 3.0–6.0, much = 6.0–12, and very much: >12) using the following formula (Equation 2):

$$\text{NBS unit} = \Delta E \times 0.92 \quad (2)$$

Statistical analysis

The IBM SPSS Statistics for Windows software, v. 20.0 (IBM Corp., Armonk, USA), was used for data analysis. The numerical data, which was based on the measurements of Ra and color stability for the 3 denture base materials, was presented as mean (*M*) and standard deviation (*SD*). The Kolmogorov–Smirnov test revealed that the tested datasets conformed to the normal distribution. A two-way analysis of variance (ANOVA) was applied to compare the results for Ra and color stability for each material and beverage. Tukey's post hoc test was applied for pairwise comparisons. The values were considered statistically significant at $p \leq 0.05$.

Results

A two-way ANOVA for Ra, followed by Tukey's post hoc test, revealed significant values for materials, beverages and time (all $p < 0.001$) (Table 2). The interaction between materials and beverages ($p < 0.001$) and the interaction between beverages and time were significant for Ra values ($p = 0.007$). However, the relationship between materials and time was not statistically significant ($p = 0.340$).

A comparison of Ra of denture base materials subjected to immersion in different beverages is outlined in Table 3. Significant differences were observed for HP between the time points in comparison to baseline (T_0) after immersion in different beverages ($p < 0.05$), with the exception of the difference between T_1 and T_0 after immersion in artificial saliva ($p = 0.573$). The time factor exhibited non-significant differences between T_1 and T_2 for all tested liquids ($p > 0.05$). The highest Ra value was observed in coffee at T_2 and T_1, followed by Pepsi at T_2 and tea immersions. Significant differences in Ra were noted for PA between the evaluated time points and baseline for all tested solutions ($p < 0.05$), with the exception of Pepsi at T_1 and T_2 ($p = 0.401$ and $p = 0.064$), and tea at T_1 ($p = 0.404$). The impact of the time interval on Ra of PA did not show significant differences after immersion in the tested solutions. The highest Ra values at T_2 were observed for coffee, followed by tea, saliva and Pepsi. Statistically significant differences in Ra were noted for all beverages when AC was examined ($p < 0.05$). However, between T_1 and T_2, a significant difference in Ra was noted only after immersion in saliva ($p = 0.012$). The highest Ra values were reported at T_2 with saliva, followed by coffee, tea and Pepsi.

A two-way ANOVA for color stability revealed significant values for materials, beverages and time (all $p < 0.001$) (Table 4). The interaction between materials and beverages, materials and time, as well as the correlation between beverages and time were significant for ΔE values ($p < 0.001$). A comparison of the color stability of denture base materials subjected to immersion in different beverages is summarized in Table 5. The results indicated significant differences in the color stability of HP after immersion in the tested liquids between baseline and all time points ($p < 0.05$). For HP, the highest color difference was recorded at T_2 with tea, followed by Pepsi and coffee. For PA and AC, significant differences in color stability were observed with all beverages ($p < 0.05$) except for T_1 in saliva (PA: $p = 0.101$; AC: $p = 0.263$). In PA at T_2, the highest color difference was documented with Pepsi, followed by the coffee and tea immersions. In AC at T_2, the most significant color change was noted with coffee, followed by Pepsi and tea.

Table 2. Results of two-way analysis of variance (ANOVA) for the surface roughness (Ra)

Source	Type III sum of squares	*df*	Mean square	*F*	*p*-value
Corrected model	182.736[a]	26	7.028	31.393	0.000*
Intercept	509.824	1	509.824	2277.224	0.000*
Materials	89.899	2	44.950	200.776	0.000*
Beverages	21.197	3	7.066	31.560	0.000*
Time	4.110	1	4.110	18.356	0.000*
Materials × beverages	12.019	6	2.003	8.947	0.000*
Materials × time	0.486	2	0.243	1.086	0.340
Beverages × time	2.777	3	0.926	4.135	0.007*
Materials × beverages × time	10.530	6	1.755	7.839	0.000*
Error	42.313	189	0.224	–	–
Total	873.143	216	–	–	–
Corrected total	225.049	215	–	–	–

* statistically significant ($p \leq 0.05$, Tukey's post hoc test); [a] $R^2 = 0.012$ (adjusted $R^2 = 0.786$); *df* – degrees of freedom.

Table 3. Comparison of the surface roughness (Ra) of denture base materials subjected to immersion in different beverages at 3 time intervals

Material	Surface roughness [µm] *M* ±*SD*								
	T_0	saliva		Pepsi		coffee		tea	
		T_1	T_2	T_1	T_2	T_1	T_2	T_1	T_2
HP	0.40 ±0.03^{A}	0.43 ±0.13^{AB}	0.62 ±0.22^{B}	0.50 ±0.16^{C}	0.91 ±0.52^{aC}	1.04 ±0.54^{D}	1.07 ±0.54^{D}	0.72 ±0.32^{E}	0.70 ±0.30^{E}
PA	1.55 ±0.13^{A}	2.25 ±0.33^{B}	2.37 ±0.37^{aB}	1.79 ±0.61^{aAC}	1.29 ±0.46^{aAC}	2.96 ±1.00^{aD}	3.68 ±0.45^{D}	1.98 ±1.00^{aAE}	3.11 ±0.62^{E}
AC	0.99 ±0.04^{A}	1.12 ±0.16^{B}	2.61 ±0.21^{aC}	2.25 ±0.50^{aD}	2.16 ±0.86^{D}	2.61 ±0.21^{aE}	2.60 ±0.25^{E}	2.44 ±0.32^{aF}	2.50 ±0.30^{F}

HP – heat-polymerized polymethyl methacrylate; PA – thermoformed polyamide; AC – acetal; T_0 – before immersion in beverages; T_1 – immersion for 30 days; T_2 – immersion for 90 days; *M* – mean; *SD* – standard deviation. The same uppercase letters show non-statistical differences between T_0, T_1 and T_2 for each beverage. The same lowercase letters indicate non-statistical differences between materials for the same time point.

Table 4. Results of two-way analysis of variance (ANOVA) for color stability

Source	Type III sum of squares	*df*	Mean square	*F*	*p*-value
Corrected model	368.532[a]	26	14.174	1304.319	0.000*
Intercept	656.336	1	656.336	60396.122	0.000*
Materials	69.386	2	34.693	3192.435	0.000*
Beverages	78.687	3	26.229	2413.602	0.000*
Time	69.276	1	69.276	6374.790	0.000*
Materials × beverages	31.959	6	5.326	490.139	0.000*
Materials × time	23.452	2	11.726	1079.037	0.000*
Beverages × time	14.432	3	4.811	442.673	0.000*
Materials × beverages × time	10.732	6	1.789	164.598	0.000*
Error	2.054	189	0.011	–	–
Total	1235.306	216	–	–	–
Corrected total	370.585	215	–	–	–

* statistically significant ($p \leq 0.05$, Tukey's post hoc test); [a] $R^2 = 0.994$ (adjusted $R^2 = 0.994$).

Table 5. Comparison of color change (ΔE) of denture base materials subjected to immersion in different beverages at 2 time intervals

Material	Color change [ΔE] *M* ±*SD*							
	saliva		Pepsi		coffee		tea	
	T_1	T_2	T_1	T_2	T_1	T_2	T_1	T_2
HP	0.78 ±0.05	0.99 ±0.06	1.15 ±0.06	1.40 ±0.19	1.10 ±0.04	1.22 ±0.03	1.09 ±0.03	1.73 ±0.08
PA	1.05 ±0.03	1.55 ±0.07	2.48 ±0.10	5.68 ±0.24	2.38 ±0.10	5.08 ±0.16	2.02 ±0.06	3.66 ±0.13
AC	0.94 ±0.08	1.36 ±0.09	1.85 ±0.12	3.76 ±0.12	2.01 ±0.08	3.99 ±0.07	1.59 ±0.09	2.41 ±0.11

A comparison of T_1 and T_2 values for the same beverage and material (horizontally) revealed significant differences for all groups ($p \leq 0.05$). A comparison of values between the materials for the same time point (vertically) demonstrated significant differences for all groups ($p \leq 0.05$).

The results indicated significant differences in ΔE between denture base materials ($p = 0.001$). Specifically, HP exhibited lower ΔE values when compared to PA and AC. A variation was observed in the conversion of mean color difference values to the NBS units, contingent upon the type of material. For HP, the change in color was slight with all tested beverages at T_1 and T_2, with the exception of tea at T_2, where a noticeable change was observed. A noticeable change at T_1 and appreciable at T_2 were evident for PA and AC across all tested beverages, except for tea at T_2, which exhibited a noticeable color change for AC. For all materials, color changes were slight for the control group, with a noticeable change observed only for PA at T_2.

Discussion

The current study evaluated the effect of Pepsi, coffee and tea on the color stability and Ra of different denture base materials. The null hypothesis of the study was rejected, as Ra and color stability were affected by the process of immersion.

The beverages selected for the present study are the most commonly consumed worldwide. Carbonated drinks, including Pepsi and Coca-Cola, are widely consumed, with more than 1.8 billion servings per day.[22] The exposure of acrylic to different beverages for a minimum of 56 days causes a clinically perceptible color change.[19]

Consequently, in this study, the immersion period was extended to 90 days. The color stability was evaluated using the spectrophotometer due to its accuracy in measuring color coordinates.[23] Certain foods and beverages cause changes in the surface properties of denture base resin.[24,25] Studies found that increased staining was correlated with an elevated Ra of dental prostheses.[25–27]

In the present study, the immersion of denture base materials in artificial saliva and beverages increased Ra of all materials. Coffee elicited the highest Ra values in all denture base materials, followed by tea for AC and PA, and Pepsi for HP. Among the tested materials, PA and AC exhibited the highest Ra values after being immersed in coffee. This phenomenon may be caused by the adsorption and absorption of coffee, which penetrated deeper than tea.[14,28] Particularly, both coffee and tea were used at high temperature in the present study, a condition that is known to promote water absorption. Moreover, the tannic acid present in tea and coffee has demonstrated to exert harmful effects on polymer surfaces.[29] Pepsi demonstrated an increase in Ra of HP and AC compared with the control group. These results are consistent with the findings of Ikram, who reported increased Ra in PMMA after immersion in a carbonated beverage (Coca-Cola).[25] The observed changes in denture base resin might have resulted from the acidity of the beverages due to the presence of phosphoric acid, which acts as a plasticizer.[30]

Intraoral dentures are subject to absorption and adsorption due to their contact with saliva, food, beverages, and denture cleansers.[24] Staining occurs due to the physical penetration of pigments between the molecular lattices or the adsorption of pigments on the surface of specimens.[14] The clinically acceptable value for color stability is 3.3.[20] Similarly to the results of the previous studies,[31–33] the present study demonstrated color changes even in specimens that were immersed in saliva (the control group). This observation may be attributable to the yellow color of mucin, a component of saliva, and the absorption of water molecules due to the polarity of acrylic resin.

In the present study, the tested beverages significantly altered the mean color stability values of HP, PA and AC at T_1 and T_2 in comparison with the control group. However, the consumption of the tested beverage for 30 days caused only a noticeable change in color for all materials, falling below the clinically acceptable value. However, after the increase in duration of immersion to 90 days, an appreciable change in the ΔE values was observed for PA and AC. The color change for HP was slight with Pepsi and coffee and noticeable with tea. Our results are in line with those of several previous studies, which indicated a correlation between the immersion time and increased discoloration of denture base materials.[28,34,35] Similarly, studies reported a decreased color stability of polyamides with an extended duration of staining solution,[7] and a heightened color stability of acrylic resin.[4,36,37] The color change of polyamide is 2–4 times more pronounced than that of PMMA.[4] The cause of the color changes in polyamide denture base material may be attributed to its hygroscopic nature, as its moisture content varies in response to the surrounding conditions.[14] In addition to its hydrophilic properties,[36] it has increased water sorption and leached plasticizer, in contrast to acrylic resin, which showed moderate sorption.[37] Another reason for the greater discoloration observed in thermoformed resin in the present study could be their higher Ra compared to HP. There is a correlation between staining and the Ra value of the denture base material.[38,39]

The highest color change of HP was observed after 90 days of immersion in tea, followed by Pepsi and coffee. This result aligns with the study by Hatim and Al-Tahho, who reported that tea induced the most significant color change among denture base materials compared with coffee and Pepsi.[13] This observation is consistent with the results of previous studies.[40,41] Um and Ruyter reported that tea and coffee caused color change after 48 h of immersion.[21] It was suggested that tannic acid present in tea and coffee is responsible for the observed denture brown discoloration, as it is water-soluble and known to trigger brown pigmentation.[15] On the contrary, some previous studies reported a significant color change with coffee compared to tea[28] and Pepsi. It should be noted that some of these studies utilized different materials than those tested in the present study.[26,27]

Pepsi and coffee caused appreciable change in the color of PA and AC, and tea caused noticeable change at T_2. The observed color change may have resulted from the low pH value of Pepsi, which adversely affected the surface integrity of the material.[42] Previous studies suggested that a low pH value of Pepsi may act as a contributing factor in color change.[13,29] Our results are in line with those of a previous study that reported a significant color change of polyamide resin after the usage of Coca-Cola and coffee.[14,43] Coffee causes staining of resin because it includes yellow colorants, in addition to caffeine and caffeic acid.[28,29,42] In the same context, a previous study has demonstrated that the discoloration caused by coffee was more than that of tea due to adsorption and absorption of colorants by resin materials, but the change in color caused by tea was only due to surface adsorption of the colorants.[31] In the present study, coffee caused the highest Ra values, which could be a contributing factor that enhanced discoloration. Another variable that may have influenced the results is the high temperature of the beverage. A previous study revealed that the immersion of HP in hot water resulted in the whitening of HP due to water absorption.[44]

Limitations

The present study tested 3 different denture base materials. In addition, the immersion protocol closely resembled the actual use of beverages. However, the

study's limitations stem from its in vitro nature, which precludes the simulation of the oral environment, including changes in temperature, pH and the presence of oral flora. Another limitation is the relatively short testing period, which simulates 3 months of actual usage. Further studies are required to test the durability of the denture materials under prolonged and simulated oral conditions. In addition to the aforementioned procedures, it is imperative to investigate the effect of denture cleansers and cleaning methods on the restoration of the original color of the tested denture base materials. Also, further research is required to evaluate the effect of other beverages and denture cleansers on other mechanical and physical properties of denture materials.

Conclusions

The findings of the present study indicate that the use of beverages resulted in a substantial increase in Ra. As time exposure to the tested beverages increased, a significant rise in color change was observed, particularly with PA and AC, while HP demonstrated greater color stability. Dentists should educate their patients about discoloration associated with certain beverages and denture base materials, which can potentially compromise the aesthetics and lead to additional costs for replacement.

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.

Use of AI and AI-assisted technologies

Not applicable.

ORCID iDs

Mai Salah El-Din https://orcid.org/0000-0002-8516-7330
Passent Ellakany https://orcid.org/0000-0002-2995-8111
Shaimaa Mohamed Fouda https://orcid.org/0000-0002-3875-4882
Mohammed Moustafa Gad https://orcid.org/0000-0003-3193-2356

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Original paper

Comparison of shear bond strength of different indirect composites to new generation polyetheretherketone materials

Zıya Seferli[1,A–F], Kubra Degirmenci[2,A,C–F], Serkan Saridag[3,A,C–F]

[1] DentAkademi, Istanbul, Turkey
[2] Department of Prosthodontics, Faculty of Dentistry, Bolu Abant İzzet Baysal University, Turkey
[3] Department of Prosthodontics, Faculty of Dentistry, Istanbul Okan University, 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

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Address for correspondence
Kubra Degirmenci
E-mail: dtkubradegirmenci@outlook.com

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Abstract

Background. The bond stability between polyetheretherketone (PEEK) materials and composites is a novel concern, and evidence regarding bond strength is limited. To date, no study has comprehensively evaluated the effect of different surface treatments, adhesive agents and composite materials on the shear bond strength (SBS) of various PEEK materials.

Objectives. The aim of the study was to compare the SBS between 2 PEEK materials and different indirect composites applied after the application of various surface pre-treatment methods and adhesives.

Material and methods. A total of 328 PEEK specimens (JUVORA™ (unfilled PEEK material, $n = 164$); BioHPP (compound containing 20% nanoceramic-filled PEEK, $n = 164$)) were divided into 4 groups according to the applied surface treatment: no treatment; air abrasion; acid etching; and acid etching + air abrasion. Subsequently, all specimens were conditioned with visio.link (VL) or Single Bond Universal (SBU). The specimens were veneered with crea.lign composite (CR) or SR Nexco composite (SR), and the bond strength values were measured. The co-variance analysis of variance (ANOVA) was performed to analyze the data ($\alpha = 0.003$).

Results. The SBS values for BioHPP specimens were significantly higher than the values for JUVORA™ specimens in the no treatment group ($p < 0.001$). The highest SBS values were detected between BioHPP and SR (22.54 ±1.22 MPa), and between JUVORA™ and SR (21.45 ±1.43 MPa) after acid etching and conditioning with VL ($p < 0.001$). The surface treatments, composites and adhesives affected the SBS between the composites and PEEK materials.

Conclusions. Following air abrasion or acid etching of surfaces, conditioning with VL and aesthetic veneering with SR may be a more reliable clinical application than other surface treatments and adhesive–composite combinations for PEEK.

Keywords: shear bond strength, polyetheretherketone, surface roughness, indirect composite, piranha acid

Highlights

- Primers significantly improve the bond strength between indirect composites and polyetheretherketone (PEEK).
- The highest bond strength was achieved when primers containing methyl methacrylate monomer were combined with high surface roughness.
- Roughening PEEK substructures with piranha acid and 110-μm Al_2O_3 particles, followed by the use of methyl methacrylate-based primers, provides the most effective bond enhancement.

Introduction

Dental materials that closely resemble tooth color have replaced metallic alloys due to increasing aesthetic expectations of the patients.[1] Therefore, clinicians prefer materials that closely match the color of natural teeth.

Polyetheretherketone (PEEK) is a high-performance polymer of the polyaryletherketone family.[2] Recently, PEEK used in orthopedics has also become popular in the field of dentistry due to its good malleability and bone-like elasticity.[3–5] Polyetheretherketone is a semi-crystalline, polyaromatic, synthetic, and polymeric material widely used as a biomaterial for custom implant abutments, frameworks of removable partial dentures, and fixed partial dentures.[6] In addition, PEEK possesses outstanding properties such as heat resistance, chemical stability, biological inertness, good biocompatibility, solvent properties, durability, excellent electrical insulation, toughness, and a better esthetic appearance than conventional metal frameworks.[7,8] Because of its low density (1.32 g/cm^3) and low elastic modulus (3–4 GPa), the use of PEEK has been preferred in the field of dentistry.[9,10] Moreover, PEEK is reinforced with either carbon or glass fibers of varying lengths, spherical ceramic filler microparticles, barium phosphate ($BaSO_4$), or titanium dioxide (TiO_2) with filler contents up to 30%.[11–14] BioHPP, a compound containing 20% nanoceramic-filled PEEK and 20% ceramic fillers, is a high-performance polymer with high biocompatibility, excellent mechanical properties, high temperature resistance, and chemical stability.[15–18] However, the clinical use of PEEK as a monolithic restoration is limited due to its low translucency and snow-white color.[19] Therefore, the material is veneered with composite resin to achieve acceptable aesthetic outcomes.[20] Yet, there are problems in the direct bonding of the material to composite veneer materials.[21] The inertness of PEEK makes it difficult to bond with composite veneer without the implementation of surface treatments and adhesive materials.[3] Earlier studies have examined the effect of various surface treatments on the bond strength of PEEK to composite materials.[22,23]

There are 2 main methods of surface treatment with PEEK: mechanical and chemical.[23,24] Air abrasion, bur grinding, laser, and plasma spray applications are mechanical treatments,[25,26] while the application of strong etching solutions is a chemical treatment.[21] Additional etching of PEEK with sulfuric acid or piranha solution (sulfuric acid + hydrogen peroxide) has been shown to significantly increase the initial bond strength of the polymer.[21,27] In a previous study, the effect of acid etching treatment following air abrasion was compared to air abrasion, acid etching, and control groups.[14] The highest bond strength values were reported for the group that underwent acid etching without mechanical treatment.[14] However, the effect of combined surface treatments on different PEEK materials has not been evaluated.

In addition, the use of solvents containing methyl methacrylate (MMA) or phosphate monomer, and low viscosity of adhesives, can contribute to the adhesion of resin–matrix composites.[28,29] Previous studies have reported that adhesive systems containing MMA are capable of establishing a sufficient bond to PEEK.[30,31] Moreover, the shear bond strength (SBS) depends on the properties of the composite veneer material.[32,33] In a previous study, the bonding ability of acid-etched PEEK surfaces to the light-curing microparticle composite (VITA VM LC) and the Sinfony composite materials without adhesive conditioning was evaluated.[20] The higher bonding values were obtained when Sinfony composite was used.[20] This phenomenon was attributed to the enhanced penetration into micropores that results from acid etching due to the low viscosity of Sinfony composite in comparison to VITA VM LC.[2,19] In addition, the composition of VITA VM LC can be responsible for the observed differences between composites, given the established role of bisphenol A-diglycidyl methacrylate (Bis-GMA) as a viscous polymer.[34]

The shear bond strength values between PEEK and composite veneer must be at least 10–12 N to be considered clinically acceptable.[35] However, the extent of knowledge concerning the potential and limitations of each treatment, with its particular specific effects, is limited, and there is a lack of clinically accepted standard protocols for enhancing PEEK frameworks to composite veneer materials.[21] There is no consensus regarding the surface treatments, adhesive agents and composites applied in PEEK materials. Therefore, the bonding properties of PEEK materials should be examined further.

The purpose of the study was to compare and evaluate the effect of surface treatments, adhesive agents and composite veneer materials on the SBS of various PEEK

materials to different composite materials. The first null hypothesis was that the use of adhesive agents and composite veneer materials would not affect the SBS of the PEEK materials. The second null hypothesis posited that there would be no differences between the SBS values of both PEEK materials.

Material and methods

In the present study, 2 different types of PEEK materials were examined: an unfilled PEEK (JUVORA™; JUVORA Ltd, Thornton Cleveleys, UK); and a compound containing 20% nanoceramic-filled PEEK (BioHPP; bredent medical GmbH & Co.KG, Senden, Germany). The characteristics of the materials used in the present study are displayed in Table 1. The sample size was calculated using the G*Power software, v. 3.0.1. The minimum number of specimens was determined to be 297, with an effect size (f) of 0.252, a power of 0.95 (1–β error probability), and a significance level of 0.05 (α error probability). Considering the results of the power analysis, a total of 328 specimens were prepared for the study.

Specimen preparation

Rectangular blocks of both types of PEEK were obtained from prefabricated PEEK discs using computer-aided design/computer-aided manufacturing (CAD/CAM). The blocks were cut with a slow-speed precision saw (IsoMet 1000 Precision Cutter; Buehler, Lake Bluff, USA). A total of 328 rectangular specimens measuring 7 mm × 7 mm × 4 mm were prepared, with 164 allocated for each type of PEEK. All specimens were embedded in acrylic resin (SCANDIQUICK; SCAN-DIA GmbH, Hagen, Germany), with a diameter of 22 mm and a height of 18 mm. The specimens' surfaces were polished underwater for 10 s using an automatic polishing device (EcoMet®/AutoMet® 250; Buehler) and silicon carbide (SiC) abrasive papers of varying grit levels (600, 800 and 1,200). Subsequently, the specimens were cleaned for 10 min using an ultrasonic machine (Transsonic T700; Elma, Singen, Germany), and the surfaces of the specimens were dried using an air pressure process.

Application of surface treatments

Both BioHPP and JUVORA™ PEEK specimens were further divided into 4 subgroups according to the surface treatment method (n = 41 specimens/group). The first group was a control group, to which no surface treatment was applied. The second group was the acid etching group. In this group, piranha solution (a mixture of 98% sulfuric acid and 30% hydrogen peroxide in a ratio of 10:3) was applied to the surfaces of the specimens using a micropipette for 30 s, followed by a rinse with distilled water for an additional 30 s. The third group was the air abrasion group, in which the surfaces of the specimens were sandblasted (Basic Classic; Renfert Richardson, Richardson, USA) with 110-mm aluminum oxide particles (Cobra; Renfert, Hilzingen, Germany) at a pressure of 0.4 MPa, perpendicularly from a 10-mm distance for 10 s. In the fourth group, both of the abovementioned surface treatments were applied. Afterward, all specimens were cleaned with distilled water in an ultrasonic machine (Transsonic T700) for 10 min and air-dried. One specimen was selected from each of the 8 PEEK subgroups with different surface treatments for scanning electron microscopy (SEM). A total of 8 specimens were examined under a scanning electron microscope (S-4800; Hitachi,

Table 1. Characteristics of the materials used in the study

Product name and manufacturer	Material type	Composition	Application steps as recommended by the manufacturer
BioHPP (bredent medical GmbH & Co.KG, Senden, Germany)	PEEK	20% nanoceramic-filled PEEK	–
JUVORA™ (JUVORA Ltd, Thornton Cleveleys, UK)	PEEK	unfilled, 100% pure PEEK	–
Piranha solution (Albar Chemistry, Kocaeli, Turkey)	acid	a mixture of 98% H_2SO_4 and 30% H_2O_2 in a ratio of 10:3	1. Apply for 30 s. 2. Rinse with distilled water for 30 s.
visio.link (bredent medical GmbH & Co.KG, Senden, Germany)	adhesive	MMA, PETA, photoinitiators	1. Apply the adhesive on the PEEK surface with a brush. 2. Place in the composite furnace for 120 s.
Single Bond Universal (3M, Seefeld, Germany)	adhesive	10-MDP, dimethacrylate resins, HEMA, filler, ethanol, water, initiators, silane	1. Apply a thin layer by rubbing for 20 s. 2. Gently air stream for 5 s. 3. Light cure for 10 s.
SR Nexco (Ivoclar Vivadent AG, Schaan, Liechtenstein)	composite resin	UDMA, aliphatic dimethacrylate	1. Light cure for 20 s. 2. Place in the composite furnace for 5 min.
crea.lign (bredent medical GmbH & Co.KG, Senden, Germany)	composite resin	Bis-GMA, 50% nanoceramic	1. Light cure for 20 s. 2. Place in the composite furnace for 5 min.

PEEK – polyetheretherketone; MMA – methyl methacrylate; PETA – pentaerythritol triacrylate; 10-MDP – 10-methacryloyloxydecyl dihydrogen phosphate; HEMA – 2-hydroxyethyl methacrylate; UDMA – urethane dimethacrylate; Bis-GMA – bisphenol A-diglycidyl methacrylate.

Ltd., Tokyo, Japan). After the respective surface treatment and sputter coating with gold alloy nanoparticles, an examination of a conductive layer of approx. 15 nm was conducted using a field emission SEM under ×1,000 magnification.

Surface topography measurements

The surface roughness (Ra) of 320 PEEK specimens was examined with a profilometer using a 90° detection device (Surtronic® S-series; Taylor Hobson, Leicester, UK). The diameter of the diamond probe tip was 2 µm. For each specimen, 5 measurements (3 vertically and 2 horizontally) were performed, with a measurement track of 6 mm. The distance between the tracks was 0.3 mm. The mean Ra of each sample was calculated.

Bonding procedure

The specimens of each of the 4 surface treatment PEEK groups were further subdivided into 2 different adhesive agent groups: VL (visio.link; bredent medical GmbH & Co.KG); and SBU (Single Bond Universal; 3M, Seefeld, Germany). Visio.link was applied to the surfaces of the PEEK specimens using a small brush and the adhesive composite furnace (Labolight DUO; GC, Leuven, Belgium), and polymerized lightly for 120 s. Single Bond Universal was applied to the surfaces of the PEEK specimens for 20 s, dried for 5 s and lightly polymerized (Elipar S10; 3M ESPE, Istanbul, Turkey) at a light intensity of 1,200 mW/cm^2 for 10 s.

After adhesive conditioning procedures, a special cylindrical mold with an inner diameter of 2.3 mm and a height of 3.0 mm was used to apply the composite to the surfaces that had been conditioned with adhesives. The mold was positioned on the PEEK specimen surface, filled with the veneering composite and lightly cured (Elipar S10) at a light intensity of 1,200 mW/cm^2 for 20 s. Subsequently, the composite veneers were polymerized using a composite furnace (Labolight DUO) for 5 min, following the standard program (190–220 mW/cm^2 depending on the wavelength). Crea.lign dental composite (CR) (bredent medical GmbH & Co.KG) was applied to the surfaces of half of the specimens from each group, and the other half of the specimens were coated with SR Nexco composite (SR) (Ivoclar Vivadent AG, Schaan, Liechtenstein). Subsequently, all specimens were stored in distilled water at 37°C for 24 h. Figure 1 presents the overview of the study design.

Bond strength measurement and failure modes

The shear bond strength between composites and PEEK materials was measured using a testing machine (Shear Bond Tester; Bisco Inc., Schaumburg, USA). The specimens were positioned and fixed in the specimen holder, ensuring that the adhesive specimen surface was parallel to the loading piston. The load, characterized by a crosshead speed of 1 mm/min, was applied to the interface of the composites and PEEK specimens until failure occurred.

The SBS values were calculated using the following formula (Equation 1):

$$\text{SBS [MPa]} = F/A \quad (1)$$

where:
F – fracture load [N];
A – bond area [mm^2].

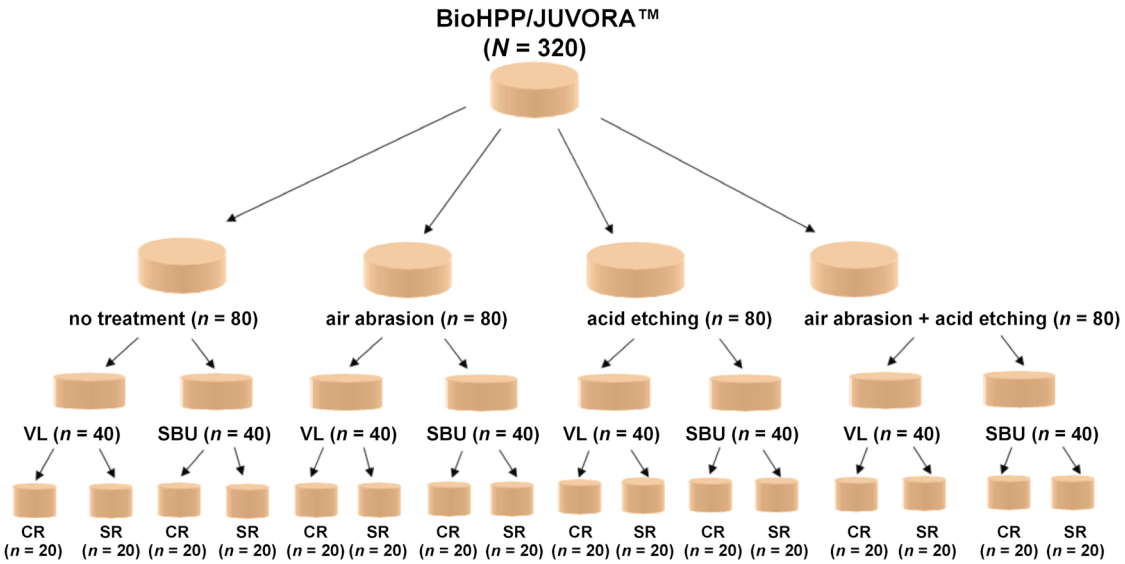

Fig. 1. Study design
VL – visio.link; SBU – Single Bond Universal; CR – crea.lign; SR – SR Nexco.

The debonded area was examined with a stereomicroscope (M3B; Wild Heerbrugg Ltd., Heerbrugg, Switzerland) at ×35 magnification for fracture analysis, and failure modes were recorded as adhesive, cohesive or mixed.

Statistical analysis

The normality of the data distribution was tested using the Shapiro–Wilk test. The Kruskall–Wallis and Mann–Whitney *U* tests were used to compare groups with non-normally distributed Ra values (Table 2). Furthermore, the analysis of covariance (ANCOVA) (Table 3) was performed with a normal distribution to state the effects of surface treatments, composite types and adhesive agents on the SBS values of PEEK materials to composite veneers among the groups. A comparison of the SBS values of PEEK materials was conducted using an independent samples *t*-test and the Holm–Bonferroni adjustment. In addition, Pearson χ^2 and Fisher's exact test were employed to determine the distribution of failure modes among the different treatment groups, with a statistical significance level set at $p < 0.05$.

Results

Surface roughness

In this study, BioHPP and JUVORA™ were compared. A statistically significant difference in the Ra values was

Table 2. Comparison of surface roughness (Ra) values between polyetheretherketone (PEEK) materials

Surface treatment	BioHPP [µm]		JUVORA™ [µm]		*p*-value
	Min–Max (*Me*)	*M* ±*SD*	*Min–Max* (*Me*)	*M* ±*SD*	
No treatment (control)	0.4–0.8 (0.5)^{aA}	0.55 ±0.08	0.4–1.2 (0.6)^{aA}	0.63 ±0.18	0.480
Acid etching	2.2–5.1 (3.4)^{bA}	3.46 ±0.75	1.6–4.6 (2.5)^{bB}	2.72 ±0.60	<0.001*
Air abrasion	1.0–2.3 (1.6)^{cA}	1.62 ±0.22	1.3–2.1 (1.6)^{cA}	1.66 ±0.20	0.281
Acid etching + air abrasion	1.2–2.3 (1.6)^{cA}	1.61 ±0.31	1.2–2.5 (1.7)^{cA}	1.67 ±0.30	0.438
p-value	<0.001*		<0.001*		–

Me – median; *M* – mean; *SD* – standard deviation; * statistically significant ($p < 0.05$, Kruskall–Wallis test and Mann–Whitney *U* test for pairwise comparison). Different lowercase letters indicate statistically significant differences between surface treatment groups (vertically). Different uppercase letters signify statistically significant differences between the PEEK materials (horizontally).

Table 3. Analysis of covariance (ANCOVA) for shear bond strength (SBS) results

Source	Type III sum of squares	*df*	Mean square	*F*	*p*-value
Corrected model	7435.491[a]	31	239.855	176.135	0.000*
Intercept	75,042.888	1	75,042.888	55,106.930	0.000*
PEEK	314.306	1	314.306	230.807	0.000*
Surface	4,620.523	3	1,540.174	1,131.010	0.000*
Adhesive	491.635	1	491.635	361.027	0.000*
Composite	380.846	1	380.846	279.670	0.000*
PEEK × surface	396.761	3	132.254	97.119	0.000*
PEEK × adhesive	172.108	1	172.108	126.386	0.000*
PEEK × composite	5.217	1	5.217	3.831	0.051
Surface × adhesive	628.496	3	209.499	153.843	0.000*
Surface × composite	100.229	3	33.410	24.534	0.000*
Adhesive × composite	0.014	1	0.014	0.010	0.921
PEEK × surface × adhesive	199.812	3	66.604	48.910	0.000*
PEEK × surface × composite	8.690	3	2.897	2.127	0.097
PEEK × adhesive × composite	11.951	1	11.951	8.776	0.003*
Surface × adhesive × composite	17.933	3	5.978	4.390	0.005*
PEEK × surface × adhesive × composite	86.970	3	28.990	21.289	0.000*
Error	392.189	288	1.362	–	–
Total	82,870.568	320	–	–	–
Corrected total	7,827.680	319	–	–	–

[a] $R^2 = 0.950$ (adjusted $R^2 = 0.945$); *df* – degrees of freedom; * statistically significant ($p < 0.05$).

observed between the surface treatment groups ($p < 0.01$) (Table 2). The values noted in the control group were lower than those in the other 3 groups for both PEEK materials ($p < 0.01$). The values obtained from the air abrasion and acid etching + air abrasion groups were lower than those from the acid etching group ($p < 0.01$). The surface roughness of BioHPP exceeded that of JUVORA™ for acid etching treatment ($p < 0.01$). Other pairwise comparisons revealed no statistically significant differences ($p > 0.05$).

Shear bond strength

The analysis of covariance revealed that the PEEK materials, surface treatments, adhesive agents, and composite materials had significant effects on the SBS values ($p < 0.001$) (Table 3). A significant two-factor interaction was observed between the PEEK materials and surface treatments ($p < 0.001$), as well as between the PEEK materials and adhesive agents ($p < 0.001$). There were borderline significant two-factor interactions between the PEEK materials and composites ($p = 0.051$). Furthermore, the three-factor interaction among the PEEK materials, adhesive agents and composites was significant ($p = 0.003$), as well as the correlation between the PEEK materials, surface treatments and bonding agents ($p < 0.001$). In addition, a significant interaction was noted among surface treatments, adhesive agents and composites ($p = 0.005$), as well as among the PEEK materials, surface treatments, adhesive agents, and composites ($p < 0.001$).

The differences between the SBS values based on the PEEK materials, composite materials and adhesives used are presented in Table 4. In the control group, the values for BioHPP specimens were significantly higher than the values for JUVORA™ specimens when the combination of the same composite material and the same adhesive was used ($p < 0.001$). In the air abrasion group, there were no significant differences between the SBS values of BioHPP and JUVORA™ specimens when VL adhesive and SR composite were used ($p = 0.006$). However, the SBS values of BioHPP were higher than those of JUVORA™ when using other adhesive and composite combinations ($p < 0.001$). When applying VL adhesive and SR composite, the values of BioHPP were not significantly different from the values of JUVORA™ after acid etching treatment ($p = 0.083$). Similarly, no significant differences were observed between PEEK materials when SBU and CR composite were applied to acid-etched surfaces ($p = 0.360$). For other combinations of bonding and composite materials in the acid etching group, the SBS values of BioHPP were significantly higher than the values of JUVORA™ ($p < 0.001$). In the acid etching + air abrasion group, the SBS values of BioHPP were significantly higher than the values of JUVORA™ when SBU and SR composite material were applied together ($p < 0.001$). The SBS values of BioHPP and JUVORA™ were not significant for the SBU and CR combination in the air abrasion + acid etching group ($p = 0.069$). However, the SBS values of JUVORA™ specimens were significantly higher than the values of BioHPP specimens for other adhesive and composite material combinations ($p < 0.001$).

Table 4. Comparison of shear bond strength (SBS) of polyetheretherketone (PEEK) materials

Surface treatment	Bonding agent	Composite	PEEK	SBS [MPa] *M* ±*SD*	*p*-value
No treatment (control)	VL	CR	BioHPP	10.13 ±1.10	<0.001*
			JUVORA™	8.06 ±0.53	
		SR	BioHPP	12.31 ±1.23	<0.001*
			JUVORA™	7.65 ±0.52	
	SBU	CR	BioHPP	10.29 ±0.67	<0.001*
			JUVORA™	5.94 ±0.36	
		SR	BioHPP	9.43 ±0.59	<0.001*
			JUVORA™	7.02 ±0.92	
Acid etching	VL	CR	BioHPP	21.41 ±1.04	<0.001*
			JUVORA™	18.66 ±0.93	
		SR	BioHPP	22.54 ±1.22	0.083
			JUVORA™	21.45 ±1.43	
	SBU	CR	BioHPP	14.69 ±0.89	0.360
			JUVORA™	15.04 ±0.81	
		SR	BioHPP	19.36 ±1.51	<0.001*
			JUVORA™	14.80 ±0.83	
Air abrasion	VL	CR	BioHPP	18.62 ±1.37	0.001*
			JUVORA™	16.28 ±1.29	
		SR	BioHPP	21.58 ±1.51	0.006*
			JUVORA™	19.36 ±1.66	
	SBU	CR	BioHPP	15.84 ±0.71	<0.001*
			JUVORA™	9.80 ±0.93	
		SR	BioHPP	17.93 ±1.07	<0.001*
			JUVORA™	11.80 ±1.03	
Acid etching + air abrasion	VL	CR	BioHPP	12.94 ±0.83	<0.001*
			JUVORA™	17.52 ±1.35	
		SR	BioHPP	14.92 ±1.29	<0.001*
			JUVORA™	21.36 ±1.13	
	SBU	CR	BioHPP	16.73 ±1.70	0.069
			JUVORA™	15.54 ±0.95	
		SR	BioHPP	22.08 ±1.53	<0.001*
			JUVORA™	18.81 ±0.74	

* statistically significant ($p < 0.05$, independent samples *t*-test; Holm–Bonferonni adjustment for multiple testing, Bonferroni adjustment value: $\alpha = 0.05/16 = 0.003$); VL – visio.link; SBU – Single Bond Universal; CR – crea.lign; SR – SR Nexco.

Scanning electron microscopy

Following surface treatments, the SEM images of BioHPP and JUVORA™ were obtained (Fig. 2–5).

Irregular structures formed by abrasive papers were observed on the surfaces of the specimens even in the absence of surface treatment (Fig. 2A,B). In the BioHPP specimens, the piranha acid-etched surfaces exhibited low porosity (Fig. 3A), while the JUVORA™ specimens demonstrated a honeycomb pattern (Fig. 3B). The regular structure underwent deterioration after air abrasion treatment, resulting in a formation of a recessed and protruding surface (Fig. 4A,B). After air abrasion and piranha acid etching, the surfaces exhibited recessed and protruded features, in addition to the presence of porous areas (Fig. 5A,B).

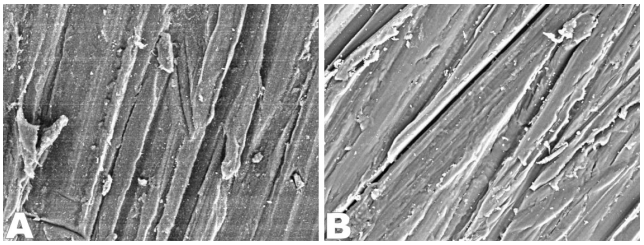

Fig. 2. Scanning electron microscopy (SEM) images of polyetheretherketone (PEEK) surfaces without pre-treatment (×1000 magnification)
A. BioHPP; B. JUVORA™.

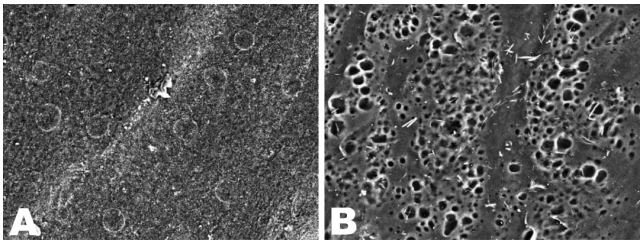

Fig. 3. SEM images of PEEK surfaces after acid etching (×1000 magnification)
A. BioHPP; B. JUVORA™.

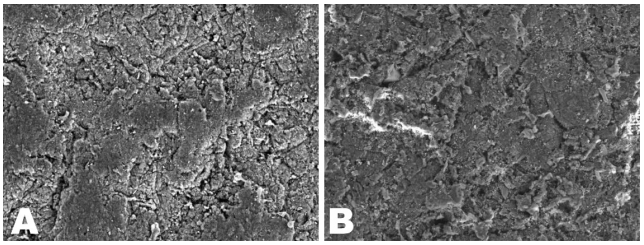

Fig. 4. SEM images of PEEK surfaces after air abrasion (×1000 magnification)
A. BioHPP; B. JUVORA™.

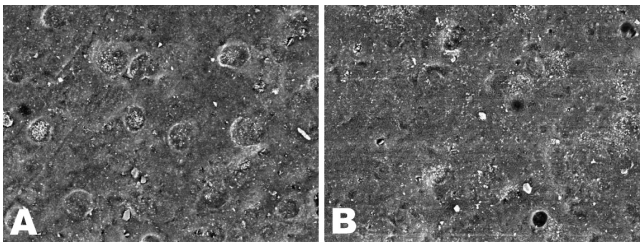

Fig. 5. SEM images of PEEK surfaces after acid etching and air abrasion (×1000 magnification)
A. BioHPP; B. JUVORA™.

Failure modes

The failure types of PEEK materials are presented in Table 5. Adhesive and mixed failures were observed; however, no instances of cohesive failure were noted within the groups (Fig. 6A,B). There were no significant differences between the failure modes of the materials for the control and acid etching + air abrasion groups ($p > 0.05$). In the air abrasion group, there were no differences in the incidence of adhesive failure between JUVORA™ and BioHPP for the SBU and CR combination ($p = 0.178$). However, adhesive failure was significantly more prevalent in JUVORA™ specimens in comparison to BioHPP for other adhesive and composite material combinations ($p < 0.05$). In the acid etching group, the incidence of adhesive failure was significantly higher in the JUVORA™ group compared to the BioHPP group for VL adhesive and SR composite ($p = 0.019$). However, no statistically significant differences were identified between the failure modes of PEEK materials when evaluated across other adhesive–composite material combinations ($p > 0.05$).

Discussion

The study demonstrated that surface treatment methods, adhesive agents and composite veneer materials had a significant effect on the SBS of both BioHPP and JUVORA™ to composites. Therefore, the first null hypothesis was rejected. Although there were no significant differences between the SBS values of BioHPP and JUVORA™ for some pairwise comparisons in the surface treatments groups, significant differences were observed between both control PEEK groups. Therefore, the second null hypothesis was partially rejected.

The SBS between the composite resin and the PEEK surfaces in the control group was significantly lower compared to the other surface-treated groups. Considering that the obtained SBS values were higher than the critical SBS value (10 MPa), it can be concluded that air abrasion, acid etching, as well as acid etching + air abrasion of both PEEK surfaces can be considered proper surface treatment methods for both BioHPP and JUVORA™.

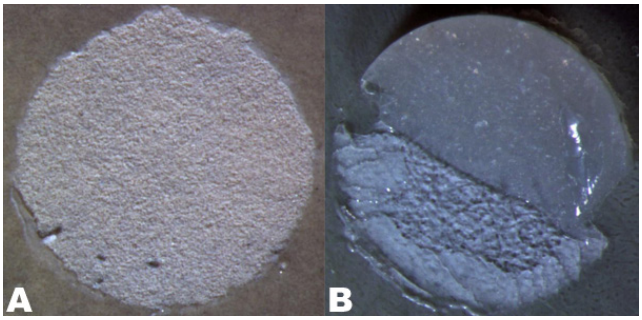

Fig. 6. Stereomicroscope images of failure modes (×35 magnification)
A. Adhesive failure; B. Mixed failure.

Table 5. Comparison of failure modes of polyetheretherketone (PEEK) materials

Surface treatment	Bonding agent	Composite	Failure mode	Failures, *n*		*p*-value
				BioHPP	JUVORA™	
No treatment (control)	VL	CR	adhesive	6	9	0.303
			mixed	4	1	
		SR	adhesive	10	10	–
			mixed	0	0	
	SBU	CR	adhesive	7	7	1.000
			mixed	3	3	
		SR	adhesive	7	8	1.000
			mixed	3	2	
Acid etching	VL	CR	adhesive	1	3	0.264
			mixed	9	7	
		SR	adhesive	1	6	0.019*
			mixed	9	4	
	SBU	CR	adhesive	6	6	1.000
			mixed	4	4	
		SR	adhesive	4	3	0.639
			mixed	6	7	
Air abrasion	VL	CR	adhesive	0	9	0.001*
			mixed	10	1	
		SR	adhesive	3	10	0.001*
			mixed	7	0	
	SBU	CR	adhesive	4	7	0.178
			mixed	6	3	
		SR	adhesive	5	9	0.048*
			mixed	5	1	
			mixed	6	7	
Acid etching + air abrasion	VL	CR	adhesive	5	5	1.000
			mixed	5	5	
		SR	adhesive	8	7	0.606
			mixed	2	3	
	SBU	CR	adhesive	8	5	0.160
			mixed	2	5	
		SR	adhesive	7	7	1.000
			mixed	3	3	

* statistically significant ($p < 0.05$, Pearson's χ^2 and Fisher's exact test).

Previous studies have examined the SBS of PEEK specimens following diverse surface treatments, including piranha acid etching and air abrasion.[27] The authors have demonstrated higher bonding values with increased Ra as compared to untreated specimens.[27] Elevated Ra values increase wettability by decreasing surface tension and increasing surface area.[7] Airborne-particle abrasion results in improved microroughness of the substrate, whereas pre-treatment with acids increases the number of functional carbon–oxygen groups in the surface layer of PEEK.[11,12] Air abrasion with 100-µm aluminum oxide particles has been stated to improve SBS of PEEK to different composite resins.[13] This finding is consistent with previous studies, which indicated that air-abraded PEEK surfaces exhibited lower SBS values between PEEK and composite veneer compared to etched PEEK surfaces.[7,28] The chemical surface treatment was more effective than the mechanical surface treatment. The present findings indicate that acid etching resulted in higher bond strength between PEEK material and composite veneer compared to the acid etching + air abrasion surface treatment method. Alumina particles attached to PEEK surfaces can reduce the mechanical interlocking between a composite veneer and PEEK. Additionally, the particles retained on the PEEK surfaces could hinder the formation of pores during acid etching treatment and reduce

the flow of an adhesive agent into the pores of PEEK surfaces, subsequently decreasing SBS values.[14] Furthermore, the SEM images of PEEK materials revealed more porous and permeable structures after acid etching (Fig. 3A,B). In this study, higher Ra values (mean (*M*): 3.46 ±0.75 μm) were observed in the acid etching group specimens as compared with other groups. In addition, the values obtained in this study were congruent with the outcomes of previous studies that examined the bond strength of PEEK substrates.[20,25]

On the other hand, it has been reported that the increase in Ra after surface treatment proved inadequate in establishing a reliable bond strength between PEEK and composite veneer materials.[26] The enhancement of bond strength between PEEK and composite veneers requires conditioning with adhesive agents. Although there were no differences between the Ra values in this study, the difference between the SBS values indicates that chemical bonding exhibits greater durability than mechanical bonding. The composition of adhesive agents is a critical factor in determining bond strength between PEEK and composites.[12] Previous studies have indicated that MMA-containing adhesive materials significantly contribute to the high bond strength.[12] Therefore, the effect of an adhesive agent on SBS may be more pronounced than the effect of surface treatment methods.[13] The chemical composition of adhesive systems plays a central role in the formation of chemical bonds between different polymers. To date, no studies have compared SBS across specimens with different bonding agents following a similar methodology, which makes it difficult to conduct a meta-analysis.[21] In this study, the best bonding potential was observed in the VL-conditioned groups containing pentaerythritol triacrylate (PETIA), MMA monomers and additional dimethacrylates in solution. It can be assumed that PETIA dissolves the surface of PEEK, whereas MMA monomers cause swelling of the dissolved PEEK surfaces, and dimethacrylate monomers connect to the composite materials with 2 carboxyl groups as the bonding site. Although the use of SBU as the coupling agent in this study resulted in improved SBS values, the results were lower when compared to VL. This finding can be explained by the fact that a functional group of the bifunctional 10-methacryloyloxydecyl dihydrogen phosphate (10-MDP) monomer is occupied by a phosphate group, which in incapable to react chemically with the PEEK substrate or veneering resin composite.[25] An exception to this phenomenon was observed in the BioHPP groups veneered with SR, wherein SBU showed a higher bond strength in comparison to groups conditioned with VL after acid etching + air abrasion treatment ($p < 0.01$). The molecular alterations of surfaces after a combination of mechanical and chemical treatment may be the underlying cause of the improvements observed in the chemical bond of 10-MDP monomer to the modified PEEK surface. Moreover, the presence of silane in SBU may facilitate the formation of bonds with the ceramic filler of BioHPP exposed after surface modification and contribute to the increased bond strength of the material.[3] Similarly, polyalkenoic acid copolymer content of SBU can improve the bond strength between PEEK and composite materials.[24]

In the present study, the SBS values ranged from 16.28 MPa to 21.58 MPa when VL was used following air abrasion treatment, which aligns with the results of previous studies.[23,25] One of these studies compared the bond strength of the veneering resin composites to PEEK after the application of different adhesives and diverse pretreatment specimens.[25] The use of VL (18.0–28.8 MPa) resulted in higher SBS when compared to Clearfil™ Ceramic Primer (2.2–9.3 MPa) that contains 10-MDP. However, the waiting time between the application of surface treatments, adhesive agents and composite veneers was not mentioned in previous studies,[23,25,31] which may be the reason for the different SBS values reported.

In this study, 2 composite veneers were used. In general, an increase in viscosity resulting from elevated filler content of dental composites may negatively affect mechanical retention.[33] Because of its low filler content (19.8%) and low molecular weight (470 g/mol) of urethane dimethacrylate (UDMA) content, SR is a low-viscosity composite.[35] Given that CR contains 50% ceramic particles, Bis-GMA, which possesses a higher molecular weight (512 g/mol), exhibits increased viscosity. In this study, specimens on which SR was applied demonstrated higher SBS values compared to CR, with the exception of 1 group. This phenomenon can be attributed to the enhanced mobility of SR into microroughness areas due to its low viscosity, which fosters good microretention. This finding is in agreement with the outcomes of the study conducted by Bötel et al.[2] Similarly, another study compared 2 different indirect resin composites, Sinfony and GC Gradia, which were applied to PEEK specimens after undergoing different surface-roughening methods.[19] The specimens veneered with low-viscosity Sinfony (50% filler rate) demonstrated higher bond strength values than the high-viscosity GC Gradia.[19]

In the present study, lower SBS values were found between JUVORA™ and SR resin for acid-etched surfaces in comparison with the corresponding values for acid-etched BioHPP surfaces. The observed difference may be caused by the presence of air voids in the acid-etched JUVORA™ surfaces, which may reduce the diffusion of monomers and composite material into the surfaces.[28] However, this difference was not observed when CR composite was used in conjunction with SBU. This discrepancy may be attributed to the higher viscosity of CR compared to SR. Increased viscosity may equally preclude the penetration into the microretention areas of all PEEK surfaces.[20] There were no significant differences between the SBS of BioHPP and JUVORA™ when air abrasion or acid etching surface treatment, VL adhesive agent, and SR composite veneer were applied. Therefore, both PEEK

materials can be applied with the same protocol, resulting in comparable bond strength.

When examining the bond strength, it is necessary to evaluate the SBS test results and analyze the fracture mode in the bonding interface. It has been claimed that cohesive and mixed failures are correlated with higher bond strength values than adhesive failures.[35] However, cohesive failures were not observed in this study, which is in agreement with another study on PEEK-indirect composite.[12] Although there were no significant differences between the SBS results of BioHPP and JUVORA™ materials when VL–SR combination was used after air abrasion or acid etching, mixed fractures were found to be significantly higher in the BioHPP material. The reason for this difference may be exposed ceramic filler and increased microretention area of the BioHPP material.

Limitations

The results of this in vitro study have limited clinical validity, as oral conditions such as saliva, mastication forces and chemical agents from food and beverages were not incorporated. To ensure the validity of the results, further clinical studies are necessary.

Conclusions

In this in vitro study, significant effects of surface treatments, adhesive agents and composite veneer materials on SBS were confirmed for 2 PEEK materials. The SBS values between the PEEK and composite veneer materials were significantly increased after the administration of surface pre-treatment procedures and adhesive agents. There were significant differences between the SBS values of both PEEK materials and the control group. When air-abraded or piranha acid-etched PEEK surfaces were conditioned with VL, there were no significant differences between the SBS of the 2 PEEK materials to the SR composite. When piranha acid-etched surfaces or air-abraded + piranha acid-etched PEEK surfaces were conditioned with SBU, no significant differences were observed between the SBS of BioHPP and JUVORA™ materials to CR composite. In general, PEEK specimens conditioned with VL exhibited significantly higher bond strength in comparison to those conditioned with SBU.

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.

Use of AI and AI-assisted technologies

Not applicable.

ORCID iDs

Zıya Seferli https://orcid.org/0000-0003-4371-6052
Kubra Degirmenci https://orcid.org/0000-0001-6429-4923
Serkan Saridag https://orcid.org/0000-0001-8767-788X

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Original paper

Effect of toothbrushing on microleakage of glass ionomer restorations with surface protection

İlay Özçelik Bulut[B–D], Ebru Hazar Bodrumlu[A,E,F]

Department of Pediatric Dentistry, Faculty of Dentistry, Zonguldak Bülent Ecevit University, 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

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Address for correspondence
Ebru Hazar Bodrumlu
E-mail: hazarebru@yahoo.com

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Abstract

Background. In the field of pediatric dentistry, preventing microleakage of glass ionomer cement (GIC) is important for clinical success. The abrasion and roughness of the surface of the restorative material that results from brushing can cause microleakage. The application of surface protection is intended to prevent this situation.

Objectives. The aim of the study was to evaluate the levels of microleakage following toothbrushing after the application of GICs with or without surface protection.

Material and methods. Cavities formed on the buccal surfaces of 180 extracted primary teeth were restored with resin-modified glass ionomer cement (RMGIC), and the teeth were divided into 3 groups according to the surface protection application, with an equal number of samples in each group ($n = 60$). The thermal cycle was applied to all samples. Subsequently, the groups were divided into 5 subgroups ($n = 12$/group) according to the brushing simulation (no brushing, and 1, 3, 6, and 12 months of brushing). The samples were stored in 2% methylene blue for 24 h and sectioned in the buccolingual direction. The presence of microleakage was determined with the use of a stereomicroscope. The data was statistically analyzed.

Results. No statistically significant differences were observed between the main groups at all brushing times ($p > 0.05$). However, higher microleakage results were obtained in the group without surface protection. When the groups were evaluated according to the duration of brushing, no statistically significant differences were identified ($p > 0.05$), but higher microleakage results were obtained in the samples that underwent brushing for 12 months.

Conclusions. Although statistically significant results were not obtained in terms of microleakage regarding surface protection application and brushing, it should be noted that coating restorations with surface protectants may contribute to a smoother surface and marginal integrity, and may be beneficial in reducing microleakage.

Keywords: dental leakage, toothbrushing, dental cements, deciduous tooth

Cite as
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Highlights

- The application of surface protectants to RMGIC restorations effectively reduces microleakage.
- No difference in microleakage was observed between nano-filled and adhesive surface protectants.
- While brushing does not significantly affect microleakage, prolonged brushing may increase surface wear and leakage in RMGIC materials.

Introduction

Dental caries is a multifactorial, preventable and common childhood disease.[1,2] The condition can cause pain, difficulty in eating, malnutrition, aesthetic problems, decreased self-confidence, and, therefore, a decrease in quality of life.[2–4] In order to prevent the occurrence or progression of dental caries, the treatment of decayed teeth should be performed promptly.[5] Amalgams, glass ionomer cements (GICs), compomers, and composite resins are used as restorative materials in the treatment of primary teeth.[6] Glass ionomer cements are used in the treatment of primary teeth and are considered an alternative restorative material that is frequently used in pediatric dentistry.[7] Glass ionomer cements were first introduced by Wilson and Kent in 1972.[6] These materials are formed by the curing reaction between powdered aluminosilicate glasses and an aqueous solution of polyacrylic acid.[8] Glass ionomer cements allow for conservative preparation. They can chemically bind to dental tissues, release fluoride, and be placed in a single step.[7] Conversely, studies have highlighted several drawbacks, such as low wear resistance, short working and long curing time, high initial moisture sensitivity, and the occurrence of microleakage.[8] To address the limitations of conventional glass ionomer cements (CGICs), resin-modified glass ionomer cements (RMGICs) have been developed. It has been documented that RMGICs have better adaptation, adhesion and aesthetic properties than CGICs.[9] Although RMGICs demonstrate resistance to early contact with water, it is not clear how sensitive these materials are to hydration or dehydration immediately after light activation.[10] Upon exposure to moisture, the mechanical resistance of GICs decreases, and the surface experiences accelerated wear.[11] The use of Vaseline®, cocoa butter, varnishes, and various surface-covering agents is recommended to prevent early contact of GICs with water. Among these, light-curing resin-containing sealants are particularly noteworthy.[11]

One of the most important factors affecting the success of restorative materials is microleakage. Microleakage is defined as the passage of bacteria, molecules, liquids, or ions between the cavity wall of the tooth and the filling material applied to it.[12] Microleakage negatively affects the success of the restorative material by causing problems such as secondary caries, sensitivity, diseases affecting the pulp, and marginal discoloration in the restoration.[13] Microleakage may occur due to thermal changes, loss of contour as a result of wear in the restorative material, mechanical stress, or a lack of adaptation of the restorative material, which can result in a gap at the tooth–material junction.[14] Restorative materials are exposed to chewing forces, dietary habits and brushing forces in the oral cavity. These factors can lead to wear of restorative materials over time and loss of anatomical form.[15] Toothbrushing has been shown to cause adverse conditions, including wear that leads to roughness and microleakage on the surface. This is due to the abrasive content of toothpastes and the mechanical effect of the brush.[16,17] It has been reported that the application of surface protection is effective in preventing microleakage by improving the mechanical and physical properties of materials.[11,18] During maturation, surface protectants isolate the GIC from saliva contamination, increase the durability of the restoration, occlude the surface cracks, and protect the restoration against abrasion.[19] However, the effect of applying surface protection to prevent microleakage as a result of the abrasive effect of toothbrushing needs to be investigated. Therefore, the aim of the study was to examine the levels of microleakage following toothbrushing after the application of GICs with or without surface protection, which are frequently used in the restorative treatment of primary teeth.

Material and methods

This study was conducted at the Department of Pedodontics of Zonguldak Bülent Ecevit University, Turkey. It was approved by the Clinical Research Ethics Committee of Zonguldak Bülent Ecevit University (protocol No. 2021-09; May 5, 2021).

A total of 180 lower and upper primary second molars, which were indicated for extraction due to infection, periodontal tissue loss or orthodontic purposes were included in the study. Teeth that were damaged during extraction, had caries on their crowns, or fractures/cracks in the dental crown before extraction were excluded from the study.

The number of samples to be used in our research was determined to have 95% test power (1–β), 95% confidence (1–α), an effect size (*f*) of 0.677, and at least 10 samples in each of the subgroups. The statistical power was calculated using the G*Power software (https://www.psychologie.hhu.de/arbeitsgruppen/allgemeine-psychologie-und-arbeitspsychologie/gpower). The study was performed

using a total of 180 samples, with 60 samples allocated to each of the main groups, and 12 samples included in each of the subgroups.

Soft tissue remnants and debris were removed from all studied teeth with a scaler. The extracted teeth were stored in distilled water until the beginning of the experimental phase. The roots of the teeth were cut out with a diamond separator (Komet USA, Rock Hill, USA), under water cooling, and apical to the cementoenamel junction. The crowns of the teeth were cut into two on the mesiodistal axis, parallel to their long axis. In all groups, only the buccal surfaces of the teeth were examined. Class V cavities were created on the buccal surfaces of the deciduous teeth, with the dimensions of 3 mm in mesiodistal width, 2 mm in occlusogingival height, and 1.5 mm in depth. All cavosurface angles were precisely measured to be 90 degrees under water cooling. The prepared cavities were restored with RMGIC (Fuji II LC; GC Corp., Tokyo, Japan).

Glass ionomer cement in capsule form, which does not require an adhesive, was prepared by mixing for 10 s in a mixer, and then applied to the prepared cavities using a capsule applier. After shaping the initial contour, the glass ionomer was polymerized by applying 470-nm wavelength light for 20 s (ELIPAR S10; 3M, Maplewood, USA). The teeth were not polished after glass ionomer polymerization. A total of 180 teeth were randomly divided into 3 equal groups.

The surfaces of the teeth in 2 of the 3 groups were treated with 2 different protective agents, while 1 group was left untreated. The present study employed 2 agents, namely a nanofilled light-curing surface protectant (Equia Forte Coat; GC Corp.) and a light-cured adhesive material (Heliobond; Ivoclar Vivadent, Schaan, Liechtenstein) for the primary teeth.

Following the formation of the groups, all teeth were stored in distilled water at 37°C for 24 h. Then, the teeth were subjected to 500 thermal cycles between 5°C and 55°C, with a 10-s transfer and 30-s holding period. After thermal cycling, each main group was randomly divided into 5 equal subgroups (n = 12/group) based on the time spent in the brushing simulator. It was determined that 1 year of toothbrushing was equivalent to 10,000 cycles, 6 months equaled 5,000 cycles, 3 months equaled 2,500 cycles, and 1 month equaled 840 cycles. The brushing simulator was applied to both groups treated with protective agents, with each subgroup undergoing equivalent brushing cycles for 1 month, 3 months, 6 months, and 1 year. The control group was not subjected to brushing. For the brushing simulation, each tooth sample was placed in the center of an acrylic block, which was prepared to fit the sample cups in the brushing simulator (DentArGe TB-6.1 Brushing Simulator; Analitik Medikal, Gaziantep, Turkey). A single tooth was embedded within each block, with the buccal surface of the teeth exposed and fixed horizontally. The brushing simulation was conducted using a children's toothpaste (Colgate-Palmolive, New York, USA) mixed with distilled water (1:1) and a children's toothbrush with medium hard bristles (Denta, Istanbul, Turkey). The brushes were replaced after 2,500 cycles. Brushing was performed for each sample under the following conditions: a vertical force of 200 g (2 N); a cycle speed of 60 mm/s; a stroke length of 20 mm; and standardized back-and-forth movement. Following the brushing simulation, the samples were removed from the sample cups, and each specimen was washed with running tap water for 20 s before being preserved in distilled water.

All teeth were then tested for microleakage. Two coats of nail polish were applied to all teeth surfaces, with the enamel surface of the restorations exposed by up to 1 mm. The teeth were stored in containers with 2% methylene blue solution at 37°C for 24 h. Following this, the teeth were removed from the solution, washed under running tap water for 5 min, and dried. Then, the samples were bisected in a buccolingual direction under water cooling. A 0.2-mm thickness diamond separator was used to examine potential microleakage. The occurrence of dye leakage in the obtained sections was examined with a stereomicroscope (Olympus SZ61; Olympus Corporation, Tokyo, Japan) at ×20 magnification. The assessment of dye leaks in the cavities was conducted using a qualitative scoring method, as outlined by Sidhu[20]:

– 0: no dye penetration;
– 1: dye penetration in less than ½ of the cavity wall;
– 2: dye penetration in more than ½ of the cavity wall;
– 3: dye penetration seen throughout the cavity wall.

The collected data was recorded digitally, and the highest score noted for each sample was evaluated.

Statistical analysis

The data analysis was performed using the IBM SPSS Statistics for Windows software, v. 23.0 (IBM Corp., Armonk, USA). The evaluation of conformity to the normal distribution was performed using the Shapiro–Wilk test. The Kruskal–Wallis test was employed to compare the microleakage values that were not suitable for normal distribution according to the groups differing by surface protection application and brushing times. The results of the quantitative data analysis were expressed as median (Me) (minimum–maximum) and mean ±standard deviation ($M \pm SD$). The significance level was set at $p < 0.05$.

Results

The microleakage scores of the samples with and without surface protectants according to the brushing time are shown in Table 1. The median microleakage score was 0 in unbrushed samples for all 3 main groups. Higher microleakage scores were observed in teeth without surface protectants, although the difference between the groups was not significant ($p > 0.05$). Furthermore, higher

microleakage levels were observed after 1, 3, 6, and 12 months of brushing in the control group. However, the difference between the time points was not statistically significant ($p > 0.05$).

The microleakage scores of the samples at varying brushing times in relation to the application of surface protectants are shown in Table 2. Higher microleakage scores were observed after 12 months of brushing for all 3 groups. However, the difference between the median values of microleakage scores according to the brushing time was not significant ($p > 0.05$) (Fig. 1–3).

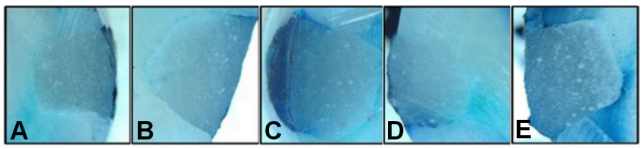

Fig. 1. Stereomicroscope images showing microleakage in untreated samples

A. Baseline (microleakage score = 0); B. After 1 month of brushing (microleakage score = 1); C. After 3 months of brushing (microleakage score = 1); D. After 6 months of brushing (microleakage score = 2); E. After 12 months of brushing (microleakage score = 3).

Table 1. Comparative evaluation of microleakage scores between samples with and without surface protectants subjected to different brushing durations

Brushing time	Surface protectant application	Samples, *n*	Microleakage score		Statistical test (Shapiro–Wilk test and Kruskal–Wallis test)	*p*-value
			M ±*SD*	*Me* (*Min–Max*)		
No brushing	no surface protection	12	0.42 ±0.67	0 (0–2)	1.247	0.536
	adhesive-containing	12	0.25 ±0.62	0 (0–2)		
	nanofilled	12	0.17 ±0.39	0 (0–1)		
1 month	no surface protection	12	0.59 ±0.79	0 (0–2)	0.747	0.688
	adhesive-containing	12	0.50 ±0.80	0 (0–2)		
	nanofilled	12	0.33 ±0.65	0 (0–2)		
3 months	no surface protection	12	0.42 ±0.67	0 (0–2)	0.285	0.867
	adhesive-containing	12	0.50 ±0.80	0 (0–2)		
	nanofilled	12	0.33 ±0.65	0 (0–2)		
6 months	no surface protection	12	0.92 ±1.08	0.5 (0–3)	0.491	0.782
	adhesive-containing	12	0.67 ±1.07	0 (0–3)		
	nanofilled	12	0.75 ±1.05	0 (0–3)		
12 months	no surface protection	12	1.33 ±1.37	1 (0–3)	0.514	0.774
	adhesive-containing	12	1.00 ±1.20	0.5 (0–3)		
	nanofilled	12	1.00 ±1.20	0.5 (0–3)		

M – mean; *SD* – standard deviation; *Me* – median.

Table 2. Comparative evaluation of microleakage scores according to the application of surface protection

Surface protectant application	Brushing time	Samples, *n*	Microleakage score		Statistical test (Shapiro–Wilk test and Kruskal–Wallis test)	*p*-value
			M ±*SD*	*Me* (*Min–Max*)		
No surface protection	no brushing	12	0.42 ±0.67	0 (0–2)	4.853	0.303
	1 month	12	0.59 ±0.79	0 (0–2)		
	3 months	12	0.42 ±0.67	0 (0–2)		
	6 months	12	0.92 ±1.08	0.5 (0–3)		
	12 months	12	1.33 ±1.37	1 (0–3)		
Adhesive-containing	no brushing	12	0.25 ±0.62	0 (0–2)	3.557	0.469
	1 month	12	0.50 ±0.80	0 (0–2)		
	3 months	12	0.50 ±0.80	0 (0–2)		
	6 months	12	0.67 ±1.07	0 (0–3)		
	12 months	12	1.00 ±1.20	0.5 (0–3)		
Nanofilled	no brushing	12	0.17 ±0.39	0 (0–1)	5.613	0.230
	1 month	12	0.33 ±0.65	0 (0–2)		
	3 months	12	0.33 ±0.65	0 (0–2)		
	6 months	12	0.75 ±1.05	0 (0–3)		
	12 months	12	1.00 ±1.20	0.5 (0–3)		

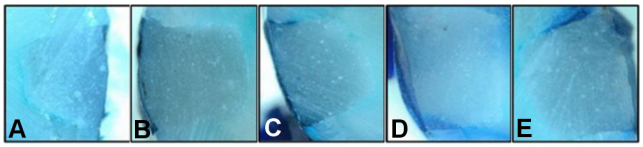

Fig. 2. Stereomicroscope images showing microleakage in samples after the application of a nanofilled surface protectant

A. Baseline (microleakage score = 0); B. After 1 month of brushing (microleakage score = 1); C. After 3 months of brushing (microleakage score = 1); D. After 6 months of brushing (microleakage score = 2); E. After 12 months of brushing (microleakage score = 2).

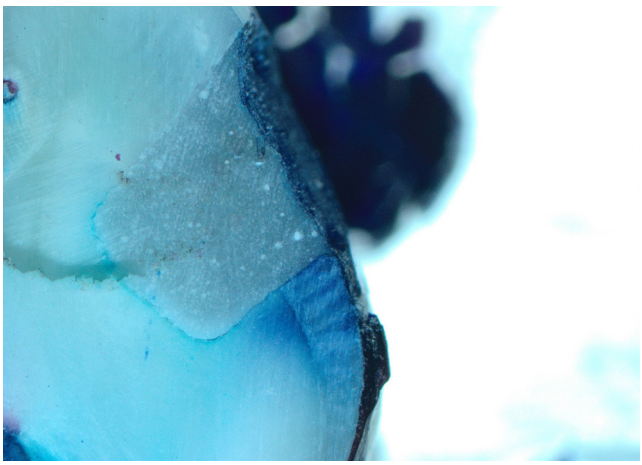

Fig. 3. Stereomicroscope image showing microleakage in a sample after the application of an adhesive-containing surface protectant

Discussion

In pediatric dentistry, composite resins, compomers, amalgams, CGICs, high-viscosity GICs (HVGICs), and RMGICs are the preferred materials in the treatment of dental caries.[21] Currently, due to the spread of minimally invasive dentistry, the preference for tooth-colored restorative materials and their ability to bond with dental tissues, GICs have become prominent in the field of pediatric dentistry.[22,23] In order to improve the properties of CGICs, various modifications have been made and RMGICs have been developed.[24] Resin-modified glass ionomer cements contain the same main components as CGICs (basic glass powder, water and polyacid), 2-hydroxyethyl methacrylate (HEMA) as a monomer component, and camphorquinone as an initiator.[25]

Glass ionomer cements are sensitive to the presence of early moisture, which can lead to water absorption and hygroscopic expansion. To prevent this, it is important to protect the cement by covering it with a suitable varnish or petroleum jelly (Vaseline®). Recently, nanofilled, self-adhesive and light-cured surface protecting agents have been developed for the use with CGICs, RMGICs, composite resins, and compomer restorations. These agents have been designed to enhance the mechanical properties of the restorations, increase their wear resistance and improve their appearance. Surface protectants contribute to the clinical success of restorations by filling small surface voids and cracks and reducing discoloration.[26–28]

The tooth surface and dental materials encounter the abrasive effects of brushing with paste.[29] The increase in surface roughness is important for the clinical life, microhardness and abrasion resistance of restorative materials, secondary caries risk, coloration, and aesthetics.[30,31] Studies have revealed that wear, roughness, color change, and microleakage occur after the application of surface protectants to GICs or other restorative materials. Additionally, studies have examined the effect of brushing on the roughness and wear resistance of restorative materials. However, there is a paucity of research examining microleakage in GICs with surface protectants after brushing. Therefore, our study evaluated the effect of toothbrushing on microleakage of RMGICs with adhesive surface protectants and nanofilleld surface protectants.

Microleakage can be observed in RMGICs. Therefore, it is recommended that a surface protectant be applied to teeth after polymerization to prevent this situation.[32] Surface protective agents, which contribute to ensuring marginal sealing and improving surface properties in restorations, are fluid materials that can penetrate gaps and restore resin-containing materials or GICs.[33,34] Oba and Aras compared polyacid-modified composite resins (PMCRs) applied in the restoration of class V cavities in extracted primary teeth and RMGICs that were covered with nanofilled surface protectants, similar to our study. The study demonstrated a reduced incidence of microleakage in RMGIC samples, suggesting that the restorations are protected with a surface-protective agent during the cement curing process, thus preventing moisture contamination and microcracking. Similarly, Agnihotri et al. reported that the application of surface protection was effective in reducing microleakage in the RMGIC group.[36] The present study found no statistically significant differences in terms of microleakage in the samples that were not brushed, between the surface-protected and unprotected groups. However, higher microleakage was noted in teeth without surface protection compared with surface-protected teeth. In agreement with the findings of previously published studies, our results indicate that the coating of the material surfaces with protective agents has a positive, even if not statistically significant, effect on microleakage. This finding could be related to the prevention of early moisture contamination and the filling of microvoids in RMGICs when surface protection was applied.

In RMGICs, it is preferred to coat the surfaces with filler-containing agents, varnishes or adhesive-containing surface protectants in order to prevent water absorption of HEMA, improve the quality of the material, and reduce dimensional changes.[37] Chuang et al. examined the microleakage of RMGIC and reported that the adhesive-containing surface protectant is the most effective in preventing microleakage.[38] A study by Ribeiro et al. using different RMGIC materials found no statistical differences in

dye uptake between RMGICs.[39] However, surface protectant application was required in all samples, and the best results were obtained with the adhesive-containing protectant.[39] Erhardt et al. reported that adhesive protectants were not effective in reducing microleakage and had a high probability of abrasion from heat exposure or intraoral abrasive forces.[40] Urquía-Morales et al. tested the effect of different surface protectants on the efficacy of composite resins in mitigating microleakage.[41] The study found that the utilization of surface protectants significantly reduced microleakage in all experimental groups compared to the control group.[41] In contrast to the aforementioned studies, Pacifici et al. evaluated HVGIC and RMGIC with a nanofilled surface protectant, an adhesive-containing surface protectant, and an unapplied surface protectant by scanning electron microscopy.[42] The authors found that regardless of the type of surface protection, it was successful for marginal sealing due to its high hydrophilicity and low viscosity.[42] In the present study, it was observed that the microleakage scores of samples that were not brushed and specimens to which surface protectants were applied yielded similar results. Furthermore, no statistical difference was detected between the groups. However, there was a discrepancy between the adhesive content and the nanofilled surface protectant with respect to the microleakage score, despite the fact that both materials yielded successful results. The present study revealed no significant difference between 2 surface protectants. The lower microleakage values of both materials were compared to the group that did not receive a surface protectant. However, the lower microleakage scores are likely attributable to the effective coverage of the surface protectants, which exhibited good fluidity and penetration on the surface of the restored teeth.

Abrasion has been reported as a undesirable condition that increases surface roughness and causes the restorative material to separate from the surface.[43] The separation of material from the surface may lead to the formation of new undesirable margins that can cause bacterial retention and subsequent microleakage.[44] Momoi et al. demonstrated that the wear rate increased significantly after brushing in CGIC, amalgam and composite resin materials.[43] When evaluating various effects of toothbrushing on microleakage, Goldstein et al. reported no statistically significant difference between the brushing group and the control group of class V composite resin restorations after using a sonic toothbrush.[45] Similar to our results, this study has shown that brushing does not have a significant effect on microleakage.[45] The prevention of early moisture contamination of materials allows for better abrasion resistance and marginal integrity, which, in turn, leads to improved sealing restorations. The application of surface protection is recommended to prevent microleakage.[46,47] Kanık compared a nanofilled surface protectant and varnish application on 2 different HVGICs with non-preserved composite resin for abrasion resistance as a result of brushing.[48] It was reported that with increasing brushing cycles, the teeth applied with varnish showed significantly more wear than the teeth applied with the nanofilled surface protectant. In the context of our study, which examined brushing simulations at different time points, no statistically significant difference in terms of microleakage was observed between the non-preserved group and the protected groups with respect to brushing times. Our observations revealed that neither brushing nor the duration of brushing exerted any influence on microleakage in all samples. A comparison of our results with other studies was precluded by the absence of research evaluating the effect of brushing on microleakage in RMGICs treated with surface protection. Although our study did not identify statistically significant differences, higher microleakage levels were observed in the group that did not utilize surface protection. Consequently, the utilization of surface protectants may enhance the wear resistance of RMGICs.

Toothbrush wear and the resulting surface roughness cause changes to the surface properties of different materials. Studies have reported that surface protectants undergo a gradual deterioration due to the effects of abrasive factors over time.[48,49] Kanık and Türkün examined the surface protective activity after brushing simulation and observed that the protective agents exhibited signs of wear.[50] In their evaluation of the effectiveness of surface protectants, Lohbauer et al. reported that nanofilled surface protectants underwent partial or complete erosion from the restoration surface at 6 months due to brush abrasion and occlusal contact.[49] While our study did not yield significant results, higher microleakage results were observed in samples that underwent brushing for 12 months when compared to the 1-, 3- and 6-month brushing periods. The observed increase in microleakage results at 12 months of brushing is likely due to the roughness and abrasion caused by the abrasive forces of toothbrushing over time, the effect on the resin matrix, and deterioration of the surface of the restorative material.

The study was conducted in vitro, under the influence of brushing only, while other conditions in the oral environment were ignored. Therefore, further clinical studies should be conducted on the topic.

Conclusions

After analyzing the collected data, it is predicted that the application of surface protectants on RMGIC restorations will reduce microleakage through the filling of microvoids and the enhancement of the wear resistance of the restorative material. The investigation revealed no statistically significant differences in microleakage outcomes between nanofilled and adhesive surface protectants, indicating that both materials are suitable for clinical use. While the impact of brushing on microleakage

is not significant, it is crucial to note that the extent of wear and leakage in RMGIC materials can increase with the increased duration of brushing time.

Ethics approval and consent to participate

The study was approved by the Clinical Research Ethics Committee of Zonguldak Bülent Ecevit University (protocol No. 2021-09; May 5, 2021).

Data availability

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

Consent for publication

Not applicable.

Use of AI and AI-assisted technologies

Not applicable.

ORCID iDs

İlay Özçelik Bulut https://orcid.org/0000-0001-9706-4868
Ebru Hazar Bodrumlu https://orcid.org/0000-0002-3474-5583

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Original paper

Evaluation of biofilm formation, adhesive strength and effectiveness of cleaning protocols on adhesive-containing acrylic resin specimens: An in vitro study

Caroline Vieira Fortes[1,A–D,F], Adriana Barbosa Ribeiro[1,A–F], Bart De Wever[2,A,C–F], Amina Sakly[2,C–F], Viviane de Cássia Oliveira[1,A–F], Lorena Mosconi Clemente[1,C–F], Evandro Watanabe[3,B–F], Cláudia Helena Silva-Lovato[1,A–F]

[1] Department of Dental Materials and Prosthesis, Ribeirão Preto School of Dentistry, University of São Paulo, Brazil
[2] Bonyf AG, Vaduz, Liechtenstein
[3] Department of Restorative Dentistry, Ribeirão Preto School of Dentistry, University of São Paulo, Brazil

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

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Address for correspondence
Cláudia Helena Silva-Lovato
E-mail: chl@forp.usp.br

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Conflict of interest
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Abstract

Background. Denture adhesives promote greater stability and retention of dentures. However, they can also facilitate biofilm formation related to oral diseases.

Objectives. The study aimed to evaluate the influence of 2 adhesives on the microbial load of mixed biofilm and adhesive strength. Additionally, the objective was to assess the effect of 3 hygiene protocols on the microbial load and cell metabolism of this biofilm.

Material and methods. The study compared Corega Ultra Cream (CCA) and OlivaFix® Gold (OFA) adhesives by evaluating the biofilm formation of *Candida albicans*, *Candida glabrata*, *Staphylococcus aureus*, and *Streptococcus mutans* by colony-forming unit (CFU), as well as adhesive strength. The implemented hygiene protocols included brushing and immersion in water (BW), 0.15% triclosan ($BT_{0.15\%}$), or 0.25% sodium hypochlorite ($BSH_{0.25\%}$). The control groups were either without adhesive (CG) or without any hygiene protocols (CGwH). The one-way and two-way analyses of variance (ANOVAs) with Tukey's post hoc test and a generalized linear model with Bonferroni adjustment were used for statistical analysis ($\alpha = 0.05$).

Results. The microbial load of *C. albicans* was higher when OFA was used ($p < 0.001$). The microbial loads of *C. glabrata* and *S. mutans* were similar between adhesives and higher in the CG ($p < 0.001$). The influence of the adhesives on the microbial load of *S. aureus* was not statistically significant ($p = 0.287$). The adhesive strength promoted by OFA was greater and more stable than when CCA was used ($p = 0.007$). The immersion in sodium hypochlorite led to a reduction in the microbial load of *C. albicans* ($p < 0.001$), *C. glabrata* ($p = 0.002$) and *S. mutans* ($p = 0.012$), independent of the adhesive. For *S. aureus*, the microbial load was lower with OFA/$BSH_{0.25\%}$ ($p = 0.022$). All hygiene protocols resulted in a decreased cell metabolism when compared to the CGwH ($p < 0.001$).

Conclusions. Brushing with $BSH_{0.25\%}$ solution was the most effective hygiene protocol, resulting in a reduction in the microbial load and metabolism. This protocol may be recommended as a first-line option for the disinfection of dentures.

Keywords: adhesives, denture, hygiene, biofilm

Highlights

- Both denture adhesives (Corega Ultra Cream and OlivaFix® Gold) promoted biofilm formation, though microbial responses varied between products.
- OlivaFix® Gold showed higher and more stable adhesive strength than Corega Ultra Cream.
- Brushing combined with immersion in 0.25% sodium hypochlorite was the most effective hygiene protocol, significantly reducing microbial load and cell metabolism.
- Triclosan and brushing with water also reduced biofilm, but less effectively than sodium hypochlorite.
- A 0.25% sodium hypochlorite solution can be recommended as the first-line disinfection protocol when cream adhesives are used.

Introduction

The rehabilitation of edentulous individuals can be achieved through the use of complete dentures.[1,2] However, the support tissues undergo continual remodeling after tooth loss, compromising retention and support for dentures, as well as affecting quality of life.[1] This problem can be addressed by using dental implants in conjunction with complete dentures.[2–5] Nonetheless, this treatment is not universally applicable due to various psychological, anatomical, systemic, and social factors.[2,3]

An alternative approach involves the use of denture adhesives,[6] which enhance retention and stability, increasing comfort, confidence, satisfaction, and, consequently, the quality of life related to oral health.[6] However, a disadvantage of this method is the difficult removal of the adhesive from the denture surface. Moreover, the repeated use of adhesives can lead to the growth of microorganisms, such as *Candida albicans*, *Candida glabrata*, *Staphylococcus aureus*, and *Streptococcus mutans*, which have been associated with the development of denture stomatitis.[7–22]

An effective hygiene method for removing adhesive residues and biofilm is essential to maintain health of the oral mucosa. However, few studies have evaluated adhesive removal methods.[23–26] The literature suggests that brushing the denture along with its immersion in 0.25% sodium hypochlorite or 0.15% triclosan provides effective anti-biofilm action.[26–37] In addition to effective hygiene methods, the incorporation of antimicrobial agents into the adhesive has also been proposed.[38] OlivaFix® Gold (bonyf AG, Vaduz, Liechtenstein) is an adhesive with 30% organic extra virgin olive oil and an absence of petroleum derivatives and zinc, making it a natural alternative on the market. A number of studies have been conducted to assess the anti-biofilm properties of OlivaFix® Gold[11,38–46] in comparison to other well-established adhesives.[6,10,12]

Further research employing a standardized methodology and utilizing the most prevalent microorganisms[10,11,14,15] in complete denture biofilm, as well as adhering to hygiene protocols accessible to patients is necessary to confirm the safe use of cream adhesives. Thus, the present study evaluated the influence of an adhesive based on olive oil on the microbial load of a mixed biofilm and adhesive strength. In addition, the study examined the effect of 3 hygiene protocols on the microbial load and cell metabolism of this biofilm. The evaluated adhesive was then compared with an adhesive that is commonly recommended in the literature. The null hypothesis posits that microbial load and adhesive strength are similar between the adhesives, as well as that the hygiene protocols have comparable effects on biofilm control.

Material and methods

Study design and setting

The materials used in the study are presented in Table 1. The quantitative response variables and variation factors were: (1) microbial load of a mixed biofilm evaluated by colony-forming units (CFUs) formed on the surface of acrylic resin specimens without (control group (CG)) or with adhesive – Corega Ultra Cream (CCA) (GlaxoSmithKline, Buenos Aires, Argentina) or OlivaFix® Gold (OFA); (2) bond strength of CCA and OFA adhesives; (3) microbial load (CFU) of the biofilm after the application of the hygiene protocols; and (4) cell metabolism (XTT assay) of the mixed biofilm formed on acrylic resin specimens with CCA and OFA adhesives, before and after the use of hygiene protocols. A group that did not undergo the brushing procedure (CGwH) was incorporated into the analysis. The results of the XTT assay were interpreted as the percentage of metabolic reduction found in the groups, considering the cell metabolism in the CG as 100%. The analyses were performed in triplicate on 3 separate occasions, and 1 specimen was used as a sterilized control (free of contamination) on each occasion.

Preparation of specimens

Circular, heat-polymerized acrylic resin specimens (12 mm × 3 mm) were obtained by the conventional technique of using metallic matrices in plaster, pressing,

Table 1. Materials used in the study

Material	Composition	Manufacturer
Corega Ultra Cream	poly(methyl vinyl ether-maleic anhydride), mixed partial sodium/calcium salt, petroleum, cellulose gum, paraffin, carboxymethylcellulose	GlaxoSmithKline, Buenos Aires, Argentina
OlivaFix® Gold	poly(methyl methacrylate), mixed calcium/sodium salt, cellulose gum, *Olea europaea* (olive) oil, hydrogenated soybean oil, silica, trihydroxystearin, menthol, lecithin, *Citrus limon* (lemon) peel oil, menthyl lactate	Bonyf AG, Vaduz, Liechtenstein
0.15% triclosan	10 mL of sodium hydroxide solution (Sigma-Aldrich, St. Louis, USA), 0.15 g of triclosan (Mix das Essências, Belo Horizonte, Brazil) (1.5 mg/mL)	Research Laboratory, Ribeirão Preto School of Dentistry, University of São Paulo, Brazil
0.25% sodium hypochlorite	active chlorine (Super Candida; Indústrias Anhembi, Osasco, Brazil)	Research Laboratory, Ribeirão Preto School of Dentistry, University of São Paulo, Brazil
Neutral soap	sodium lauryl sulfate, diethanolamine, cocamidopropyl betaine, methylparaben, polyquaternium 7, citric acid, polyethylene glycol, pearlescent base, perfume, water	Perol Comercial e Industrial, Ribeirão Preto, Brazil
TEK® soft	soft brush with 26 tufts of nylon bristles (0.25 mm in diameter and 10 mm in height)	Johnson & Johnson, São José dos Campos, Brazil
Thermopolymerizable acrylic resin	resin (powder): polymethyl methacrylate, benzoyl peroxide, biocompatible pigments monomer: methyl methacrylate monomer, inhibitor	Clássico, Campo Limpo Paulista, Brazil
Artificial saliva	4 g of carboxymethylcellulose, 60 g of sorbitol, 1 g of potassium chloride, 1 g of sodium chloride, 50 mg of magnesium chloride, 400 mg of potassium phosphate, 2 mg of Nipagin (methyl 4-hydroxybenzoate) in 1 L of distilled water (pH = 7)	Research Laboratory, Ribeirão Preto School of Dentistry, University of São Paulo, Brazil

polymerization, and finishing.[32] The roughness (Ra) of the surface of the specimens was standardized between 2.7 μm and 3.7 μm with a profilometer (Surftest SJ-201P; Mitutoyo Corporation, Kawasaki, Japan).[33] Subsequently, 27 specimens were randomly distributed into 3 groups (n = 9 per group): non-adhesive group (CG); CCA group; and OFA group. The specimens were placed in a beaker with 200 mL of distilled water for sterilization in a microwave oven set at 650 W for 6 min (model Perfect; Panasonic, Tokyo, Japan).[33] After cooling, the specimens were distributed into 24-well plates (Techno Plastic Products AG, Trasadingen, Switzerland). A quantity of 0.080 g of the adhesive was applied homogeneously to the surface of the acrylic resin samples to form a thin layer, which was then disinfected using ultraviolet-C (UV-C) light with a power of 60 W for 20 min in a laminar flow chamber (Pa 400-ECO; Pachane, São Paulo, Brazil). Following the disinfection process, the adhesive was distributed into 24-well tissue culture plates.

Analysis of biofilm formation on acrylic resin surfaces

Exponential growth phase cultures of *C. albicans* (ATCC 10231), *C. glabrata* (ATCC 2001), *S. aureus* (ATCC 25923), and *S. mutans* (ATCC 25175) were obtained. Subsequently, 1.5 mL of brain heart infusion (BHI Broth; HiMedia Laboratories Pvt. Ltd., Thane, India) inoculated with yeast and bacteria in the concentrations of 1×10^5 cells/mL and 1×10^6 cells/mL, respectively, was added to the specimens, which were then incubated as previously described.[33] After biofilm maturation, the specimens were rinsed 3 times in phosphate-buffered saline (PBS) and inserted into polypropylene test tubes (Techno Plastic Products AG) with 10 mL of Letheen Broth (HiMedia Laboratories Pvt. Ltda.). Then, the specimens were sonicated at 40 kHz and 200 W (Clean 9CA; Altsonic, Ribeirão Preto, Brazil) and vortexed (Phoenix™ AP 56; Phoenix Industria e Comercio de Equipamentos Cientificos, Ltda, Araraquara, Brazil). The suspension was seeded in the specific culture media for the growth of the microorganisms. The biofilm formation was quantified as CFU/mL and presented as $\log_{10}$.[33]

Evaluation of the adhesive strength of cream adhesives

To assess the adhesive strength, cylindrical specimens (n = 15, 25 mm × 35 mm) were made using a previously described conventional technique with minor modifications.[10] A handle was attached to the upper part of the specimens and connected to the tow bar of the mechanical testing machine (EMIC DL 2000; Instron Brasil Equipamentos Científicos Ltda, São José dos Pinhais, Brazil). The specimens were then measured according to the ISO 10873 recommendations.[47] To simulate the presence of mucosa, a piece of pig skin with the same diameter as the surface was fixed with cyanoacrylate-based instant adhesive (Loctite® Super Bonder®; Henkel Ltda., São Paulo, Brazil).[48] Subsequently, the pig skin-covered surface was moistened with 5 mL of artificial saliva for 1 min, and 0.5 g of cream adhesive was evenly applied. Another acrylic resin specimen was then positioned in contact with this surface, according to the manufacturer's instructions. The adhesives were compressed with a force of 12 N (1.2 kg of weight) for 30 s.[48] The adhesive strength was measured immediately (T0), after 5 min (T5m) and after 4 h (T4h) of application. The assembly was moved in a tensile mode at 1 mm/min, and the maximum force was calculated in Newtons (N).

Evaluation of the effect of hygiene protocols on mixed biofilms

The antibiofilm efficacy of the hygiene protocols on the microorganisms in biofilms formed on the surfaces of acrylic resin specimens with CCA or OFA was determined by means of microbial load (CFU/mL) and metabolic activity (XTT assay) evaluation. Three replicate inter-assays were performed at 3 independent times. Seventy-two specimens (12 mm × 3 mm) were randomly distributed among the following regimens: no hygiene protocol (CGwH); brushing and immersion in water (BW); brushing and immersion in 0.15% triclosan ($BT_{0.15\%}$); and brushing and immersion in 0.25% sodium hypochlorite ($BSH_{0.25\%}$).

In order to implement the hygiene protocols, 2 specimens were removed from the culture plate and placed within orifices, prepared in plexiglass plates (Policarbonato; Day Brasil, Barueri, Brazil), with the dimensions corresponding to those of the specimens. The specimens were manually brushed by the same operator using a dental brush[49] (TEK® soft; Johnson & Johnson, São José dos Campos, Brazil) and 1 drop of neutral soap, with standardized movements and pressure. The brushing movement was executed in the same direction for 20 s on both the upper and posterior surfaces of the specimen. Afterward, the specimens were washed 3 times with PBS and immersed in 10 mL of the hygiene solutions for 10 min. Then, the samples were rinsed thrice in PBS and transferred to tubes containing 10 mL of Letheen Broth.[33] To analyze the residual microbial load, the procedures for seeding in agar medium and CFU counting were performed as previously described.

Analysis of cell metabolism

The XTT colorimetric assay was used for the analysis of cell metabolism.[33] Briefly, after the formation of biofilms, 60 specimens were allocated according to hygiene protocols and transferred to sterile 24-well culture plates containing tetrazolium salt. Following a 2-h incubation period at 37°C, the absorbance of the formazan product was measured in triplicate using a microplate reader (Multiskan GO; Thermo Fisher Scientific, Vantaa, Finland) at 492 nm.

Statistical analysis

The data was tested for normality (Shapiro–Wilk test) and heterogeneity (Levene's test). The effect of the adhesives on biofilm formation and adhesive strength was analyzed using one-way analysis of variance (ANOVA), two-way ANOVA and Tukey's post hoc test. The generalized linear model with Bonferroni adjustment, two-way ANOVA and Tukey's post hoc test were used to compare the effects of the antibiofilm action of the hygiene protocols. All statistical tests were performed using the IBM SPSS Statistics for Windows software, v. 25.0 (IBM Corp., Armonk, USA), considering $\alpha = 0.05$.

Results

Biofilm formation

The biofilm formation of *C. albicans* was higher in the presence of OFA compared to the CG and CCA, which demonstrated similar results. There were no significant differences between the adhesives for *C. glabrata* and *S. mutans*; however, both presented higher values compared to the CG. *Staphylococcus aureus* was not influenced by the presence or type of the adhesive (Table 2).

Adhesive strength

The adhesive strength exhibited a significant interaction with time ($p = 0.007$). At the initial time point (T0), the bond strength was higher for CCA. However, at T5m and T4h, OFA values were elevated. For CCA, the adhesive strength increased over time. For OFA, the adhesive strength increased between T0 and T5m, and reached comparable levels at T5m and T4h (Fig. 1).

Effect of hygiene protocols on mixed biofilms

The implementation of hygiene protocols resulted in a reduction of the microbial load for all microorganisms compared to the CGwH, irrespective of the adhesive used. The $BSH_{0.25\%}$ protocol demonstrated the greatest efficacy, causing the inhibition of *C. albicans* ($p < 0.001$),

Table 2. Comparison of the microbial load (log_{10}) on the surface of specimens with and without adhesives

Adhesive	*C. albicans* [CFU]	*p*-value	*C. glabrata* [CFU]	*p*-value	*S. aureus* [CFU]	*p*-value	*S. mutans* [CFU]	*p*-value
CG	4.41 ±0.48[a]		4.40 ±0.38[a]		7.15 ±1.01		5.66 ±0.37[a]	
CCA	4.02 ±0.42[a]	<0.001*	4.93 ±0.54[b]	<0.001*	6.77 ±0.96	0.287	6.45 ±0.33[b]	<0.001*
OFA	5.07 ±0.43[b]		5.39 ±0.39[b]		7.54 ±1.22		6.76 ±0.51[b]	

* statistically significant ($p < 0.05$, one-way ANOVA); CG – control group without adhesive; CCA – Corega Ultra Cream; OFA – OlivaFix® Gold. Data presented as mean ± standard deviation (*M* ±*SD*). Different lowercase letters show statistical differences between the adhesives for the same microorganism.

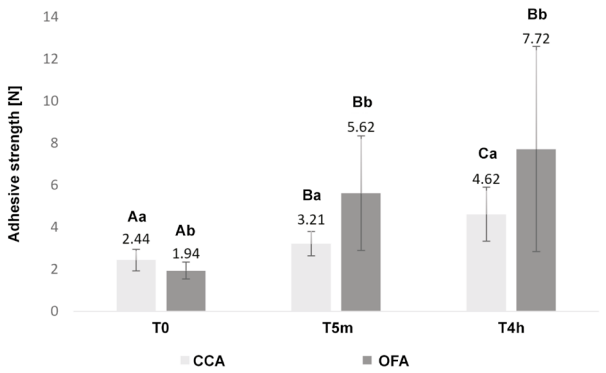

Fig. 1. Comparison of the adhesive strength of Corega Ultra Cream (CCA) and OlivaFix® Gold (OFA) at different time points

T0 – baseline; T5m – after 5 min; T4h – after 4 h. Different lowercase letters indicate statistically significant differences between the adhesives for the same time point, while different capital letters indicate statistically significant differences between the time points for the same adhesive ($p < 0.05$, one-way ANOVA and Tukey's post hoc test).

Table 3. Comparative analysis of *C. albicans* count (log_{10}) based on different adhesives and hygiene protocols

Adhesive		CGwH	BW	$BT_{0.15\%}$	$BSH_{0.25\%}$
CCA	*M* ±*SD*	4.03 ±0.41^{Aa}	2.53 ±1.00^{Ba}	1.48 ±0.86^{Ba}	#
	Me	4.14	2.66	1.61	
	CI	3.73–4.32	1.82–3.24	0.86–2.09	
OFA	*M* ±*SD*	5.07 ±0.44^{Aa}	2.21 ±0.93^{Ba}	0.60 ±1.02^{Ca}	#
	Me	5.09	2.55	0.00	
	CI	4.75–5.38	1.54–2.87	−0.12–1.32	
p-value*		0.065	1.000	0.236	–

microbial load reduced to 0; * generalized linear model with Bonferroni adjustment; *Me* – median; *CI* – confidence interval; CGwH – control group without hygiene protocols; BW – brushing and immersion in water; $BT_{0.15\%}$ – brushing and immersion in 0.15% triclosan; $BSH_{0.25\%}$ – brushing and immersion in 0.25% sodium hypochlorite. Different lowercase letters indicate differences between the adhesives for the same group. Different capital letters show differences between the groups for the same adhesive. For CCA: CGwH×BW: $p < 0.001$; CGwH×$BT_{0.15\%}$: $p < 0.001$; BW×$BT_{0.15\%}$: $p = 0.060$. For OFA: CGwH×BW: $p < 0.001$; CGwH×$BT_{0.15\%}$: $p < 0.001$; BW×$BSH_{0.25\%}$: $p < 0.001$.

Table 4. Comparative analysis of *C. glabrata* count (log_{10}) based on different adhesives and hygiene protocols

Adhesive		CGwH	BW	$BT_{0.15\%}$	$BSH_{0.25\%}$
CCA	*M* ±*SD*	4.93 ±0.54^{Aa}	2.52 ±0.97^{Ba}	2.41 ±1.21^{Ba}	#
	Me	4.80	2.65	2.37	
	CI	4.54–5.32	1.82–3.21	1.54–3.27	
OFA	*M* ±*SD*	5.39 ±0.40^{Aa}	2.39 ±0.21^{Ba}	1.06 ±1.15^{Cb}	#
	Me	5.57	2.38	0.95	
	CI	5.11–5.67	2.24–2.53	0.23–1.88	
p-value		1.000	1.000	0.005*	–

microbial load reduced to 0; * statistically significant ($p < 0.05$, generalized linear model with Bonferroni adjustment). Different lowercase letters indicate differences between the adhesives for the same group. Different capital letters show differences between the groups for the same adhesive. For CCA: CGwH×BW: $p < 0.001$; CGwH×$BT_{0.15\%}$: $p < 0.001$; BW×$BT_{0.15\%}$: $p = 1.000$. For OFA: CGwH×BW: $p < 0.001$; CGwH×$BT_{0.15\%}$: $p < 0.001$; BW×$BT_{0.15\%}$: $p = 0.006$.

C. glabrata ($p = 0.002$) and *S. mutans* ($p = 0.012$), and significantly reducing *S. aureus* ($p = 0.022$) when associated with OFA. For *C. albicans* and *C. glabrata*, the $BT_{0.15\%}$ protocol was more efficient with OFA (Table 3,4). For *S. aureus*, all protocols were statistically different from each other, and the most significant reduction was promoted by $BSH_{0.25\%}$, followed by $BT_{0.15\%}$ and BW. Triclosan caused a decrease in *S. aureus* CFUs with OFA (Table 5). For *S. mutans*, $BT_{0.15\%}$ was more effective than BW for both cream adhesives and resulted in the inhibition of *S. mutans* with OFA ($p = 0.012$) (Table 6).

Cell metabolism

The impact of hygiene protocols on cell metabolism was found to be statistically significant ($p = 0.000$) (Fig. 2). The study revealed no difference between the adhesives ($p = 0.124$) and no interaction between the hygiene protocols and adhesives ($p = 0.260$). The microorganisms exhibited no evidence of cell metabolism with $BSH_{0.25\%}$. The use of triclosan and BW yielded analogous outcomes, leading to a more pronounced reduction in metabolism when compared to the CGwH.

Table 5. Comparative analysis of *S. aureus* count (log_{10}) based on different adhesives and hygiene protocols

Adhesive	CGwH	BW	$BT_{0.15\%}$	$BSH_{0.25\%}$
CCA	6.77 ±0.97^{Aa}	5.24 ±0.44^{Ba}	3.66 ±1.38^{Ca}	0.54 ±1.24^{Da}
OFA	7.55 ±1.23^{Aa}	5.24 ±0.55^{Ba}	2.68 ±0.57^{Cb}	0.00 ±0.00^{Da}
p-value	0.062	0.994	0.020*	0.193

* statistically significant ($p < 0.05$, two-way ANOVA). Data presented as *M* ±*SD*. Different lowercase letters indicate differences between the adhesives for the same group. Different capital letters show differences between the groups for the same adhesive. For CCA: CGwH×BW: $p = 0.002$; CG×$BT_{0.15\%}$: $p < 0.001$; CG×$BSH_{0.25\%}$: $p < 0.001$; BW×$BT_{0.15\%}$: $p = 1.001$; BW×$BSH_{0.25\%}$: $p < 0.001$; $BT_{0.15\%}$×$BSH_{0.25\%}$: $p < 0.001$.
For OFA: CGwH×BW: $p < 0.001$; CGwH×$BT_{0.15\%}$: $p < 0.001$; CGwH×$BSH_{0.25\%}$: $p < 0.001$; BW×$BT_{0.15\%}$: $p < 0.001$; BW×$BSH_{0.25\%}$: $p < 0.001$; $BT_{0.15\%}$×$BSH_{0.25\%}$: $p < 0.001$.

Table 6. Comparative analysis of *S. mutans* count (log_{10}) based on different adhesives and hygiene protocols

Adhesive	CGwH	BW	$BT_{0.15\%}$	$BSH_{0.25\%}$
CCA	6.46 ±0.33^{Aa}	4.33 ±0.43^{Ba}	1.10 ±1.54^{Ca}	#
OFA	6.76 ±0.57^{Aa}	3.77 ±0.37^{Ba}	0.00 ±0.00^{Cb}	#
p-value	0.349	0.087	0.001*	–

microbial load reduced to 0; * statistically significant (p < 0.05, two-way ANOVA and Tukey's post hoc test). Data presented as *M* ±*SD*. Different lowercase letters indicate differences between the adhesives for the same group. Different capital letters show differences between the groups for the same adhesive. For CCA: CGwH×BW: $p < 0.001$; CGwH×$BT_{0.15\%}$: $p < 0.001$; $BT_{0.15\%}$×$BSH_{0.25\%}$: $p < 0.001$.
For OFA: CGwH×$BT_{0.15\%}$: $p < 0.001$; CGwH×$BT_{0.15\%}$: $p < 0.001$; BW×$BT_{0.15\%}$: $p < 0.001$.

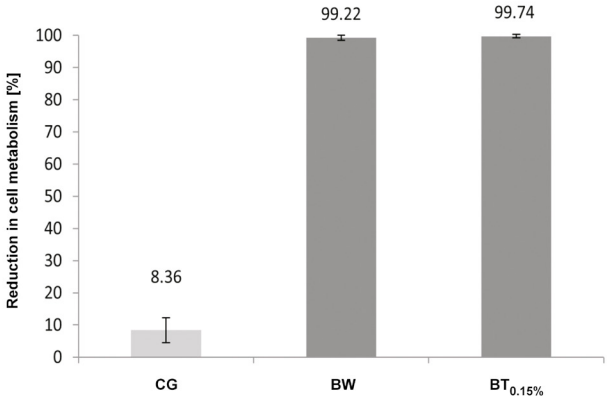

Fig. 2. Comparison of the reduction in cell metabolism of the mixed biofilm after the application of hygiene protocols

CG – control group without adhesive; BW – brushing and immersion in water; $BT_{0.15\%}$ – brushing and immersion in 0.15% triclosan.

Discussion

The null hypothesis was rejected due to the observed difference between the cream adhesives in terms of biofilm formation and adhesive strength, as well as between the hygiene protocols. The results of this study confirm the tendency for greater biofilm accumulation when the adhesive is associated with the prosthesis. However, the findings also reveal that biofilms can be controlled through brushing and the use of sodium hypochlorite. Consequently, the patient's quality of life can be ensured through the retention facilitated by the adhesive, while concurrently preserving the health of the tissues by the effective regulation of biofilm promoted by hygiene methods.

Candida albicans and *C. glabrata* are frequently isolated in individuals with denture stomatitis, especially in immunocompromised individuals.[14,15,22] Furthermore, *C. albicans* develops a dense, multilayered biofilm with intricate hyphae to support the adhesion of *C. glabrata*.[16] In the present study, the *C. albicans* count was higher with OFA when compared to the CCA and CG. At the same time, there was no difference in the biofilm formation of *C. glabrata* between the 2 adhesives. This result may be related to adhesion and cell surface hydrophobicity (CSH), which can suffer environmental variations.[13] In a study with a limited number of *C. glabrata* isolates, the CSH was comparable to that of *C. albicans*. However, when many *C. glabrata* isolates were analyzed in comparison to *C. albicans*, the CSH of *C. glabrata* exhibited enhanced resistance to the same conditions,[14] suggesting that *C. glabrata* may not be as sensitive or susceptible to environmental factors.

The mean CFU count of *C. albicans* associated with CCA was analogous to the CG, suggesting that this adhesive did not promote the proliferation of this microorganism. However, it did not hinder its growth, which is consistent with the findings of several studies.[8–10] Another study reported that CCA caused 42% inhibition in the growth of *C. albicans* using a 1% solution of adhesive in a liquid culture medium, which was more diluted than in our study.[7]

A higher *C. albicans* count was observed for the OFA adhesive. This may be related to a highly viscous film formation on the specimens, which possibly affected the adhesion capacity of yeasts. These findings contradict those reported by Azevedo et al.[50] The authors conducted a crossover clinical study with 23 patients using 3 groups of cream adhesives: a control (Kukident Pro); an experimental type (OFA); and a placebo (Vaseline). The experimental adhesive demonstrated superior *C. albicans* growth inhibition and prolonged effectiveness in comparison to the control and placebo groups ($p < 0.001$).[50]

Staphylococcus aureus forms a strong biofilm on denture surfaces.[17] The effective control measures are highly necessary due to antibiotic resistance.[51] The results demonstrated that the microbial load of *S. aureus* remained consistent across different adhesives, corroborating the observations reported by Costa et al.[10] and Ozkan et al.[15] The latter study, a clinical investigation, confirmed that there was no difference in the CFU count of *S. aureus* isolated from biofilms of complete dentures, both with and without adhesive.[15]

Streptococcus mutans is a precursor of biofilm formation, which can alter the local environment by forming an extracellular polysaccharide matrix-rich and low pH milieu, thereby creating a favorable niche for other acidogenic and aciduric species to colonize hard surfaces, such as dentures.[52] This is clinically significant because denture wearers are typically elderly patients who are more likely to develop systemic infections.[21] In this study, the CFU count of *S. mutans* was higher for adhesives than for the CG. These results highlight the need for meticulous removal of adhesives. However, Chen et al. evaluated the growth of *S. mutans* following the use of 3 denture adhesives (Polident cream, Protefix® cream and Protefix® powder) and did not observe any differences between the adhesives when compared to the control group.[19] Additionally, 3 commercial adhesives (CCA, Fixodent Pro Original and Biotene Denture Grip) showed antimicrobial effects against *S. mutans*.[20] The observed discrepancy between the results of the present study and those of other studies may be due to methodological differences.

For CCA, the maximum adhesive strength was reached after 4 h, which is consistent with the findings of the study by Costa et al.[10] With regard to OFA, the comparison of results is limited due to the paucity of literature on the subject. However, the manufacturer stipulates an adhesive retention period of up to 24 h. The results of this study could be attributable to variations in composition. Briefly, carboxymethylcellulose (CMC) and poly(methyl vinyl ether-co-maleic acid) (PVM-MA) are classified as short-acting and long-acting salts, respectively.[53] The CMC

compound exhibits strong initial retention, but due to its high level of solubility, its effectiveness is rapidly diminished.[53] The CCA adhesive contains both PVM-MA and CMC, while the OFA adhesive contains PVM-MA.

A number of studies have evaluated different hygiene protocols and found positive results regarding adhesive removal.[23–25] However, these studies did not observe favorable outcomes in terms of the antimicrobial effect.[23–25] Thus, the findings of our study are promising, as the $BT_{0.15\%}$ and $BSH_{0.25\%}$ protocols promoted a reduction in the microbial load when compared to the CGwH.

Triclosan is a synthetic, lipid-soluble antimicrobial agent of the broad spectrum that has the capacity to inhibit enzymes responsible for fatty acid biosynthesis.[34] The agent induces K^+ extravasation, leading to cell lysis through its effects on RNA and protein synthesis.[36] It can be used as an alternative to hypochlorite for allergic patients and is recommended for wearers of partial dentures.[32] In the present study, $BT_{0.15\%}$ was more effective when used with OFA. The effect of BW was analogous to that of $BT_{0.15\%}$ against *C. albicans* and *C. glabrata* when used in conjunction with CCA. This phenomenon may be attributed to the mechanical brushing procedure, which can disorganize the biofilm[32,33] and remove the adhesive component.

Sodium hypochlorite, an oxidizing agent, interferes with the integrity of the cytoplasmic membrane due to its high pH.[33] This property renders it effective in sanitizing complete dentures.[26–34] Although one of the disadvantages of sodium hypochlorite is its unpleasant odor, it was well accepted by patients at a concentration of 0.25% and can serve as a positive control in the evaluation of other solutions.[29–33] The results of this study demonstrated a reduction in mitochondrial activity of metabolically active cells, which aligns with the findings on microbial load. Sodium hypochlorite completely inhibited cell metabolism,[33] while triclosan or water caused a significant decrease in metabolic activity (99.74% and 99.22%, respectively). However, a direct comparison with the extant literature is precluded by the dearth of studies in the field.[38] A notable finding in the CGwH sample is an 8.36% reduction in metabolism, indicating that the adhesives provided a slight imbalance in the metabolic activity of microorganisms without compromising their viability.

Limitations

The present study was subject to certain limitations. First, an adhesive removal test was not conducted, which would have complemented the obtained results. Second, alternative techniques for assessing biofilm quantity, such as fluorescence microscopy, were not employed. This underscores the necessity for further research on the subject. However, the obtained results can inform clinical decision-making regarding the selection of the most suitable adhesive, based on the adhesive strength and hygiene method to be employed with each material.

Conclusions

The formation of biofilms was favored for both cream adhesives; however, the OFA adhesive demonstrated greater bond strength and stability with the mucosa. Brushing and immersion in 0.25% sodium hypochlorite resulted in a more significant reduction in the microbial load and cell metabolism when compared to the use of 0.15% triclosan.

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.

Use of AI and AI-assisted technologies

Not applicable.

ORCID iDs

Caroline Vieira Fortes https://orcid.org/0000-0003-1570-1435
Adriana Barbosa Ribeiro https://orcid.org/0000-0003-3108-8589
Bart De Wever https://orcid.org/0000-0002-5312-3237
Amina Sakly https://orcid.org/0000-0002-0204-6737
Viviane de Cássia Oliveira https://orcid.org/0000-0002-0734-0652
Lorena Mosconi Clemente https://orcid.org/0000-0002-7701-9311
Evandro Watanabe https://orcid.org/0000-0001-5674-2589
Cláudia Helena Silva-Lovato https://orcid.org/0000-0003-1629-2207

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Original paper

Comparison of the efficacy of simple and combined oral rinses with chlorhexidine digluconate against selected bacterial and yeast species: An in vitro study

Rafał Wiench[1,A–F], Anna Kuśka-Kiełbratowska[1,B–D,F], Małgorzata Kępa[2,A–D], Zuzanna Grzech-Leśniak[3,A–D,F], Maciej Jabłoński[1,A–C], Jan Kiryk[4,D–F], Kinga Grzech-Leśniak[5,6,A–F], Dariusz Skaba[1,A–C,E,F]

[1] Department of Periodontal Diseases and Oral Mucosa Diseases, Faculty of Medical Sciences in Zabrze, Medical University of Silesia, Katowice, Poland
[2] Department of Microbiology and Virology, Faculty of Pharmaceutical Sciences in Sosnowiec, Medical University of Silesia, Katowice, Poland
[3] Student Science Club, Wroclaw Medical University, Poland
[4] Department of Dental Surgery, Faculty of Dentistry, Wroclaw Medical University, Poland
[5] Laser Laboratory, Department of Dental Surgery, Faculty of Dentistry, Wroclaw Medical University, Poland
[6] Department of Periodontics, Virginia Commonwealth University (VCA) School of Dentistry, Richmond, 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

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Address for correspondence
Kinga Grzech-Leśniak
E-mail: kinga.grzech-lesniak@umw.edu.pl

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Abstract

Background. Chlorhexidine digluconate (CHG) is considered the most effective and safe antimicrobial agent in dentistry. Recently, it has often been produced in the form of preparations with additional substances that may modify its effect.

Objectives. The aim of the present study was to compare the efficacy of various simple and combined CHG rinses against selected bacterial and yeast strains.

Material and methods. This research followed the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines, using the disk diffusion method. The study was carried out on the following reference strains: *Staphylococcus aureus* ATCC 43300; *Streptococcus pyogenes* ATCC 19615; *Pseudomonas aeruginosa* ATCC 27853; *Enterococcus faecalis* ATCC 29212; *Candida albicans* ATCC 10231; *C. glabrata* ATCC 15126; *C. krusei* ATCC 14243; and *C. parapsilosis* ATCC 22019. The disinfection efficacy of 9 commercial mouthwashes with CHG was assessed (4 simple preparations, with different concentrations (0.5%, 0.2%, 0.12%, and 0.05%), and 5 combined preparations (0.2% CHG with adjuvants)) by comparing the size of the growth inhibition zones (GIZs) of microorganisms after 24 h of incubation.

Results. Growth inhibition zones were observed around all tested substances, for all assessed strains. In simple preparations, the greatest reduction in growth was observed for Gram-positive bacteria. Statistically significantly smaller GIZs were recorded for *P. aeruginosa* and all *Candida* strains. The size of GIZ also depended on the CHG concentration used. In combined preparations, the greatest reduction in growth was also observed for Gram-positive bacteria (especially large GIZs for *S. aureus* when using 0.2% CHG with colostrum). Statistically significantly smaller GIZs were observed for *P. aeruginosa* and all yeasts. None of the evaluated adjuvants impaired the disinfecting effect of CHG.

Conclusions. The evaluated combined preparations of CHG showed disinfecting efficacy against selected bacterial and fungal strains comparable to that of simple formulations. The combination of 0.2% CHG with colostrum showed the additive synergism of antimicrobial activity against the *S. aureus* ATCC 43300 strain.

Keywords: chlorhexidine, mouthwash, infection control, mouth rinse, antiseptic

Highlights

- Chlorhexidine digluconate (CHG) is a highly effective antimicrobial agent, particularly against Gram-positive bacteria.
- Research shows that higher CHG concentrations (0.5%) deliver the strongest antibacterial and antifungal effects.
- Adjuvants, such as colostrum and hyaluronic acid, do not compromise the effectiveness of CHG. In fact, these additives have been shown to preserve, or even enhance the antimicrobial activity of CHG. Specifically, the combination of CHG and colostrum has demonstrated synergistic effects, significantly increasing its efficacy against *Staphylococcus aureus*.
- Clinical relevance: The development of combined CHG formulations offers not only effective disinfection, but also additional therapeutic benefits.

Introduction

Chlorhexidine (CHX) is an organic chemical compound, a biguanide derivative, which serves as a synthetic antiseptic. Pharmacologically, it mostly occurs in the form of digluconate, diacetate or dihydrochloride salt. The most often used disinfectant is chlorhexidine digluconate (CHG).[1]

The antibacterial spectrum of CHG is broad, and covers both vegetative forms of Gram-positive and Gram-negative bacteria, some viruses (herpes virus, human immunodeficiency virus (HIV), influenza virus, and cytomegalovirus (CMV)), and yeasts as well as dermatophytes.[2–4] However, it does not act on bacterial spores at room temperature and on some small viruses (papillomavirus, enterovirus and poliovirus).[5]

Depending on the CHG concentration used and the environmental factors (pH, the temperature and the exposition time), the mechanism of action of this compound varies from bacteriostatic to bactericidal. A cation molecule of CHG is attracted to the negatively charged surface of the microbial cell. Afterward, it connects with the bacterial membrane (phosphate and 2-keto-deoxy-caprylic groups in lipopolysaccharides, and carboxyl groups in proteins), thereby changing its integrity. That leads to increased cell membrane permeability and the loss of low-mass molecules (mainly potassium ions – K^+), and inhibits the activity of some membrane enzymes. It is a reversible mechanism of CHG at a bacteriostatic stage. Increasing the concentration of CHG leads to greater damage to the cell membrane, resulting in the loss of cellular components, such as nucleic acids. The effects of the substance become irreversible with the loss of approx. 15% of nucleotides. Cytoplasmic elements are also precipitated by forming complexes with phosphorylated compounds, e.g., adenosine triphosphate (ATP). Chlorhexidine digluconate can be chemisorbed onto the hydroxyapatite surface or may interact as an ion, forming non-resorbable compounds with phosphates in the mouth, hydroxyapatite, dental plaque, and the carboxyl groups in collagen found in dentin and the connective tissue. These bonded compounds can gradually release CHG over time, prolonging its antimicrobial effect (even up to 12 h), which negatively impacts the possibility of forming biofilm by bacteria and fungi.[5–7]

The oral cavity is the initial segment for both the digestive tract and the respiratory system, and it is a reservoir for numerous microorganisms. Oral microbiome includes bacteria such as streptococci, staphylococci, bacteria of the genera *Lactobacillus*, *Neisseria*, *Prevotella*, *Porphyromonas*, *Veillonella*, *Actinomyces*, and many others.[8–10] Among the fungi, the predominate yeast species are *Candida albicans*, *C. dubliniensis* and *C. glabrata*.[11,12] When the body balance is disturbed, those organisms may transform into pathological flora.

Streptococci are responsible for the inflammation of the throat and tonsillitis, while *Staphylococcus aureus* is very often isolated in the inflammation of the labial commissures and skin (contagious impetigo). In the etiology of this disease, also *Streptococcus pyogenes* and *Pseudomonas aeruginosa* should be considered.[13] Yeast infections are most common in the oral cavities of immunocompromised patients.[14–16] Controlling such inflammation is an extremely important element of successful dental treatment. Antiseptics exhibiting a broad antimicrobial spectrum are used for this purpose, of which CHG is one of the most popular. Inflammation is manifested clinically as edema, bleeding, pain, damage to the epithelium of the oral mucosa, and difficulties in food intake and hygiene maintenance.[17–19] That is why there are various types of combined preparations, which contain CHG in different concentrations, as well as additional active ingredients exhibiting anti-inflammatory, analgesic, local anesthetic, and coating properties, along with accelerating tissue healing, sealing blood vessels and strengthening natural immune mechanisms. Such commercial products are dedicated to the complex, one-step treatment of the oral cavity, causative (the elimination of the etiological microbial flora) and symptomatic.

The issues studied so far include the sensitivity of microorganisms to various concentrations of CHG,[20–24] the disinfecting efficacy of CHG in comparison with other oral disinfectants (e.g. Listerine®, essential oils, cetylpyridinium chloride (CPC))[25–28] or the combination

of CHG with other substances with antimicrobial activity but different mechanisms of action, e.g., CHG with CPC, colloidal solutions containing nanoparticles, hydrogen peroxide, or essential oils.[29–33] However, there is a lack of studies that would unambiguously show the mechanism of action of CHG combined with adjuvants with an anti-symptomatic effect (additive/super-additive synergism or competence/functional/chemical antagonism). Therefore, the purpose of the present study was to compare the efficacy of various simple and combined CHG rinses against selected bacterial and yeast strains.

Material and methods

Organisms and growth conditions

This research followed the European Committee for Antimicrobial Susceptibility Testing (EUCAST) guidelines (v. 9.0, January 2019), using the disk diffusion method.[34]

The study was carried out on 4 reference strains of bacteria: *Staphylococcus aureus* ATCC 43300; *Streptococcus pyogenes* ATCC 19615; *Pseudomonas aeruginosa* ATCC 27853; and *Enterococcus faecalis* ATCC 29212, which are frequently detected in the classic form of impetigo lesions of red lips and corners of the mouth (angular cheilitis).[11,13] Besides, the 4 most common species causing oral candidiasis were used in the experiment, namely *Candida albicans* ATCC 10231, *C. glabrata* ATCC 15126, *C. krusei* ATCC 14243, and *C. parapsilosis* ATCC 22019.[11,12] The species were taken from the American Type Culture Collection (ATCC, Manassas, USA); they belong to the strain bank of the Department of Microbiology and Virology, Faculty of Pharmaceutical Sciences in Sosnowiec, Medical University of Silesia, Katowice, Poland. They were stored at –80°C in tryptic soy broth with the addition of glycerol.

The cultures of each species were placed separately on a Petri dish of a 90-millimeter diameter, with agar supplemented with 5% defibrinated sheep blood (bioMérieux, Marcy-l'Étoile, France), 4 mm deep (approx. 25 mL). The agar surface was dry and homogeneous. After 24 h of incubation at 37°C in aerobic conditions, a sample of colonies was removed from the surface of the plate and suspended in a sterile saline solution (0.9% NaCl; B. Braun, Melsungen, Germany). The number of viable cells in the suspension was counted using a Densi-La-Meter II densitometer (Erba Lachema, Prague, Czech Republic) at a wavelength of 525 nm. We used an optical density of McFarland of 0.5, which corresponds to approx. 1.2×10^8 CFU (colony-forming units)/mL for bacteria and 1.5×10^6 CFU/mL for yeasts.

Then, 15 min after preparation, 1 mL of the prepared *Candida* spp. suspension (for each strain separately) was inoculated with a sterile pipette onto the surface of Sabouraud dextrose agar (SDA) with chloramphenicol (bioMérieux). The suspensions of the *S. aureus*, *P. aeruginosa* and *E. faecalis* species were inoculated on the surface of the Mueller–Hinton agar (MHA) (bioMérieux), and the suspension of the *S. pyogenes* strain on the surface of the Mueller–Hinton agar enriched with 5% defibrinated sheep blood (MHF) (bioMérieux). A total of 48 plates were prepared, 6 for each tested strain.

The plates were marked with numbers I–VIII, as follows:

I. *S. aureus* ATCC 43300;
II. *S. pyogenes* ATCC 19615;
III. *P. aeruginosa* ATCC 27853;
IV. *E. faecalis* ATCC 29212;
V. *C. albicans* ATCC 10231;
VI. *C. glabrata* ATCC 15126;
VII. *C. krusei* ATCC 14243;
VIII. *C. parapsilosis* ATCC 22019.

Materials and analyzed substances

In total, 264 sterile paper disks (Oxoid™ blank antimicrobial susceptibility disks; Oxoid Ltd., Basingstoke, UK), 6 mm in diameter, were prepared for the study. According to the previously prepared templates, 33 disks were assigned for each tested strain, 5 or 6 per plate.

Study and control groups

The test groups were comprised of the disks located on the periphery of the plates. They were soaked in 9 tested preparations – oral hygiene solutions from Curasept (Saronno, Italy) (Fig. 1A) – by applying 20 µL of each liquid to appropriate disks.

To blind the trial, this part of the experiment was only performed by a microbiologist unfamiliar with the assessed preparations. The test tubes with the analyzed substances were marked with the following numbers (Fig. 1B):

1. 0.2% CHG solution;
2. 0.5% CHG solution;
3. 0.12% CHG solution;
4. 0.05% CHG solution;
5. a liquid containing 0.2% CHG solution + colostrum + polyvinylpyrrolidone/vinyl acetate (PVP/VA) copolymer;
6. a liquid containing 0.2% CHG solution + chlorobutanol (ChB);
7. a liquid containing 0.2% CHG solution + hyaluronic acid (HA);
8. a liquid containing 0.2% CHG solution + *Hamamelis*; and
9. a liquid containing 0.2% CHG solution + HA + phyto DNA.

Liquids 1–4, containing only CHG at various concentrations, were placed on the plates with 5 disks, while fluids 5–9, containing 0.2% CHG solution with various assessed additives, were placed on the plates with 6 disks. The control (marked with the letter K) was a paper disk

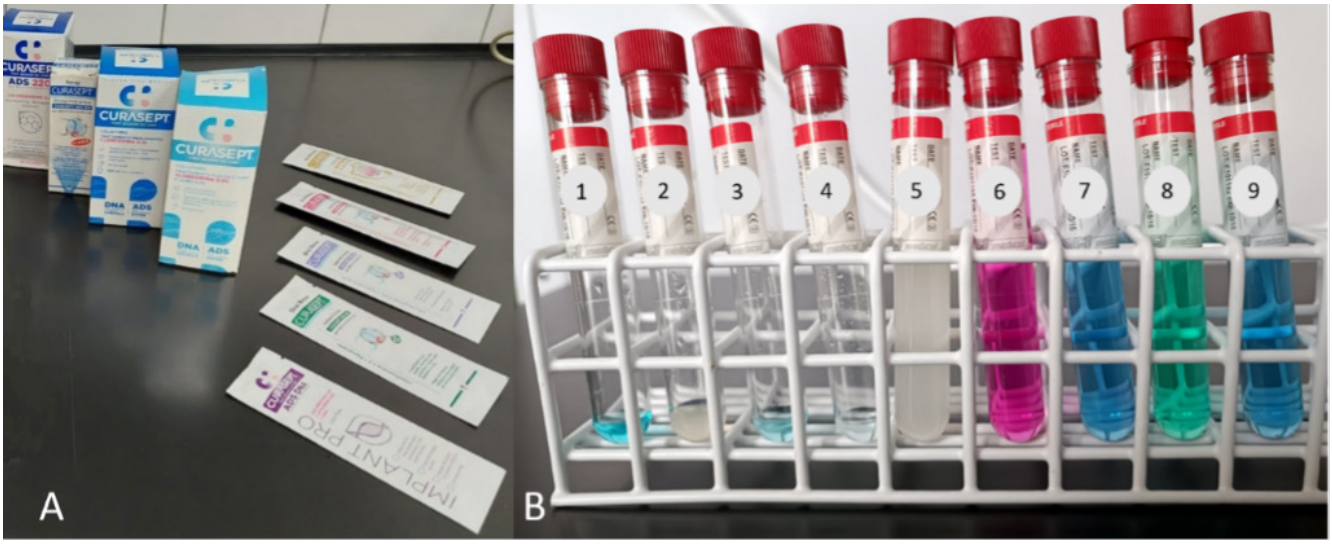

Fig. 1. Oral rinses tested

A – 9 tested oral hygiene preparations from Curasept (Saronno, Italy); B – tubes with the evaluated substances marked with numbers to blind the sample to the microbiologist.

soaked in 20 µL of sterile saline (0.9% NaCl), placed in the center of the plate, regardless of the reference used (Fig. 2).

The maximum number of disks on a 90-millmeter plate was 6. This number of disks prevents the possible overlapping of growth inhibition zones (GIZs) after incubation. In addition, the distances between the disks, following the EUCAST guidelines, were ≥20 mm, which meant that the individual preparations did not affect each other. All disks were placed on the plates within 15 min of inoculation. A sterile 1-centimeter fragment of the ruler scale was also placed in a specific place in each dish to assess the diameter of GIZ based on the photographic documentation taken for the test.

The cultures were incubated at 35 ±2°C under aerobic conditions in a MIR-262 laboratory incubator (Sanyo E&E Europe, Etten-Leur, the Netherlands). The experiment for each strain was performed in triplicate (Fig. 3).

After 24 h of incubation, the Petri dishes were removed from the incubator and photographed with a DMC-G80 Lumix camera (Panasonic, Osaka, Japan) equipped with a Lumix H-FS12060 12–60-millimeter micro HD lens (Panasonic) from a fixed distance of 30 cm at an angle of 90° to the surface. The camera was mounted on a tripod (Fig. 4).

The diameters of GIZs around individual disks were read after collecting all planned photos. The analysis was performed using the ImageJ – Fiji, v. 1.53j (National Institutes of Health (NIH), Bethesda, USA) software platforms. The measurement was carried out after calibrating the size from the left to the right border of GIZ parallel to the base of the photo so that the line passed through the center of the diffusion disk. The diameters were provided in millimeters. The obtained results were saved in an Excel spreadsheet (Microsoft, Redmond, USA), and then subjected to statistical evaluation.

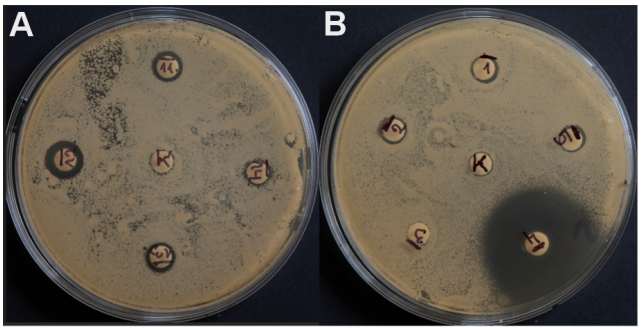

Fig. 2. Petri dishes with diffusion disks

A – prepared in a five-disk template: 4 disks with chlorhexidine digluconate (CHG) at various concentrations and a central control disk soaked in sterile saline; B – prepared in a six-disk template: 5 disks with the substances containing 0.2% CHG solution with various assessed additives and a central control disk soaked in sterile saline.

Fig. 3. Incubation (the experiment was performed in triplicate for each strain)

Fig. 4. Laboratory stand

Statistical analysis

The results were presented as mean and standard deviation ($M \pm SD$). Statistical differences were assessed using the analysis of variance (ANOVA) and the Newman–Keuls post-hoc test. A p-value ≤0.05 was considered to indicate a statistically significant difference.

The statistical analysis was performed using Statistica, v. 7.1 PL (StatSoft Poland, Krakow, Poland).

Results

The research results obtained in the first part of the experiment illustrate the effect of the preparations containing only CHG, at various concentrations, on the growth of selected reference bacteria and fungi as compared to the control sterile saline (0.9% NaCl). In the case of all tested strains, a homogeneous, confluent (uncountable) increase in CFU was observed on the surface of the media in Petri dishes after 24 h of incubation (Fig. 5). In the present study, undisturbed microbial growth around all central paper disks (control) was particularly visible. A completely different pattern was observed around the paper disks soaked in 0.2%, 0.5%, 0.12%, and 0.05% CHG solutions. For all tested strains, clear GIZs were observed after 24 h of incubation. However, these zones had different sizes.

Among bacteria, the highest average value of zone diameter, obtained from 3 measurements, was recorded

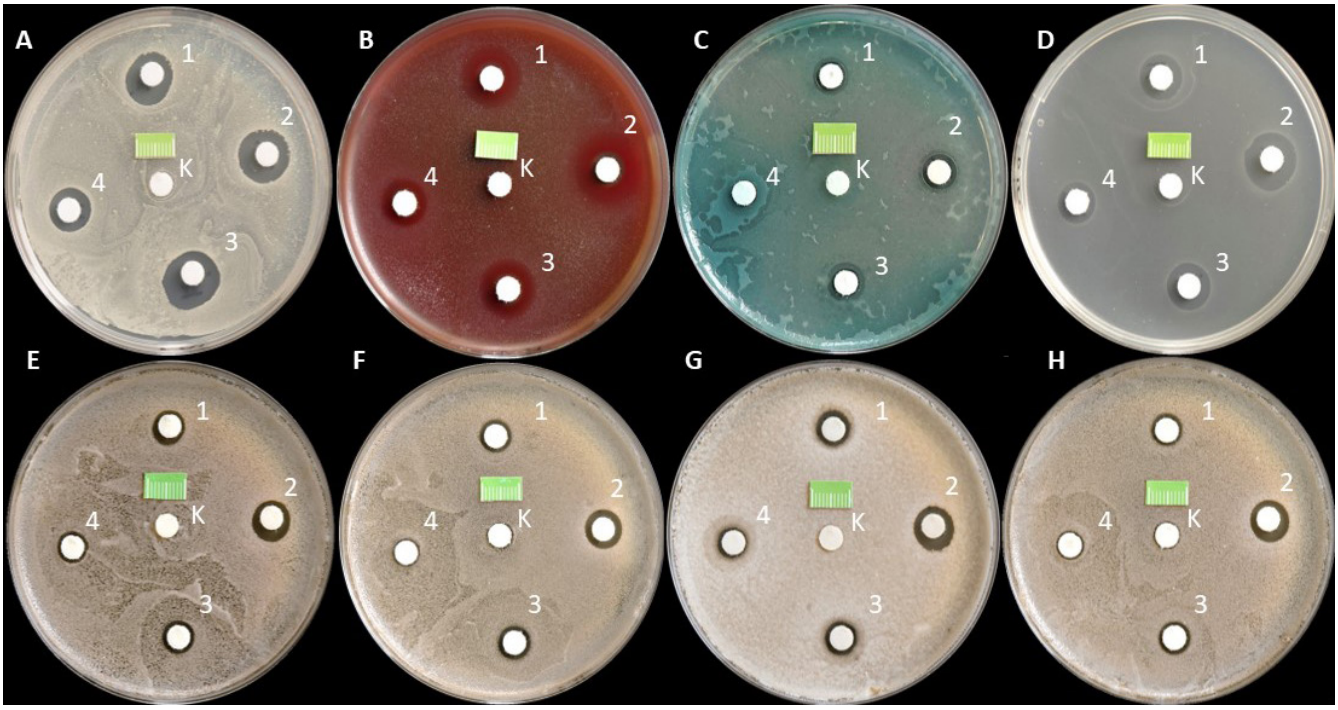

Fig. 5. Results obtained in the first part of the experiment

The effect of the preparations containing only chlorhexidine digluconate (CHG), at various concentrations – 0.2% (1), 0.5% (2), 0.12% (3), and 0.05% (4) – on the growth of selected standard bacteria and yeasts as compared to the control (K) sterile saline (0.9% NaCl).
A – *Staphylococcus aureus* ATCC 43300; B – *Streptococcus pyogenes* ATCC 19615; C – *Pseudomonas aeruginosa* ATCC 27853; D – *Enterococcus faecalis* ATCC 29212; E – *Candida albicans* ATCC 10231; F – *C. glabrata* ATCC 15126; G – *C. krusei* ATCC 14243; and H – *C. parapsilosis* ATCC 22019 (1 of 3 samples).

for 0.5% CHG solution for the *S. pyogenes* ATCC 19615 strain, amounting to 17.81 mm. The following places were taken by *S. aureus* ATCC 43300 – 15.25 mm, *E. faecalis* ATCC 29212 – 13.48 mm and *P. aeruginosa* ATCC 27853 – 9.34 mm. These results were statistically significantly higher than those obtained for other concentrations ($p < 0.001$) There were no statistically significant differences between the concentrations of 0.2% and 0.12% for individual bacterial strains ($p > 0.05$). The lowest results were obtained for 0.05% CHG solution: *S. pyogenes* ATCC 19615 – 12.24 mm; *S. aureus* ATCC 43300 – 10.83 mm; *E. faecalis* ATCC 29212 – 10.33 mm; and *P. aeruginosa* ATCC 27853 – 7.23 mm. The latter result was statistically significantly different in comparison with the control (6 mm – the width of the diffusion disk itself) ($p < 0.001$) (Table 1, Fig. 6).

Statistically significant differences were noted in the effect of individual simple CHG rinses on the assessed bacterial strains. They were most effective against *S. pyogenes* ATCC 19615 ($p < 0.001$), followed by *S. aureus* ATCC 43300 ($p < 0.001$), *E. faecalis* ATCC 29212 ($p < 0.001$) and *P. aeruginosa* ATCC 27853 ($p < 0.01$) (Fig. 6).

Among yeasts, the highest average value of zone diameter, obtained from 3 measurements, was recorded for 0.5% CHG solution for the *C. albicans* ATCC 10231 strain, and it was 10.86 mm. The following places were taken by *C. krusei* ATCC 14243 – 10.59 mm, *C. glabrata* ATCC 15126 – 10.43 mm and *C. parapsilosis* ATCC 22019 – 10.24 mm. These results were statistically significantly higher than those obtained for other concentrations ($p < 0.001$). No statistically significant differences were noted between the concentrations of 0.2% and 0.12% for individual *Candida* strains ($p > 0.05$). The lowest result

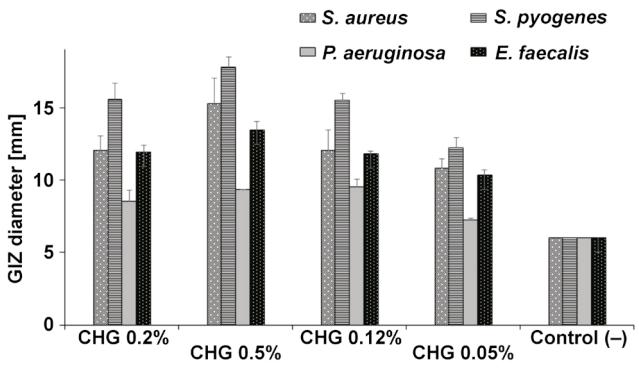

Fig. 6. Efficacy of the preparations containing only chlorhexidine digluconate (CHG), at various concentrations, on the growth of selected reference bacteria as compared to the control (physiological saline)

GIZ – growth inhibition zone.

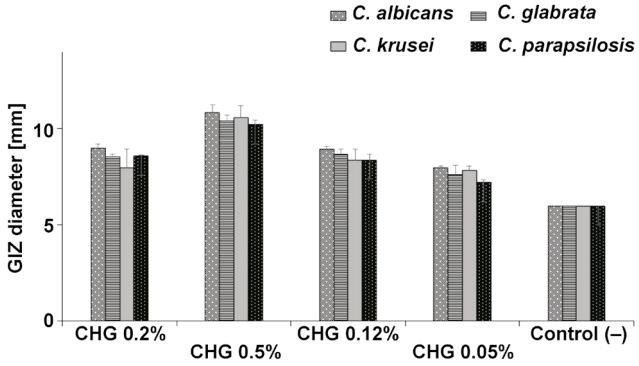

Fig. 7. Efficacy of the preparations containing only chlorhexidine digluconate (CHG), at various concentrations, on the growth of selected reference yeasts as compared to the control (physiological saline)

Table 1. Average size of the microbial growth inhibition zone (GIZ) [mm] for selected reference bacteria with regard to the preparations containing only chlorhexidine digluconate (CHG) at various concentrations

Bacteria	Measurement	CHG 0.2%	CHG 0.5%	CHG 0.12%	CHG 0.05%	Control (–)
S. aureus	1	13.14	18.46	11.66	11.13	6
	2	11.25	13.41	10.92	10.12	6
	3	11.70	13.89	13.62	11.25	6
	M ±*SD*	12.03 ±0.99	15.25 ±2.79	12.07 ±1.40	10.83 ±0.52	6 ±0
S. pyogenes	1	15.72	17.93	15.39	13.02	6
	2	14.35	17.06	15.98	11.06	6
	3	16.60	18.43	15.05	11.74	6
	M ±*SD*	15.56 ±1.13	17.81 ±0.69	15.47 ±0.47	12.24 ±0.68	6 ±0
P. aeruginosa	1	7.82	9.35	9.89	7.27	6
	2	8.39	9.36	9.77	7.31	6
	3	9.32	9.30	8.89	7.11	6
	M ±*SD*	8.51 ±0.76	9.34 ±0.03	9.52 ±0.55	7.23 ±0.11	6 ±0
E. faecalis	1	11.47	13.97	11.76	10.06	6
	2	12.11	12.87	12.01	10.74	6
	3	12.27	13.59	11.71	10.18	6
	M ±*SD*	11.95 ±0.42	13.48 ±0.56	11.83 ±0.16	10.33 ±0.36	6 ±0

M – mean; *SD* – standard deviation.

Table 2. Average size of the microbial growth inhibition zone (GIZ) [mm] for selected reference yeasts with regard to the preparations containing only chlorhexidine digluconate (CHG) at various concentrations

Yeasts	Measurement	CHG 0.2%	CHG 0.5%	CHG 0.12%	CHG 0.05%	Control (–)
C. albicans	1	8.99	10.59	8.85	7.98	6
	2	9.20	11.32	8.89	7.91	6
	3	8.78	10.68	9.11	8.08	6
	M ±*SD*	8.99 ±0.21	10.86 ±0.40	8.95 ±0.14	7.99 ±0.09	6 ±0
C. glabrata	1	8.65	10.07	9.00	7.41	6
	2	8.64	10.66	8.49	8.18	6
	3	8.39	10.55	8.57	7.33	6
	M ±*SD*	8.56 ±0.15	10.43 ±0.31	8.69 ±0.27	7.64 ±0.47	6 ±0
C. krusei	1	8.16	11.03	8.81	7.92	6
	2	6.98	9.85	7.74	7.61	6
	3	8.86	10.88	8.60	8.03	6
	M ±*SD*	8.00 ±0.95	10.59±0.64	8.38 ±0.57	7.85 ±0.22	6 ±0
C. parapsilosis	1	8.57	10.22	8.37	7.36	6
	2	8.51	10.48	8.69	7.11	6
	3	8.67	10.01	8.11	7.19	6
	M ±*SD*	8.58 ±0.08	10.24 ±0.24	8.39 ±0.29	7.22 ±0.13	6 ±0

for yeasts of 7.22 mm was recorded for the *C. parapsilosis* ATCC 22019 strain for 0.05% CHG solution, which was statistically significantly different in comparison with the control ($p < 0.01$) (Table 2, Fig. 7).

However, there were no statistically significant differences in the effect of individual simple mouth rinses on the assessed *Candida* strains (Fig. 7).

The research results obtained in the second part of the experiment illustrate the effect of various commercial combined preparations with a fixed 0.2% CHG solution together with bovine colostrum, ChB, HA, *Hamamelis* extract, and HA with fragments of DNA obtained from plant sources on the growth of selected reference bacteria and yeasts as compared to the negative control (C–),

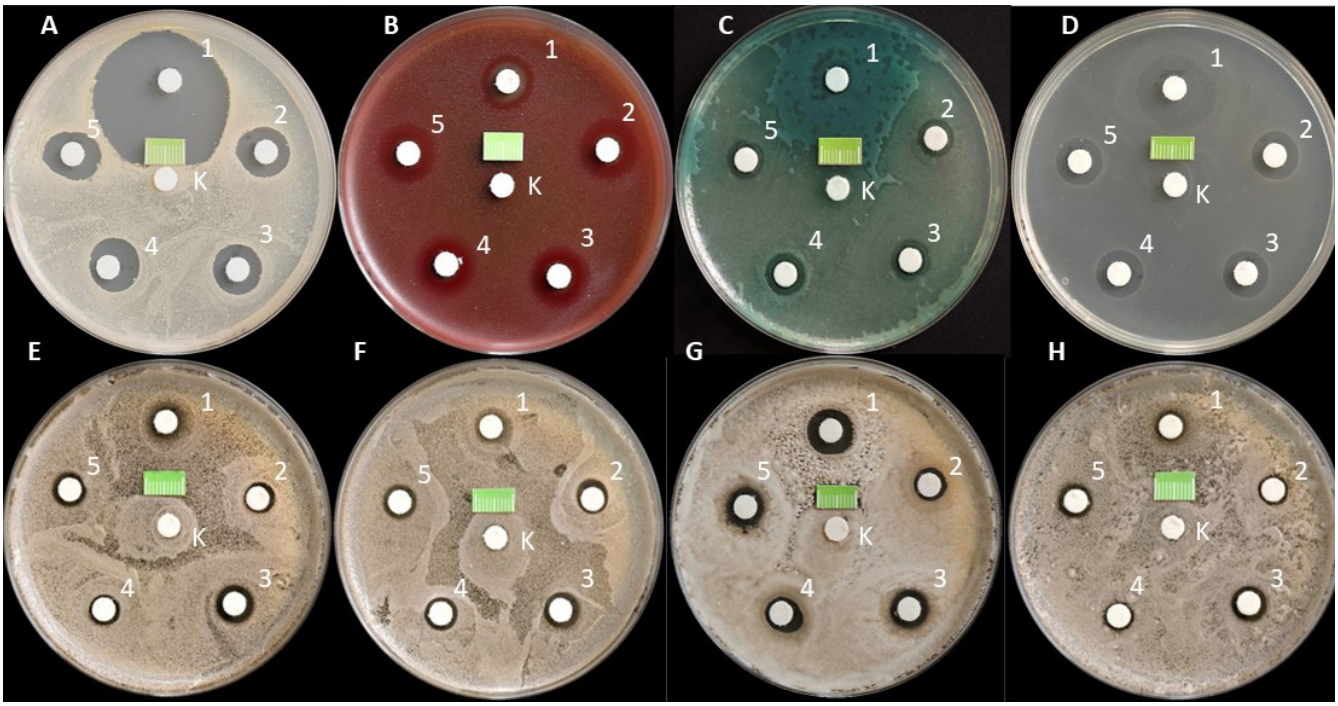

Fig. 8. Results obtained in the second part of the experiment

The effect of the preparations containing 0.2% chlorhexidine digluconate (CHG) solution in combination with bovine colostrum (1), chlorobutanol (ChB) (2), hyaluronic acid (HA) (3), *Hamamelis* extract (4), and HA along with fragments of DNA of plant origin (5) on the growth of selected standard bacteria and yeasts as compared to the control (K) sterile saline (0.9% NaCl).

A – *S. aureus* ATCC 43300; B – *S. pyogenes* ATCC 19615; C – *P. aeruginosa* ATCC 27853; D – *E. faecalis* ATCC 29212; E – *C. albicans* ATCC 10231; F – *C. glabrata* ATCC 15126; G – *C. krusei* ATCC 14243; and H – *C. parapsilosis* ATCC 22019 (1 of 3 samples).

saline. Furthermore, in the statistical analysis of this part of the study, the positive control (C+) was used, which was 0.2% CHG solution taken from the first part of the study (Fig. 8).

In all tested strains, after 24 h of incubation, a uniform, confluent (uncountable) increase in CFU was observed on the surface of the media in Petri dishes. Undisturbed colony growth was observed around all the central paper disks constituting the study controls. A different picture was observed around the paper disks soaked in the evaluated combined CHG solutions. For all tested strains, clear GIZs were observed after 24 h. These zones had significantly different sizes.

Among bacteria, the highest average value of zone diameter, obtained from 3 measurements, was recorded for 0.2% CHG + colostrum for the *S. aureus* ATCC 43300 strain, amounting to 33.06 mm. This result was statistically significantly higher as compared to the results obtained for other bacteria ($p < 0.001$).

Significant GIZs were also recorded for *S. pyogenes* ATCC 19615. Depending on the substance added, the values ranged from 16.47 mm for 0.2% CHG + *Hamamelis* to 14.86 mm for 0.2% CHG + colostrum. For the *E. faecalis* ATCC 29212 strain, slight differences in the size of GIZs were noted, with the best result of 12.42 mm for 0.2% CHG + HA, and the worst result (11.60 mm) for 0.2% CHG + colostrum. The results for all assessed solutions were statistically significantly different, and also significantly different in comparison with the control ($p < 0.001$). The smallest diameters of GIZs were recorded for *P. aeruginosa* ATCC 27853, for which the lowest result of 7.42 mm was read for the 0.2% CHG + colostrum regimen. This result was statistically significantly different in comparison with the control ($p < 0.01$) (Table 3, Fig. 9).

Statistically significant differences were noted in the effect of individual combined CHG rinses on the assessed bacterial strains. They were most effective against *S. pyogenes* ATCC 19615, followed by *S. aureus* ATCC 43300, *E. faecalis* ATCC 29212 and *P. aeruginosa* ATCC 27853 (Fig. 9).

Among yeasts, the highest average value of zone diameter, obtained from 3 measurements, was recorded for 0.2% CHG + colostrum for the *C. krusei* ATCC 14243 strain, and it was 12.56 mm. The following places were taken by *C. albicans* ATCC 10231 – 9.86 mm for 0.2% CHG + HA, *C. parapsilosis* ATCC 22019 – 9.81 mm for 0.2% CHG + HA + DNA and *C. glabrata* ATCC 15126 – 9.74 mm for 0.2% CHG + HA. These results were statistically significantly different from those obtained for the negative control ($p < 0.001$), but not significantly different from those obtained for the positive control ($p > 0.05$) (Table 4, Fig. 10).

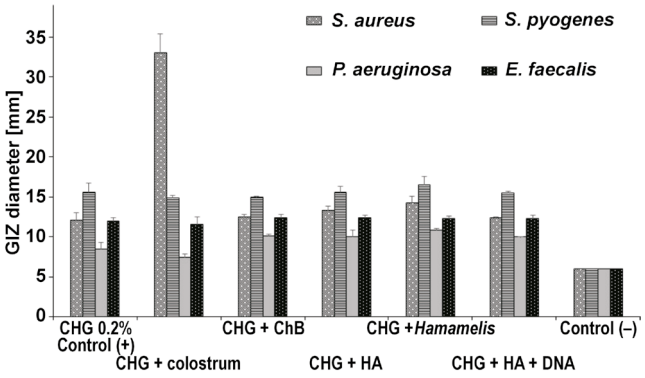

Fig. 9. Efficacy of the preparations containing complex chlorhexidine digluconate (CHG) solutions on the growth of selected reference bacteria as compared to the control (physiological saline)

Table 3. Average size of the microbial growth inhibition zone (GIZ) [mm] for selected reference bacteria with regard to the preparations containing complex chlorhexidine digluconate (CHG) solutions

Bacteria	Measurement	CHG + colostrum	CHG + ChB	CHG + HA	CHG + *Hamamelis*	CHG + HA + DNA	Control (–)
S. aureus	1	35.22	12.86	13.01	13.87	12.35	6
	2	33.40	12.30	12.99	15.22	12.29	6
	3	30.57	12.29	13.87	13.74	12.55	6
	M ±*SD*	33.06 ±2.34	12.48 ±0.33	13.29 ±0.50	14.28 ±0.82	12.40 ±0.14	6 ±0
S. pyogenes	1	15.24	14.92	15.89	17.28	15.15	6
	2	14.60	15.12	16.12	15.21	15.49	6
	3	14.74	14.81	14.73	16.91	15.72	6
	M ±*SD*	14.86 ±0.34	14.95 ±0.16	15.58 ±0.75	16.47 ±1.10	15.45 ±0.29	6 ±0
P. aeruginosa	1	7.16	10.11	10.83	11.03	9.88	6
	2	7.95	10.30	9.11	10.76	9.99	6
	3	7.14	10.05	10.05	10.67	10.05	6
	M ±*SD*	7.42 ±0.46	10.15 ±0.13	9.99 ±0.86	10.82 ±0.19	9.97 ±0.09	6 ±0
E. faecalis	1	12.63	12.48	12.71	12.60	11.89	6
	2	11.16	12.74	12.18	12.20	12.30	6
	3	11.01	11.81	12.38	12.12	12.69	6
	M ±*SD*	11.60 ±0.90	12.34 ±0.48	12.42 ±0.27	12.31 ±0.26	12.29 ±0.40	6 ±0

Table 4. Average size of the microbial growth inhibition zone (GIZ) [mm] for selected reference yeasts with regard to the preparations containing complex chlorhexidine digluconate (CHG) solutions

Yeasts	Measurement	CHG + colostrum	CHG + ChB	CHG + HA	CHG + *Hamamelis*	CHG + HA + DNA	Control (–)
C. albicans	1	8.70	8.59	10.52	8.30	9.25	6
	2	10.12	8.58	9.61	8.19	9.37	6
	3	8.00	8.36	9.44	7.93	9.19	6
	M ±*SD*	8.94 ±1.08	8.51 ±0.13	9.86 ±0.58	8.14 ±0.19	9.27 ±0.09	6 ±0
C. glabrata	1	7.58	8.00	9.30	8.16	9.39	6
	2	7.46	8.50	9.86	9.04	9.60	6
	3	7.05	8.35	10.06	8.93	9.34	6
	M ±*SD*	7.36 ±0.28	8.28 ±0.26	9.74 ±0.39	8.71 ±0.48	9.44 ±0.14	6 ±0
C. krusei	1	11.72	8.53	10.41	9.02	10.60	6
	2	11.96	8.80	10.75	8.51	9.23	6
	3	14.01	10.14	10.36	8.68	10.37	6
	M ±*SD*	12.56 ±1.26	9.16 ±0.86	10.51 ±0.21	8.74 ±0.26	10.07 ±0.73	6 ±0
C. parapsilosis	1	8.62	8.67	9.40	8.50	9.72	6
	2	8.41	8.56	9.55	8.87	10.03	6
	3	7.93	8.31	9.39	8.37	9.68	6
	M ±*SD*	8.32 ±0.35	8.51 ±0.18	9.45 ±0.09	8.58 ±0.26	9.81 ±0.19	6 ±0

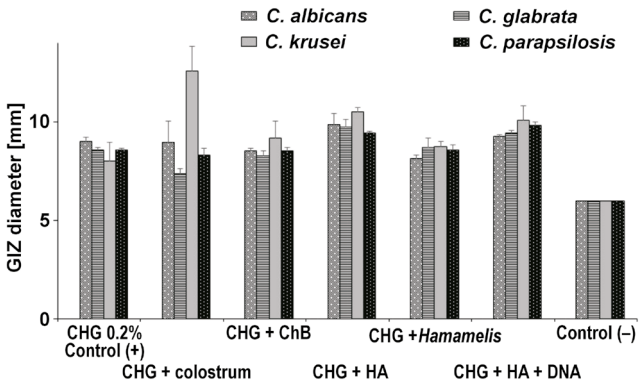

Fig. 10. Efficacy of the preparations containing complex chlorhexidine digluconate (CHG) solutions on the growth of selected reference yeasts as compared to the control (physiological saline)

Discussion

This study aimed to compare the efficacy of different simple and combined CHX mouthwashes against selected bacterial and yeast strains. The study was designed to include reference strains representing microorganisms commonly inducing frequent infections, like oral candidiasis (*Candida* spp.),[11,12,14] impetigo, angina (*S. aureus*, *S. pyogenes*, *P. aeruginosa*),[13] and endodontic infections (*E. faecalis*).[35] The treatment of these infections involves the usage of antiseptics, especially CHG. Furthermore, the selected strains represented microorganisms exhibiting variable morphological and physiological features. Three microorganisms, i.e., *S. aureus*, *S. pyogenes* and *E. faecalis*, are Gram-positive bacteria, and the other one – *P. aeruginosa* – is a Gram-negative bacterium. The cells of those microbes differ in terms of the thickness, structure and composition of the cell wall, as well as the presence of the polysaccharide coating.[36] The presence of a very thick, mucous alginate coating in *P. aeruginosa* is associated with, among others, particularly large resistance to disinfectants and antibiotics.[37] The environmental conditions of the selected bacteria are also distinct, specifically the demands regarding nutrients and oxygen. Staphylococci (*S. aureus*), streptococci (*S. pyogenes*) and enterococci (*E. faecalis*) are facultative anaerobes. On the other hand, the metabolism of *P. aeruginosa* is strictly aerobic. The microorganisms used in the research also differed significantly in cell size. Yeasts are 25–50 times bigger than bacteria. They belong to eukaryotes and have a more complex structure.[36] Their cells have a distinctly formed nucleus surrounded by a nuclear membrane and numerous cell organelles. *Candida* cells are surrounded by a thick, hardly permeable cell wall composed of beta-glucan, mannoproteins and chitin. The abovementioned differences between the organisms were intended to ensure the comprehensiveness of the observations and their future clinical usefulness.

Moreover, the experiment was planned to be carried out in stages. The first part assessed simple preparations in a liquid formula, at various CHG concentrations. In addition, the most common CHG concentrations in commercial and dental preparations were compared, i.e., 0.05%, 0.12%, 0.2%, and 0.5%. The selected products were obtained from a European manufacturer with the broadest portfolio. The results obtained in this phase confirmed the strong effect of CHG on Gram-positive bacteria, even at low concentrations (0.05%), whereas the efficacy against Gram-negative bacteria was worse. The greatest disinfection effectiveness was recorded for the highest concentration (0.5%), regardless of the evaluated microbe strain. However, no difference in

efficacy between the 0.2% and 0.12% concentrations was observed, regardless of the bacterial strain assessed. Other authors report similar results. Leshem et al. demonstrated that CHG had low effectiveness against *P. aeruginosa* obtained from skin injuries.[37] In their study, the minimum inhibitory concentration (MIC) for this strain was 1,024 μg/mL,[37] although in the works of other authors, the efficacy of solutions of 8–10 μg/mL was reported.[38–40] Mengistu et al. presented only a minimal disinfecting effect of 0.05% CHG solution against many bacterial strains, such as *Actinobacter*, *Klebsiella pneumonia*, *Enterobacter, Pseudomonas*, and *Proteus*.[41]

It was also shown in the present study that all tested *Candida* strains were sensitive to CHG, even at the lowest analyzed concentration. Their vulnerability was significantly higher than that of the Gram-positive bacteria, but comparable to that of the Gram-negative bacterium. It was proved that CHX impairs the yeast cell wall through binding with cell wall beta-glucan, causing temporary or permanent damage, depending on the concentration.[42] The highest studied concentration worked the best, just like in the case of bacteria. However, no difference was noted between the 0.2% and 0.12% concentrations, regardless of the yeast strain assessed. Another study using the MTT test showed a high effectiveness of 0.2% CHG against the *Candida* strains, with the viability of fungal cells decreasing along with an increase in the application time of the disinfectant.[43]

Furthermore, it was shown that the antifungal properties of CHX against *C. albicans* were stronger as compared to some polyene antibiotics, such as nystatin or amphotericin B.[5,44] It should be remembered that in complex candidiasis treatment, CHX and nystatin should not be used together, since they induce a pharmacological interaction responsible for the reduction of their efficacy.[45,46] Nevertheless, positive interactions between miconazole and CHX have been proven, enabling the simultaneous use of these agents during the local therapy of oral candidiasis.[47] Beneficial effects have also been observed for the topical use of CHX combined with fluconazole; such a regimen decreases the mass and growth of *Candida* single-strain biofilm.[48]

The second stage of the study investigated the disinfecting properties of 5 combined preparations containing 0.2% CHG solution and different supplements added to facilitate the symptomatic treatment of infections through several different mechanisms of action (anti-inflammatory, analgesic, local anesthetic, tissue healing acceleration, blood vessel sealing, and stengthening natural immune mechanisms). This part of the experiment was based on the knowledge that combined preparations, apart from the expected drug-specific reactions, sometimes show qualitative and quantitative deviations in their activity. These phenomena include the synergism and antagonism of action. Synergism is a phenomenon in which individual ingredients support each other, boosting the pharmacological effects. The effect of such an interaction is either the addition (additive synergism) or potentiation of action (super-additive synergism) of simultaneously used substances. Antagonism involves inhibiting or eliminating the pharmacological effects of simultaneously used ingredients. There are 3 types of antagonism distinguished: competitive (when the substances compete for the same receptor); functional (when the substances have an adverse mechanism of action); and chemical (when the substances react with each other and create a weaker or biologically inactive compound).

Given the cationic structure of the molecule, numerous antagonist actions of CHG with the compounds containing organic anions (detergents, natural soaps) and inorganic anions (nitrates, chlorides, phosphates, carbonates, and sulfates) are known. In such cases, ionic inactivation and salt precipitation occur. Also, alginates, carboxymethylcellulose and tragacanth can inactivate CHG, mainly through the absorption mechanism.[5] Combining CHX and sodium hypochlorite causes the precipitation of a flocculent sediment containing insoluble magnesium (Mg), iron (Fe) and calcium (Ca) salts, and the precipitate parachloroaniline (PCA).[49,50] Similar antagonistic mechanisms of action exhibit CHX and ethylenediaminetetraacetic acid (EDTA); their interaction produces pink inactive precipitates.[51] It has also been shown that most of non-ionic surfactants significantly decrease the effectiveness of CHX.[5]

What is also of great importance for the activity of CHX is the pH of the substance and the environment in which the substance is used. The most stable are the aqueous solutions of CHG with pH ranging from 5 to 8, whereas the most beneficial environmental pH is from 5.5 to 7, corresponding to the pH of the oral cavity.[52] A well-examined substance increasing the antimicrobial properties of CHG is diethyl alcohol, which is why it is often added in small amounts to commercially available products.[53,54] However, none of the preparations analyzed in our experiment contained this additive. Nevertheless, all of the tested combined preparations showed good disinfecting properties against bacteria and yeasts.

Similar to the results obtained by other authors and in the first part of our study, the second phase of our experiment confirmed greater efficacy of CHG in combined preparations against Gram-positive bacteria than against Gram-negative microorganisms. The preparations presented the strongest effect against streptococcus, and slightly weaker against staphylococcus and cocci. Alansari et al. demonstrated strong antibacterial activity of CHX-loaded halloysite nanotubes against *S. aureus*, *Streptococcus pneumoniae* and *Streptococcus agalactioe* on acrylic plates.[55] Manuschai et al. showed great effectiveness of 1% and 2% CHG solutions in eliminating the dual-species biofilm of *C. albicans* and *Streptococcus mutans*.[56] The effective biocidal action of CHG against Gram-positive bacteria results from the electrical charge of the

cell wall of these microorganisms. It has a much higher negative charge in comparison with Gram-negative bacteria, so the strength of the interaction is greater.[5] Thus, the MICs for these bacteria are less than 10 μg mL; for Gram-negative bacteria, the values are more variable and seldom below 50 μg/mL.[48,49] Some Gram-negative microbes, e.g., *Mycobacterium tuberculosis*, are insensitive to CHX action.[53,54] In our study, the effect of the CHG + colostrum preparation against *S. aureus* ATCC 43300 was noteworthy (33.06 mm) and indicated strong additive synergism. In this case, the GIZ was more than double that of the 2nd most potent combined preparation – CHG + chamomile extract (16.47 mm for *S. pyogenes*). All other combination formulations tested showed disinfecting potency similar to that of the simple 0.02% CHG formulation (the positive control group of this study) against each of the evaluated bacterial strains. None of the combined preparations had an action statistically significantly weaker than the positive control. Silva et al. compared the effectiveness of 3 plant-derived compounds in combination with CHX.[30] They showed, in contrast to our study, significant enhancement of the antibacterial activity of all their combinations, as indicated by reduced MIC values.[30] Hegde and Kamath demonstrated greater efficacy of a 0.12% CHX mouth rinse as compared to a combination (CHX and sodium fluoride) mouth rinse and a green tea extract (0.5%) mouth rinse in reducing the salivary count of *S. mutans* and *Lactobacillus*, with the latter two being similar in terms of efficacy.[57]

When evaluating the efficacy of CHX combined formulations against yeasts, their lower antimicrobial activity, comparable to that in the case of *P. aeruginosa* ATCC 27853, is noteworthy. None of the assessed rinses showed exceptionally greater activity, and only in the case of *C. krusei* were the values of all tested preparations statistically significantly higher than for the positive control. On the other hand, it should also be noted that for the *C. glabrata* and *C. parapsilosis* strains, none of the rinses showed statistically significantly lower efficacy. The study results obtained by Korbecka-Paczkowska and Karpiński, who assessed the antifungal activity of 15 different commercial oral rinses against 12 strains of *Candida* spp., showed a good disinfecting effect and a moderate antibiofilm effect of 0.12% CHG and CHG with CPC.[58] Statistically significant differences were noted between the rinses. The rinse with octenidine dihydrochloride had the strongest effect against *Candida* spp.[58] Also, a study by Fathilah et al. confirmed good efficacy of CHX and CPC against *Candida tropicalis* and *C. krusei*.[59] In this study, the combination of CHX and CPC doubled the inhibitory effect against *Candida* spp. expressed by MIC.[59] Handschuh Briones et al. assessed the efficacy of 6 oral rinses, including two 0.1% and 0.12% CHX solutions, and 2 CHX solutions with CPC, against 10 strains of *Candida* spp. and other yeasts (*Rhodotorula*).[60] All tested preparations achieved a good disinfecting effect. Contrary to the results of Fathilah et al.,[59] there were no statistically significant differences in the effect of CHX with CPC as compared to CHX alone.[60]

In the future, we would like to expand the study to include wild-type strains obtained from the oral swabs of patients presenting with various forms of oral candidiasis and impetigo. This would allow us to confront the obtained laboratory results with clinical needs, and to prepare guidelines and procedures for the pharmacological management of these difficult, chronic and recurrent oral diseases.

In conclusion, it should be noted that the analyzed combined preparations proved good disinfecting properties against bacteria and yeasts. Preparing commercial dental rinses with additives affecting various elements of symptomatic treatment and exhibiting an anti-causative effect did not result in the loss of pharmacological efficacy of CHG and allows a comprehensive treatment effect to be achieved. Using a combined preparation will also limit the patient's purchases to only one product, saving money. The large range of products offered enables the patient to choose the rinse best suited to their therapeutic needs, depending on the predominant clinical symptoms (pain, swelling, irritation). Often, the additives used also favorably alter the taste of the mouthwash (CHX is a relatively bitter substance), ensuring greater willingness on the part of the patient to maintain the prescribed therapeutic regimen.

Conclusions

The evaluated combined preparations of CHG showed disinfecting efficacy against selected bacterial and fungal strains comparable to that of simple formulations. The combination of 0.2% CHG with colostrum showed the additive synergism of antimicrobial activity against the *S. aureus* ATCC 43300 strain.

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.

Use of AI and AI-assisted technologies

Not applicable.

ORCID iDs

Rafał Wiench https://orcid.org/0000-0001-5673-7413
Anna Kuśka-Kiełbratowska https://orcid.org/0000-0001-8708-470X
Małgorzata Kępa https://orcid.org/0000-0002-4013-6688
Zuzanna Grzech-Leśniak https://orcid.org/0000-0001-6109-6809
Maciej Jabłoński https://orcid.org/0000-0003-3752-3828
Jan Kiryk https://orcid.org/0000-0002-5442-1412
Kinga Grzech-Leśniak https://orcid.org/0000-0002-5700-4577
Dariusz Skaba https://orcid.org/0000-0001-8732-251X

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Original paper

Do bleaching dentifrices associated with a low-concentration hydrogen peroxide gel affect the surface properties and mineral content of enamel?

Maiara Rodrigues de Freitas[1,A,B], Kusai Baroudi[2,A–C], Giuseppe Minervini[3,A,B], Ana Carolina Andreucci[1,A–C], Maria Júlia de Carvalho Feijó de Moura[1,A–C], Rodrigo Barros Esteves Lins[4,C,D], Flavio Henrique Baggio Aguiar[5,B,D,E], Rayssa Ferreira Zanatta[6,B,E,F], Priscila Christiane Suzy Liporoni[1,E,F]

[1] 1st Department of Dentistry, University of Taubaté (UNITAU), Brazil
[2] Department of Clinical Sciences, College of Dentistry, Ajman University, UAE
[3] Multidisciplinary Department of Medical-Surgical and Odontostomatological Specialties, University of Campania Luigi Vanvitelli, Naples, Italy
[4] School of Dentistry, Federal University of Alagoas (FOUFAL), Brazil
[5] School of Odontology of Piracicaba, State University of Campinas (UNICAMP), Brazil
[6] Department of Operative Dentistry and Dental Materials, School of Dentistry, Federal University of Uberlândia (UFU), Brazil

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

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Address for correspondence
Giuseppe Minervini
E-mail: giuseppe.minervini@unicampania.it

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Abstract

Background. Comparing the new and existing products is essential to identify the one that minimizes risks to the dental structures while effectively fulfilling its intended purpose.

Objectives. The aim of the present study was to evaluate possible changes in the surface properties, mineral loss and color of bovine enamel subjected to bleaching dentifrices used in combination with a low-concentration hydrogen peroxide (HP) bleaching gel.

Material and methods. Bovine tooth substrates disinfected with thymol were used to make 112 circular samples with a diameter of 4 mm. After the samples were embedded in transparent acrylic resin, they were polished with grit of decreasing granulation and divided into 8 groups ($n = 14$ per group), according to the bleaching treatment (Opalescence Go (OpGo) – 10% HP or immersion in buffered water (BW) – control) and the toothpastes used (OMW – Oral-B 3D Mineral White Clean; CLW – Colgate Luminous White Advanced; STW – Sensodyne True White; or CT – Colgate Total 12). The bleaching gel was used for 30 min daily for 10 days. The samples were brushed using an electric brush and a slurry (3:1 ratio) for 120 s twice a day, with an interval of 12 h, with the first brushing immediately after the bleaching treatment. Prior to the commencement of the treatment, the initial microhardness, surface roughness and color data was evaluated.

Results. For microhardness, a reduction in values was observed for all groups, except for the control (CT + salt), whereas for roughness, there was an increase in the final values for all groups. A significant difference in the post-treatment values was observed only for the lightening treatment factor ($p = 0.0079$).

Conclusions. There was a reduction in enamel microhardness for all groups, except for the group that used a non-bleaching dentifrice and was treated with BW.

Keywords: microhardness, roughness, over-the-counter products

Highlights

- Demand for smile and teeth-whitening treatment is rapidly increasing, making patient education more important than ever.
- Dentists should guide patients on the safe use of over-the-counter whitening products and recommend appropriate toothpastes.
- The combination of a whitening toothpaste and bleaching treatment does not improve results and may damage enamel by increasing surface roughness.
- Activated charcoal toothpastes – even when used alone – can increase tooth surface roughness, leading to greater biofilm accumulation.

Introduction

Tooth whitening is based on the oxidation reaction of hydrogen peroxide (HP). This reaction releases free radicals of low molecular weight, capable of penetrating the dental structures and causing the chemical degradation of the chromogens. The action of HP promotes the reduction of chromophore molecules, modifying the refractive index of light and causing the lightening effect.[1,2] Bleaching can be done in-office, using high-concentration HP gels or at home with lower-concentration gels. However, even though whitening is considered the most cost-effective procedure for treating chromatic changes, access to is restricted to part of the population, as it requires professional consultation and supervision.[3] In this context, alternative methods for tooth whitening are frequently desired by patients. Over-the-counter (OTC) products comprise toothpastes, whitening tapes, whitening pens, and mouthwashes, and are sold without professional prescription.[4,5]

Dentifrices with whitening properties are in high demand among patients.[3] They contain chemical and optical agents, usually with a high amount of abrasives and detergents as compared to non-whitening toothpastes.[6] The combination of different abrasives is usually responsible for removing stains,[3] but their continuous use may be related to the abrasive wear of the tooth surface,[7] which can cause a progressive dental structure loss. Consequently, dentin hypersensitivity may develop in teeth with non-carious cervical lesion or gingival ressession.[8,9] Bleaching procedures can also increase the surface roughness of enamel, dentin and composite restorations,[10–12] as well as enhance the cytotoxicity of certain restorative materials when they come into contact with saliva.[13–17]

According to the literature, the effectiveness of OTC whitening products is controversial. Karadas and Duymus reported a positive effect of a toothpaste with a whitening effect on removing superficial enamel stains.[18] Rached Dantas et al. showed opposite results, concluding that whitening dentifrices were not effective in terms of staining removal.[19] A recent systematic review showed that whitening dentifrices could produce some level of whitening effect on enamel, yet with considerable adverse effects, with low to moderate quality of evidence.[20] Most of the patients do not have knowledge about the deleterious effects the prolonged use of these products can generate.

Beside dentifrices, strips with low-concentration HP gels are often available as an OTC option for tooth whitening. Recent evidence suggests that their use presents lower whitening efficacy than dentist-supervised at-home bleaching, although with a lower incidence of tooth sensitivity and gingival irritation.[21] The associated use of whitening dentifrices and HP strips might induce further adverse effects on enamel, which are yet to be defined. The industry innovations regarding OTC products always demand updated research to evaluate their adverse effects on the dental structures. In addition to abrasives, some whitening dentifrices have active ingredients in their composition, such as HP or activated carbon, and the side effects on enamel related to their constant use have not been established yet. Comparing the new and existing products is essential to identify the one that minimizes risks to the dental structures while effectively fulfilling its intended purpose.

The aim of the present study was to evaluate the effects of the concomitant use of different whitening dentifrices and a low-concentration HP gel on the surface properties, mineral loss and color change (ΔE) of bovine enamel.

Material and methods

Sample size calculation

The sample size was calculated based on the study by Borges et al., considering the microhardness values for calculating *f* (effect size).[22] The G*Power software, v. 3.1 (Heinrich-Heine-Universität, Düsseldorf, Germany), was used with a 95% significance level and 80% test power. The calculation obtained was 14 samples per group.

Sample preparation

Sound bovine incisor teeth, freshly extracted and acquired, were cleaned with periodontal curettes to remove

any gum tissue residues adhered to the surface, and polished with a rubber cup and pumice paste and water, using Robinson brushes (Microdont, São Paulo, Brazil) in low rotation. Subsequently, 0.9% saline solution was used to store the teeth under refrigeration until used.

The crown was separated from the root at the cementoenamel junction (CEJ) with a diamond disk (KG Sorensen, Cotia, Brazil), and then, a circular enamel/dentin sample with a diameter of 4 mm was obtained using a diamond trephine mill. Subsequently, the enamel and dentin thickness were standardized at 2 mm (1 mm of enamel and 1 mm of dentin). The sample surface was polished in a circular polishing machine (Aropol; Arotec, Cotia, Brazil) with a speed of 600 rpm and constant irrigation, with 600-, 800- and 1,200-grit silicon carbide sandpaper (Extec Corp., Enfield, USA) for 60 s, 90 s and 120 s, respectively, resulting in parallel surfaces.

Study groups

The samples were stratified and divided into 8 groups (n = 14), considering their initial Knoop microhardness (KMH) values for enamel. The initial KMH of all specimens was measured using a microdurometer with a Knoop indenter (HMV-2T; Shimadzu, Kyoto, Japan), with a load of 50 g and a residence time of 15 s, following ISO 28399 (2011). Three indentations were made for each sample, and the average with regard to them was considered. The samples showing outliers in 20% were excluded.

The group division followed the whitening treatment and the toothpaste products. For the whitening treatment variable, the use of a low-concentration HP gel (Opalescence Go (OpGo), 10% HP; Ultradent Products, Inc., Indaiatuba, Brazil) was considered, with the negative control (buffered water (BW), pH 7.0). For the dentifrice factor, the dentifrices used were as follows: OMW – Oral-B 3D Mineral White Clean (Procter & Gamble Company, Cincinnati, USA); CLW – Colgate Luminous White Advanced (Colgate–Palmolive Company, New York, USA); STW – Sensodyne True White (GSK, Philadelphia, USA); and CT – Colgate Total 12 (non-whitening; Colgate–Palmolive Company). The composition of all dentifrices is presented in Table 1. Figure 1 shows the group division and the study flowchart.

Table 1. Composition of the dentifrices tested

Dentifrice	Composition
OMW – Oral-B 3D White Mineral Clean (Procter & Gamble, Cincinnati, USA)	sodium fluoride (1,100 ppm), sorbitol, hydrated silica, disodium pyrophosphate, sodium lauryl sulfate, cellulose gum, sodium hydroxide, sodium saccharin, carbomer, charcoal powder, mica, limonene, sucralose, titanium dioxide, polysorbate 80, water, aroma
CLW – Colgate Luminous White Advance (Colgate–Palmolive Company, New York, USA)	sodium monofluorophosphate (1,000 ppm fluoride), hydrogen peroxide 3%, PVP-hydrogen peroxide, propylene glycol, calcium pyrophosphate, glycerin, sodium lauryl sulfate, silica, tetrasodium pyrophosphate, sodium saccharin, disodium pyrophosphate, sucralose, eugenol, PVP, PEG-12, PEG/PPG-116/66 copolymer, BHT, flavor
STW – Sensodyne True White (GSK, Philadelphia, USA)	sodium fluoride (1,426 ppm), potassium nitrate 5%, sorbitol, glycerin, hydrated silica, pentasodium triphosphate, cocamidopropyl betaine, titanium dioxide, xanthan gum, sodium hydroxide, sodium saccharin, PEG-6, water, aroma
CT – Colgate Total 12 (Colgate–Palmolive Company)	sodium fluoride (1,450 ppm fluoride), triclosan 0.3%, glycerin, sorbitol, hydrated silica, sodium lauryl sulfate, carrageenan, sodium saccharin, sodium hydroxide, PVM/MA copolymer, white dye CI 77891, water, flavor

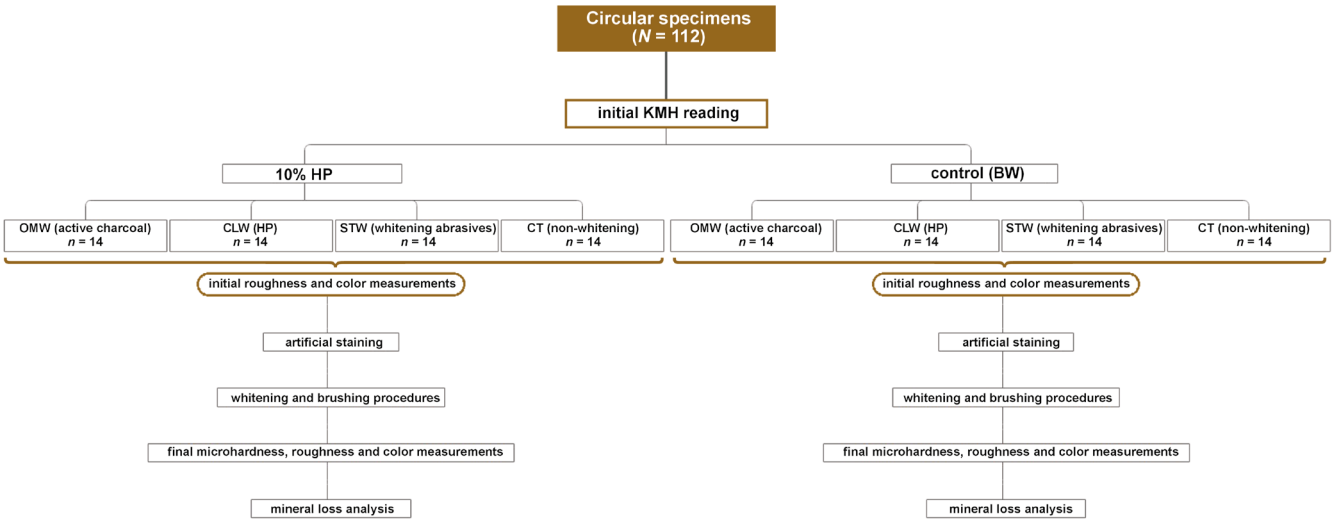

Fig. 1. Group division and study flowchart

KMH – Knoop microhardness; HP – hydrogen peroxide; BW – buffered water; dentifrices: OMW – Oral-B 3D Mineral White Clean; CLW – Colgate Luminous White Advanced; STW – Sensodyne True White; and CT – Colgate Total 12.

Initial roughness and color measurements

The initial roughness values were obtained with a roughness tester (Surftest SJ-301; Mitutoyo, Tokyo, Japan) and measured by the parameter Ra. Three readings were made per sample and their average value was considered according to the provisions of ISO 28399 (2011).

The initial color data was collected using a colorimetric reflectance spectrophotometer (CM-2600d, Konica Minolta, Osaka, Japan), according to the CIE L* a* b* system (Commission internacionale de l'eclairage – CIE, International Commission on Illumination). In this, the L* axis represents the degree of luminosity and varies from 0 (black) to 100 (white), the a * axis represents the degree of the green/red color and the b * axis – the degree of the blue/yellow color. The initial color coordinates were measured with the equipment adjusted to the use of the D65 light source, with 100% ultraviolet, and the specular component included (SCI) mode. The observer's angle was adjusted to 2° and the reading area was 12.56 mm^2 (considering π = 3.14 and the radius of 2 mm), since the reading was made considering the internal diameter of 4 mm of the sample. To standardize the position of the samples in the spectrophotometer, each sample was marked with a diamond tip #1012 (KG Sorensen) on one of its sides, and a mark was made on the equipment.

Artificial sample pigmentation

The samples were immersed in a black tea coloring solution for extrinsic staining. The solution was prepared with 1.6 g of black tea (Leão Junior S.A., Curitiba, Brazil) in 100 ml of boiling distilled water (100°C), brewed for 5 min. The solution was renewed every 24 h and the samples were immersed for 6 days.[23] After the pigmentation process, the samples were submerged for 7 days in artificial saliva, which was daily changed.

Whitening and brushing procedures

In the bleached groups, the whitening gel was used according to the manufacturer's instructions in terms of time and number of applications. A 1-millimeter-thick layer of gel was applied over the samples for 30 min, totaling 9.4 mm^3, once a day for 10 days. In the control groups, the samples were immersed in BW, with pH 7.0, for 30 min per day for 10 days, instead of the bleaching treatment. Afterward, the samples were washed with mineral water and submerged in artificial saliva (Byofórmula, São José dos Campos, Brazil) for 30 min.

Regarding the abrasion protocol with the tested dentifrices, they were applied as a slurry (3:1 with artificial saliva). The samples were brushed with the toothpaste corresponding to each group twice a day, with the first brushing immediately after the bleaching procedure, and the other one 12 h later. The sample was in contact with the slurry for 120 s, comprising 15 s of abrasion with an electric brush (Procter & Gamble) and 105 s of immersion in the slurry.

After the first brushing, the samples were washed with mineral water and submerged in artificial saliva for 12 h, when they were again brushed with the toothpaste corresponding to each group. In the intervals, the samples were kept immersed in artificial saliva. The artificial saliva used was composed of sodium carboxymethylcellulose (CMC, 10 g/L), sorbitol (30 g/L), potassium chloride (1.2 g/L), monobasic potassium phosphate (3.42 g/L), calcium chloride dihydrate (1.46 g/L), magnesium chloride (52 g/L), sodium chloride (84 g/L), sodium benzoate (1 g/L), sodium fluoride (1.25 g/L), methylparaben (1.5 g/L), and distilled water.

Final microhardness, roughness and color measurements

The final KMH and Ra measurement was made 7 days after the bleaching/abrasive procedures to enable the rehydration and stabilization of the samples. The samples were submerged in artificial saliva, which was changed daily.

The final color measurement was also made 7 days after the bleaching/abrasive procedures were completed, for rehydration and color stabilization. The same parameters were used as in the initial readings, and the final color was defined by calculating the variation of L* (ΔL), a* (Δa) and b* (Δb). The total color change was calculated by parameter ΔE, using the following formula (Equation 1):

$$\Delta E = (\Delta L^2 + \Delta a^2 + \Delta b^2)^{½} \quad (1)$$

where:
ΔE – color change;
ΔL* – difference in lightness;
Δa* – difference in the green–red axis; and
Δb* – difference in the blue–yellow axis.

Mineral loss analysis

Five samples per group were randomly selected and were subjected to the micro-energy dispersive X-ray (μEDX) analysis, evaluating the mineral loss as the ratio between calcium (Ca) and phosphorus (P). The readings were performed using an energy dispersive X-ray fluorescence spectrometer (μEDX-1300; Shimadzu). The device is equipped with a rhodium (Rh) X-ray tube and a silicon (Si) detector, cooled by liquid nitrogen (N), and is associated with a computer and specific software for processing the collected data. The samples were placed on a glass plate sequentially in the order of each group. In each specimen, 3 readings were performed on the enamel surface with a voltage of 15kV and current of 100 μA, for

100 s with dead time 25%. The equipment was calibrated with stoichiometric hydroxyapatite, a certified commercial reagent ($Ca_{10}(PO_4)6(OH)_2$ – synthetic hydroxyapatite, 99.999% purity grade, lot 10818HA; Sigma-Aldrich, St. Louis, USA), as a reference. The measurements were made using the fundamental emission parameters characteristic of the elements Ca and P. The element oxygen (O) was used as a chemical balance.

Statistical analysis

After analyzing data normality, the KMH values were subjected to the two-way repeated measures analysis of variance (ANOVA) with the Bonferroni correction. For the Ra values, the Friedman and Kruskal–Wallis tests were used, and the color data was analyzed with the one-way ANOVA and Tukey's test (ΔL and ΔE), or the Kruskal–Wallis test and Dunn's test (Δa and Δb). For the EDX values, the data underwent a descriptive analysis. The confidence level was set at $\alpha = 0.05$.

Results

The results of the analysis of surface microhardness are available in Table 2. After bleaching, the samples showed a significant difference in microhardness after the abrasive protocol, regardless of the dentifrice tested ($p < 0.05$). When the samples were not bleached, there was a significant difference only in the case of the abrasive protocol carried out with the toothpaste containing charcoal ($p < 0.05$). A statistically significant difference was found comparing the bleached and non-bleached groups, regardless of the toothpaste used. The dentifrices showed no statistically significant difference between each other in the final values. When whitening was used concomitantly with the toothpaste with charcoal, a significant difference was found as compared to the use of charcoal alone, without bleaching taking place.

As the roughness data did not meet the normality criteria, non-parametric tests were applied; the Friedman test was used to compare between the time points, and for groups, the Kruskal–Wallis test was employed. There were statistically significant differences between the baseline and final values in the bleached groups using the OMW and CLW toothpastes ($p < 0.05$) (Table 3).

Regarding ΔE, there were no statistically significant differences between the tested groups ($p > 0.05$) (Table 4).

The Ca/P data revealed no differences between the dentifrices studied ($p > 0.05$). Figure 2 shows the absolute mean values of the 5 samples tested in each group, assessing the mineral loss as the ratio between CA and P for the treatment performed.

Table 2. Knoop microhardness (KHN) values [HK] obtained at different times of evaluation

Group	Baseline value	Final value	*p*-value (time)
BW + OMW	265.85 ±36.1^{Aa}	258.61 ±32.9^{Bab}	0.049*
BW + CLW	258.87 ±40.5^{Aa}	253.88 ±40.8^{Aab}	0.184
BW + STW	257.85 ±36.4^{Aa}	253.40 ±29.9^{Aab}	0.173
BW + CT	268.56 ±45.3^{Aa}	272.07 ±40.5^{Aa}	0.204
OpGo + OMW	254.18 ±38.6^{Aa}	232.75 ±33.7^{Bb}	<0.001*
OpGo + CLW	256.61 ±36.2^{Aa}	240.47 ±34.8^{Bb}	<0.001*
OpGo + STW	258.42 ±34.5^{Aa}	243.35 ±39.5^{Bb}	0.004*
OpGo + CT	258.12 ±33.6^{Aa}	237.42 ±34.6^{Bb}	<0.001*

Data presented as mean ± standard deviation (*M* ±*SD*).
OpGo – Opalescence Go, a low-concentration HP gel (10% HP);
* statistically significant. Different letters in superscript indicate statistically significant differences at 5%, according to two-way repeated measures ANOVA and the Bonferroni post-hoc test: uppercase letters – between different times of evaluation (baseline and final) (lines); lowercase letters – between different groups at each time point (columns).

Table 3. Surface roughness values [Ra] obtained at different times of evaluation

Group	Baseline value	Final value	*p*-value (time)
BW + OMW	0.40 (0.2;1.1)^{Aa}	0.56 (0.2;1.4)^{Ab}	0.782
BW + CLW	0.43 (0.1;0.7)^{Aa}	0.48 (0.4;1.1)^{Ab}	0.166
BW + STW	0.40 (0.2;2.2)^{Aa}	0.71 (0.3;1.5)^{Aab}	0.166
BW + CT	0.51 (0.2;2.0)^{Aa}	0.65 (0.3;1.1)^{Aab}	0.166
OpGo + OMW	0.52 (0.2;1.7)^{Aa}	0.93 (0.6;2.3)^{Ba}	<0.001*
OpGo + CLW	0.41 (0.1;0.8)^{Aa}	0.68 (0.5;1.5)^{Bab}	0.001*
OpGo + STW	0.42 (0.3;0.9)^{Aa}	0.68 (0.4;1.1)^{Aab}	0.052
OpGo + CT	0.41 (0.1;1.0)^{Aa}	0.57 (0.4;2.1)^{Aab}	0.052

Data presented as mean (minimum–maximum) (*M* (min;max)).
* statistically significant. Different letters in superscript indicate statistically significant differences at 5%, according to the Friedman and Kruskal–Wallis tests: uppercase letters – between different times of evaluation (baseline and final) (lines); lowercase letters – between different groups at each time point (columns).

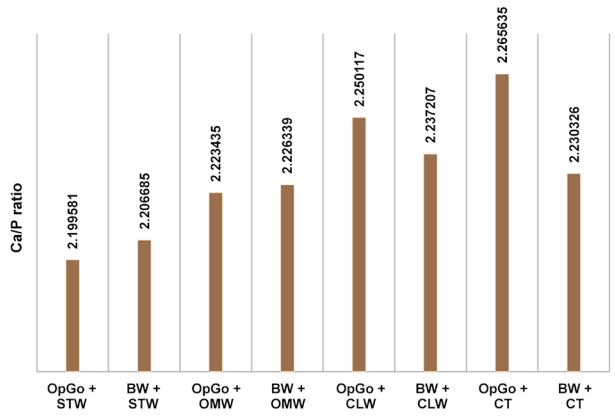

Fig. 2. Mineral loss as the ratio between calcium (Ca) and phosphorus (P) for the treatment performed

Table 4. Color change values (ΔL, Δa, Δb, ΔE)

Group	Color parameters			
	ΔL	Δa	Δb	ΔE
BW + OMW	16.97 ±7.1[a]	−2.15 (−3.2;−0.9)[a]	−5.23 (−17.4;6.8)[a]	18.03 ±7.6[a]
BW + CLW	15.52 ±5.7[a]	−1.65 (−5.2;−1.3)[a]	−7.55 (−12.7; .5)[a]	17.71 ±4.4[a]
BW + STW	16.64 ±5.0[a]	−1.71 (−4.3;−0.2)[a]	−6.83 (−18.8;−4.0)[a]	19.81 ±4.9[a]
BW + CT	17.04 ±5.8[a]	−2.17 (−4.3;−0.3)[a]	−7.51 (−9.1;−3.1)[a]	18.00 ±5.1[a]
OpGo + OMW	16.77 ±4.0[a]	−1.63 (−4.6;0.2)[a]	−6.90 (−15.0;−3.8)[a]	20.17 ±3.9[a]
OpGo + CLW	17.09 ±7.4[a]	−2.26 (−5.1;0.5)[a]	−8.99 (−11.8;4.4)[a]	19.13 ±6.1[a]
OpGo + STW	17.45 ±5.8[a]	−1.55 (−4.4;−0.4)[a]	−7.53 (−14.3;5.7)[a]	20.68 ±2.6[a]
OpGo + CT	15.83 ±5.2[a]	−2.29 (−3.7;−1.2)[a]	−8.39 (−14.6;3.2)[a]	19.54 ±4.9[a]

Data presented as *M* ±*SD* for ΔL and ΔE, and as *M* (min;max) for Δa and Δb.
Different lowercase letters in superscript indicate statistically significant differences between different groups for each color parameter (columns) at 5%, according to the one-way ANOVA and Tukey's post-hoc test (ΔL and ΔE), or the Kruskal–Wallis test and Dunn's post-hoc test (Δa and Δb).

Discussion

This in vitro study evaluated the effects of whitening strips with 10% HP associated or not with brushing with toothpastes containing different active ingredients on enamel. Regarding toothpastes, apart from active ingredients, they also presented differences in their formulation; however, the results were interpreted considering that differences in performance between the formulations were due exclusively to their operating principles (charcoal, low-concentration HP and whitening abrasives).

Regarding microhardness, the differences found for all the samples bleached with 10% HP were significant in relation to the dentifrices tested; for roughness, there were significant differences for the groups of samples bleached with 10% HP, and brushed with the toothpastes containing activated charcoal (OMW) and a low concentration of HP (CLW); the color analysis being the exception, since between the baseline and final values, there were no differences, regardless of the dentifrice used. As mentioned above, for the samples brushed with the toothpaste containing HP, the difference was statistically significant. There is evidence that HP can increase enamel roughness due its ability to demineralize hydroxyapatite crystals,[22] and the association of the gel and the toothpaste containing this ingredient induced a more pronounced adverse effect. There is no specific clinical protocol described to overcome this, and patients should be aware that increased roughness can enhance biofilm formation and staining.

When the toothpastes were used immediately after whitening with 10% HP, a decrease in the surface microhardness values of tooth enamel was observed when compared to the groups where there was no whitening, with the exception of the dentifrice containing activated charcoal, which showed a reduction in microhardness ($p < 0.05$).

The methodology in the present study was designed to simulate the performance of supervised home whitening treatment, in which the patient uses a tray pre-loaded with the gel, and, in an attempt to enhance the effect of this whitening product, without the guidance and permission of their dentist, makes use of toothpastes freely available in the market that are advertised as making the teeth whiter.

In this study, the reduction in enamel microhardness promoted by the whitening gel was less than 10%, which is below the safety limit allowed by the American Dental Association (ADA). In fact, previous data showed that the use of HP does not promote significant changes in the histomorphology and microhardness of enamel,[24,25] and even if any changes occur with regard to the initial microhardness and roughness, they are reversed by the action of saliva within a certain time.[26] As the toothpaste was used immediately after removing the gel, there may not have been enough time for saliva to produce its remineralizing effect.

It is argued that the viscosity of the whitening gel may be directly related to the deleterious effects.[27] The pH of Opalescence Go used in this study, according to the manufacturer, is neutral. Therefore, any expected deleterious effect on enamel was minimal, which corroborates previous studies.[27–29] Bistey et al. reported that, in addition to these factors, structural changes to the enamel surface also depend on the contact time, with considerable alterations occurring when the time of exposure to HP exceeds 60 min.[30] The exposure time used in this study was 30 min, which may also have been the reason for not promoting changes in the studied surface properties.

However, there was a statistically significant difference in microhardness in the group with no bleaching, using the toothpaste containing activated charcoal. Charcoal is an abrasive that can be manufactured from a variety of carbon-rich materials, including walnut shells,

coconut shells, bamboo, peat, and wood.[31] When it is used for toothbrushing, it is manufactured as a fine powder of varying abrasiveness, depending on the source and the methods used to prepare and grind it.[32] The charcoal-based toothpaste used in this study has also other abrasives in its formulation, such as silica, titanium dioxide and mica, enhancing the abrasive potential of this toothpaste, thus making it more harmful to enamel. It is known that the abrasiveness offered by toothpastes is normally the determining factor for the removal of extrinsic stains and, consequently, the whitening sensation promoted.[33,34] However, the findings of this study indicate that the combined effects of toothpaste abrasiveness and pH can lead to greater tooth tissue loss when applied to enamel with a softened surface layer.

Although the toothpaste used in this study contains fluoride, its high adsorption capacity raises concerns about the actual availability and benefits of fluoride and other active ions in the dentifrice, as these may have been absorbed by charcoal.[32] Furthermore, despite fluoride sodium present in the composition of the toothpaste used in this study, it is reported in the literature that only 8% of charcoal toothpastes contain fluoride.[31,32] Fluorides that are present in conventional toothpastes have an acknowledged anti-cariogenic potential, and offer protection, even if limited, against erosion and cariogenic microorganisms, forming fluoride precipitates of calcium (CaF_2).[35] These benefits are not available if fluoride inactivation by charcoal occurs, leaving the tooth more susceptible to acid attacks.

Brushing with the toothpaste containing a low concentration of the active ingredient HP immediately after using the whitening gel resulted in significant changes in the surface roughness of enamel. This tested toothpaste, in addition to having 3% HP, contains a combination of abrasives (sodium and tetrasodium pyrophosphate and silica), which may have been responsible for the significant increase in roughness.

The μ-EDX analysis indicates the Ca/P ratio in the dental structures. The literature shows that a sound enamel has similar Ca and P concentrations.[26] In this study, the analysis was conducted after the procedures, and the results indicated no significant changes to enamel in all groups after the bleaching treatment and the abrasion with the tested dentifrices.

Finally, regarding color alteration, there were no significant differences between the groups, indicating that the toothpastes tested did not enhance color alteration when used with the bleaching tray tested. All kinds of treatment produced high ΔE values, indicating effective whitening; however, these results should be interpreted with caution, as the staining protocol used may have overestimated the extent of extrinsic staining. Future studies should include polishing of the enamel surface previous to the initial color measurement to remove loosely bounded staining, and be closer to clinical conditions.

The aim of this study was to evaluate the concomitant effect of the whitening treatment and the abrasive action of toothpaste; therefore, the brushing period was limited to the recommended period of the whitening treatment used, i.e., 10 days. Hence, the results obtained after longer periods of use could demonstrate greater deleterious effects on the surface of tooth enamel. Even though it is an in vitro study, the findings show that the use of toothpastes without guidance from a dental surgeon, especially when it is carried out concomitantly with supervised whitening treatment, can be harmful to enamel. As a limitation, this study was conducted in vitro, using artificial saliva in the bleaching and abrasion protocol; therefore, the presence of the acquired pellicle was not considered. Additionally, although the staining protocol employed is commonly cited in the literature, it may overestimate the physiological staining that occurs in the oral cavity.

Conclusions

Within the limitations of this study, it might be concluded that the association of whitening dentifrices with a low-concentration HP gel does not improve bleaching effectiveness, and might induce more negative effects on the enamel surface. Thus, this combination might not be clinically viable, and patients shall be advised of possible side effects when using OTC whitening products without a proper supervision of a dentist.

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.

Use of AI and AI-assisted technologies

Not applicable.

ORCID iDs

Maiara Rodrigues de Freitas https://orcid.org/0000-0002-4383-7228
Kusai Baroudi https://orcid.org/0000-0002-1009-0869
Giuseppe Minervini https://orcid.org/0000-0002-8309-1272
Ana Carolina Andreucci https://orcid.org/0000-0001-9489-8277
Maria Júlia de Carvalho Feijó de Moura https://orcid.org/0009-0000-1844-568X
Rodrigo Barros Esteves Lins https://orcid.org/0000-0002-8224-6578
Flavio Henrique Baggio Aguiar https://orcid.org/0000-0003-3389-5536
Rayssa Ferreira Zanatta https://orcid.org/0000-0001-5230-1508
Priscila Christiane Suzy Liporoni https://orcid.org/0000-0001-6787-1167

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Original paper

Surface and optical properties of multilayer zirconia after toothbrushing

Lívia Fiorin[A–D], Stephanie Francoi Poole[B–D], Alia Oka Al Houch[B–D], Adriana Cláudia Lapria Faria[C–E], Ricardo Faria Ribeiro[C,E,F], Renata Cristina Silveira Rodrigues[A,C–F]

Department of Dental Materials and Prosthodontics, Ribeirão Preto School of Dentistry, University of São Paulo, Ribeirão Preto, Brazil

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

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Address for correspondence
Renata Cristina Silveira Rodrigues
E-mail: renata@forp.usp.br

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Conflict of interest
None declared

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Abstract

Background. The impact of toothbrushing on the surface and optical properties of multilayer zirconia is unknown.

Objectives. The aim of this in vitro study was to evaluate the effect of finishing procedures on the surface roughness (SR) and relative translucency (RT), as well as the effect of toothbrushing on SR, RT, color difference (ΔE_{00}), and gloss (Δgloss) of multilayer zirconia stabilized with 5 mol% of yttrium oxide (5Y-TZP) following polishing or glazing.

Material and methods. Thirty specimens were fabricated from the cervical layer of pre-sintered blocks of 5Y-TZP. The specimens were divided into 3 groups ($n = 10$/group): control (C); polishing (P); and glazing (G). The surface roughness was evaluated with a confocal laser microscope, and the RT, ΔE_{00} and Δgloss were assessed with the use of a spectrophotometer. A total of 50,000 cycles of toothbrushing (2 Hz, 2.5 N) were performed using a dentifrice slurry. The linear mixed-effects model and Bonferroni test ($\alpha = 0.05$) were employed to analyze SR and RT. The color change and Δgloss were assessed using the one-way analysis of variance (ANOVA) and Tukey's post hoc test ($\alpha = 0.05$).

Results. The finishing procedures had an influence on SR and RT. The polishing process did not affect SR and increased RT, while the glazing process resulted in an increase in SR and a decrease in RT in the multilayer 5Y-TZP. The impact of toothbrushing on SR was not significant ($p = 0.052$). However, decreased RT was observed in the P group ($p < 0.05$), while an increase in RT was noted in the G group ($p < 0.001$). Additionally, the G group presented the highest mean values for ΔE_{00}, as well as for the difference in lightness (ΔL*), the red/green axis (Δa*) and the yellow/blue axis (Δb*). No statistically significant differences were observed among the groups for Δgloss ($p = 0.646$).

Conclusions. The polishing process increased RT and resulted in the lowest ΔE_{00} values after toothbrushing. In contrast, the glazing procedure increased SR and decreased RT, while also promoting the most prominent variation in color parameters after toothbrushing. Toothbrushing with a conventional dentifrice did not influence SR and gloss; however, it led to clinically observable color variations and affected RT of the multilayer 5Y-TZP polycrystals.

Keywords: toothbrushing, color, dental polishing, dental aesthetics, yttria-stabilized tetragonal zirconia

Highlights

- Finishing procedures and toothbrushing significantly influence the properties of multilayer zirconia stabilized with 5 mol% of yttrium oxide (5Y-TZP).
- Polishing improved translucency, maintained surface smoothness, and showed minimal color variation after brushing.
- Glazing increased roughness, reduced translucency, and resulted in the greatest color change after brushing.
- Although brushing did not alter surface roughness or gloss, it caused perceptible changes in color and translucency.
- Color variations in all groups exceeded clinical acceptability limits, emphasizing the importance of selecting appropriate finishing procedures to ensure the long-term esthetic stability of zirconia restorations.

Introduction

Dental zirconia has been increasingly used as a prosthetic restorative material in computer-aided design (CAD) and computer-aided manufacturing (CAD/CAM). The material is widely utilized for tooth- and implant-supported restorations,[1] and can be implemented in 2 different forms: porcelain-veneered zirconia; and monolithic zirconia.[2–4] Failures such as chipping and porcelain fracture have been reported in porcelain-veneered zirconia restorations.[2–5] Monolithic zirconia restorations, which are anatomically contoured restorations fabricated by CAD/CAM, have been proposed to prevent veneer chipping or porcelain fracture, and provide excellent strength with minimal tooth reduction.[3,6–8] The quality of zirconia-based restorations also depends on the bonding effectiveness of the zirconia surface and the adhesive system. Some authors have reported that bonding effectiveness can be enhanced through the use of single-component universal adhesive[9] and laser.[10]

Zirconia has been marketed with different yttria content. Zirconia partially stabilized with 3 mol% of yttrium oxide (3Y-TZP) exhibits high opacity and is intended for manufacturing frameworks for porcelain-veneered zirconia restorations. Zirconia partially stabilized with 4 mol% of yttrium oxide (4Y-TZP) presents an increase in the yttria content, and, in consequence, increased amount of cubic phase, grain size and translucency. Zirconia stabilized with 5 mol% of yttrium oxide (5Y-TZP) exhibits the highest yttria content, with a maximum of 53% of the cubic phase, which is isotropic. In addition, cubic crystals are larger than tetragonal crystals, which has been shown to reduce the number of grain boundaries that are the source of light scattering. This decrease leads to enhanced translucency in anterior monolithic zirconia restorations.[3,4] In addition, the color of the prosthetic restoration is an important aspect that significantly affects the success of the treatment.[11] Monolithic zirconia exhibits a whitish shade; however, matching the aesthetics of natural teeth remains a challenge.[12–14] To address this need, a novel multilayer monolithic zirconia has been developed.

Multilayer zirconia can have a polychromic or hybrid composition. Polychromic multilayer zirconia presents a color gradient by adding different pigments in the cervical and incisal layers of the 5Y-TZP block, while hybrid multilayer zirconia comprises different generations of zirconia in the dentine (3Y-TZP or 4Y-TZP) and enamel (5Y-TZP) layers of the hybrid block.[8,15] A previous study investigated the microstructure, as well as the physical and mechanical properties of polychromic multilayer zirconia and concluded that this material is suitable for fixed prosthetic restorations.[16] Monolithic restorations manufactured with the use of polychromic multilayer 5Y-TZP require finishing procedures to improve aesthetics,[17] ensure color stability,[18] reduce surface roughness (SR),[19] minimize biofilm formation,[20] and mitigate antagonist wear.[21] Although manufacturers recommend both polishing and glazing for monolithic zirconia restorations, there is no well-established method for finishing procedures, and their impact on color difference remains unclear.[17,22] It has been established that 4Y-TZP presents a less enduring glazing layer against toothbrushing compared to reinforced glass ceramics.[23]

Toothbrushing with a dentifrice is an important component of oral hygiene. However, this process can result in a superficial abrasion and cause color differences in restorative materials.[24–27] Color differences must be evaluated using perceptibility and acceptability thresholds as quality control tools to predict the clinical performance of these restorative materials.[28,29] A number of studies have investigated the effects of toothbrushing on the color difference and SR of 3Y-TZP[25–27,30,31] and 5Y-TZP.[27,32] However, the studies that evaluated 5Y-TZP used extrinsic staining, and there is no data concerning the effects of toothbrushing on multilayer 5Y-TZP.

Therefore, the purpose of the present study was to evaluate the effect of finishing procedures on SR and relative translucency (RT), as well as the influence of toothbrushing on SR, RT, color difference (ΔE_{00}), and gloss (Δgloss) of multilayer 5Y-TZP following polishing or glazing. The research hypotheses were as follows: (1) the finishing procedures (polishing or glazing) would have an effect on SR and RT of multilayer 5Y-TZP; and (2) toothbrushing would have an impact on SR, RT, ΔE_{00}, and Δgloss of polished and glazed multilayer 5Y-TZP.

Material and methods

Thirty specimens were fabricated from the cervical layer of 5Y-TZP (ceramill® zolid fx multilayer, LOT 1708000; Amann Girrbach AG, Koblach, Austria) (Table 1). Pre-sintered blocks of 5Y-TZP were cut with a diamond disk (Diamond Wafering Blade; Allied High Tech Products, Inc., Cerritos, USA) in a high precision cutter (IsoMet 1000 Precision Cutter; Buehler, Lake Bluff, USA) under water cooling. The specimens were manually finished with 25-µm grit sandpaper (211Q; 3M ESPE, St. Paul, USA) (pre-sintered specimens' dimensions: 6.0 mm × 6.0 mm × 1.8 mm), and then sintered in a furnace (inFire HTC Speed; Dentsply Sirona, Charlotte, USA) with a maximum temperature of 1,450°C according to the manufacturer's instructions (post-sintered specimens' dimensions: 5.0 mm × 5.0 mm × 1.5 mm).

The sample size was calculated based on the results of a previous study.[27] Specimens were divided into 3 groups (n = 10/group) according to the finishing procedure used: control (C); polishing (P); and glazing (G). The finishing procedures were carried out by a single trained operator (LF). Polishing was performed using a medium (W16DC Diacera, LOT 447317; EVE Ernst Vetter GmbH, Keltern, Germany) and fine (W16DCmf Diacera, LOT 446560; EVE Ernst Vetter GmbH) diamond polisher in a slow-speed dental handpiece (Micromotor; Dabi Atlante, Ribeirão Preto, Brazil) operating at 10,000 rpm for 30 s.[19,33,34] The diamond polisher was positioned on the device for standardization[35–37] and replaced after polishing 5 specimens.[31,36] Glazing was performed by applying a single layer of glaze paste (InSync® Glaze Paste, LOT 172108; Jensen Dental, North Haven, USA) with a brush, followed by firing in a furnace (sinter press alumini; EDG, São Carlos, Brazil) according to the manufacturer's instructions. The thickness of the specimen was measured with a digital caliper (Absolute Digital Pachymeter; Mitutoyo Corporation, Kawasaki, Japan) before and after glazing to standardize the thickness of the glaze layer (100 µm).[27,36]

A confocal laser scanning microscope (CLSM) (LEXT OLS4000; Olympus Corporation, Tokyo, Japan) was used to evaluate the topography and SR before and after 50,000 toothbrushing cycles. A representative image of the surface topography was selected based on the repetitive pattern identified for each group. The surface roughness values (µm) were obtained by analyzing the entire scanned surface using the software dedicated for the CLSM (LEXT OLS4000; Olympus Corporation).

The color difference, RT and gloss were analyzed using a calibrated spectrophotometer (Delta Vista 2.0; Delta Color, São Leopoldo, Brazil) at baseline and after 50,000 toothbrushing cycles. The International Commission on Illumination (CIE) L*, a* and b* color coordinates of the specimens were evaluated. The CIEDE2000 color difference (ΔE_{00}) was calculated based on the previously described formula.[38,39] The relative translucency was obtained by computing the lightness (L*), red/green axis (a*) and yellow/blue axis (b*) values against the white (W) and black (B) backgrounds by using the following formula (Equation 1):

$$RT = \sqrt{(L_B - L_W)^2 + (a_B - a_W)^2 + (b_B - b_W)^2} \quad (1)$$

The difference in gloss was calculated using the CIEDE2000 color system based on the following formula (Equation 2):

$$\Delta gloss = [(gloss2^*) - (gloss1^*)] \quad (2)$$

where:
gloss1* – baseline gloss value;
gloss2* – gloss value measured after toothbrushing.

The specimens were brushed using a linear brushing machine (P200 brushing machine; Biopdi, São Carlos, Brazil) equipped with soft bristle toothbrush heads (J&J REACH Eco; Johnson & Johnson, New Brunswick, USA) using a conventional dentifrice (Colgate Maximum Caries Protection; Colgate-Palmolive, New York, USA) slurry (Table 1). Dentifrice slurries were prepared by mixing distilled water (mL) with a dentifrice (g)[25,27,30] at a proportion of 2:1 in a vacuum mixer for 2 min. The machine was set at a rate of 180 toothbrushing cycles per minute,[25] with a vertical load of 2.5 N[23,25,32] until 50,000 toothbrushing cycles were completed,[27,32] which simulated 5 years of toothbrushing.[23,27]

Statistical analysis

All statistical analyses were performed using the IBM SPSS Statistics for Windows software, v. 20.0 (IBM Corp., Armonk, USA). The Shapiro–Wilk test evaluated the data

Table 1. Characteristics of the materials used in the study

Material	Composition	Manufacturer
ceramill® zolid fx multilayer (5Y-TZP)	$ZrO_2 + HfO_2 + Y_2O_3 \geq 99.0\%$ Y_2O_3: 8.5–9.5% $HfO_2 \leq 5\%$ $Al_2O_3 \leq 0.5\%$ other oxides ≤1%	Amann Girrbach AG, Koblach, Austria
Colgate Maximum Caries Protection	calcium carbonate, aqua, glycerin, sodium lauryl sulfate, sodium monofluorophosphate (1,450 ppm fluoride), cellulose gum, aroma, tetrasodium pyrophosphate, sodium bicarbonate, benzyl alcohol, sodium saccharin, sodium hydroxide	Colgate-Palmolive, New York, USA

5Y-TZP – zirconia stabilized with 5 mol% of yttrium oxide.

for normality. Given that the data presented normal distribution, the results of SR and RT were analyzed using repeated measures analysis of variance (ANOVA) and Bonferroni post hoc test ($\alpha = 0.05$). The data was compared between groups (baseline values) and within groups (before and after toothbrushing) on the same specimens. The results for ΔE_{00} and Δgloss were analyzed using the one-way ANOVA and Tukey's post hoc test ($\alpha = 0.05$).

Results

The finishing procedures had an influence on SR and RT. The recorded baseline mean values indicate that polishing did not influence SR and increased RT, while glazing resulted in an increase in SR and a decrease in RT of multilayer 5Y-TZP (Table 2). The surface roughness of the C and P groups was comparable ($p < 0.05$). The glazing group presented higher SR compared to the C and P groups ($p < 0.001$ for both). With respect to RT, the C group exhibited lower mean values than the P group ($p < 0.05$) and higher results in comparison to the G group ($p < 0.001$).

Figure 1 displays representative images of surface topography obtained by means of a laser confocal microscope. At baseline, the G group demonstrated the most irregular surface, while the P group exhibited the most regular one. Toothbrushing had no discernible effect on the surfaces of the C and G groups. However, a reduction in scratches was observed on the surface of the P group.

The impact of toothbrushing on SR was not significant ($p = 0.052$). However, it resulted in a decrease in RT of the P group ($p < 0.05$) and an increase in RT of the G group ($p < 0.001$) (Table 2).

The mean (*M*) and standard deviation (*SD*) values of the ΔE_{00}, ΔL*, Δa*, Δb*, and Δgloss for all groups are presented in Table 3. The G group showed the highest mean ΔE_{00} value. The positive values of ΔL* indicate that toothbrushing increased lightness for all groups. The Δa* and Δb* presented negative values for the C and P groups, and positive values for the G group.

Statistically significant differences were noted among the groups for ΔE_{00}, ΔL*, Δa*, and Δb* values ($p < 0.05$), and there was no difference for the Δgloss parameter ($p = 0.646$). The G group presented the highest mean values of ΔE_{00}, ΔL*, Δa*, and Δb*. The C and P groups exhibited similar mean values of ΔE_{00} ($p = 0.064$), Δa* ($p = 0.337$) and Δb* ($p = 0.344$). Intermediate mean values of ΔL* were noted in the C group, while the ΔL* values in the P group were the lowest (Table 4).

Table 2. Comparison of surface roughness (SR) and relative translucency (RT) in the study groups at baseline and after toothbrushing

Variable	Group	Baseline	After toothbrushing
Surface roughness [μm]	C	1.55 ±0.09^{aA}	1.50 ±0.08^{A}
	P	1.22 ±1.74^{aA}	1.29 ±0.46^{A}
	G	9.77 ±0.41^{bA}	9.61 ±1.38^{A}
Relative translucency	C	3.94 ±0.61^{aA}	3.92 ±0.78^{A}
	P	4.65 ±0.58^{bA}	3.45 ±0.90^{B}
	G	2.37 ±0.95^{cA}	4.59 ±0.87^{B}

C – control group; P – polishing group; G – glazing group. Data presented as mean ± standard deviation (*M* ±*SD*). Different lowercase letters indicate statistical differences between the groups based on the finishing procedures (vertically) ($p < 0.05$). Different uppercase letters denote statistical differences between the baseline and post-brushing values for a specific group (horizontally) ($p < 0.05$).

Table 3. Comparison of differences in color, lightness and gloss of specimens in the study groups

Variable	Group	*M*	*SD*	*Me*	*CI*	Lower limit	Upper limit
ΔE_{00}	C	4.41	0.68	4.4680	3.92; 4.89	2.62	5.02
	G	6.19	1.21	6.1285	5.33; 7.06	3.79	7.71
	P	3.48	0.63	3.4296	3.03; 3.92	2.73	4.68
ΔL*	C	5.02	0.76	5.1650	4.48; 5.56	3.16	5.92
	G	6.65	1.22	6.4650	5.77; 7.52	4.48	8.78
	P	3.43	1.02	4.3600	2.69; 4.16	1.85	4.84
Δa*	C	−0.50	0.50	−0.4900	−0.86; −0.15	−1.46	0.20
	G	1.18	0.71	1.2250	0.68; 1.69	−0.41	2.10
	P	−0.87	0.48	−0.7950	−1.21; −0.52	−1.67	0.24
Δb*	C	−1.19	0.76	−1.1650	−1.73; −0.65	−2.38	0.16
	G	2.25	0.89	2.4700	1.61; 2.89	0.19	3.38
	P	−0.69	0.74	−0.6300	−1.22; −0.15	−1.87	0.27
Δgloss	C	20.03	2.83	20.4800	18.01; 22.06	15.97	25.30
	G	20.97	3.16	20.0700	18.70; 23.23	17.74	28.85
	P	23.77	15.43	26.4150	12.74; 34.81	0.19	44.19

ΔE_{00} – color difference; ΔL* – difference in lightness; Δa* – difference in the red/green axis; Δb* – difference in the yellow/blue axis; *M* – mean; *SD* – standard deviation; *Me* – median; *CI* – confidence interval.

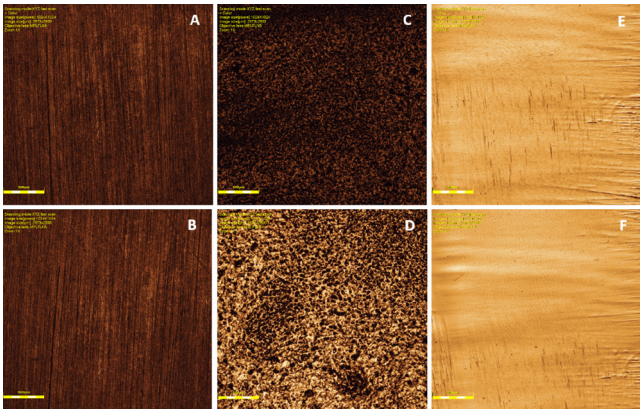

Fig. 1. Representative micrographs illustrating the topography of all groups (×10^7 magnification)

A. Control group at baseline; B. Control group after toothbrushing; C. Glazing group at baseline; D. Glazing group after toothbrushing; E. Polishing group at baseline; F. Polishing group after toothbrushing.

Table 4. Pairwise comparison of the study groups regarding the differences in color and lightness of specimens after toothbrushing

Variable	Comparison	Mean difference	Standard error	*p*-value	95% *CI*	
					lower limit	upper limit
ΔE_{00}	C × G	−1.78	0.39	<0.001*	−2.76	−0.81
	C × P	0.93	0.39	0.064	−0.04	1.90
	G × P	2.71	0.39	<0.001*	1.74	3.69
ΔL*	C × G	−1.62	0.45	0.004*	−2.76	−0.49
	C × P	1.59	0.45	0.005*	0.46	2.72
	G × P	3.22	0.45	<0.001*	2.09	4.35
Δa*	C × G	−1.68	0.25	<0.001*	−2.32	−1.05
	C × P	0.36	0.25	0.337	−0.26	1.00
	G × P	2.05	0.25	<0.001*	1.42	2.68
Δb*	C × G	−3.44	0.35	<0.001*	−4.33	−2.55
	C × P	−0.51	0.35	0.344	−1.39	0.37
	G × P	−2.93	0.35	<0.001*	−3.82	−2.04

* statistically significant ($p < 0.05$, Tukey's post hoc test).

Discussion

The first research hypothesis was accepted because different finishing procedures influenced the baseline SR and RT values of multilayer 5Y-TZP. Glazing resulted in the most irregular surface, high mean values of SR and the lowest RT, while polishing promoted a regular surface and the highest RT.

The second research hypothesis was partially accepted because toothbrushing influenced ΔE_{00} and RT but did not have an impact on SR and gloss of polished and glazed multilayer 5Y-TZP. In order to ensure the long-term clinical success of aesthetic restorations, it is essential that the restorative materials present color stability and reliable color matching with natural dentition. Color differences can be evaluated with a spectrophotometer and calculated using appropriate mathematical formulas, with values for clinically acceptable color differences being reported. A previous study suggested that the CIEDE2000 color system more accurately represents the human perception of color difference compared to the CIE L*a*b* color space,[28] which justifies the use of the CIEDE2000 in this study for the evaluation of visual tolerances. For the CIEDE2000, the 50:50% perceptibility threshold in dentistry was determined to be $\Delta E_{00} = 0.8$ units, whereas the 50:50% acceptability threshold was found to be $\Delta E_{00} = 1.8$ units.[28,29] In this study, the observed mean values for color differences after toothbrushing in all groups exceeded the acceptability thresholds. The mean values of ΔE_{00} for the P, C and G groups can be interpreted as moderately, clearly and extremely unacceptable, respectively.[28] The high values for ΔE_{00} can be attributed to the considerable increase in lightness, indicating that the multilayer 5Y-TZP became luminous after toothbrushing, regardless of the finishing procedure used. Similarly, some authors have reported that monolithic zirconia presented a significant color difference after toothbrushing, with this difference being more pronounced than that observed for glass ceramic.[25,27] Eldwakhly et al. investigated the color differences of dental ceramic specimens subjected to different staining solutions and found that glass ceramic presented the lowest color difference when compared to monolithic zirconia, resin nanoceramic and hybrid ceramic, while monolithic zirconia demonstrated the most substantial color variation.[14]

It has been reported that 5Y-TZP and 3Y-TZP exhibited different behaviors after toothbrushing, indicating that the chemical composition and crystallographic phase content may influence the color difference of monolithic zirconia.[27] In this study, the multilayer 5Y-TZP presented high mean values of color difference, suggesting that the percentage of cubic phase content may have influenced this outcome.

Regarding finishing procedures, Lee et al. investigated the effect of polishing and glazing on intrinsically colored 3Y-TZP that underwent toothbrushing and found that glazed 3Y-TZP presented lower values of color difference after toothbrushing than polished 3Y-TZP.[26] In contrast, this study observed that the polished multilayer 5Y-TZP demonstrated lower values of color difference than the glazed multilayer 5Y-TZP. These divergent results can be explained by the method used to color the monolithic zirconia. In the case of intrinsically colored 3Y-TZP, the glazing layer can play a protective role, promoting color maintenance.[26] Ataol et al. evaluated the effect of substructure thickness and finishing procedure (polishing or glazing) on the color difference before and after cementation in 3Y-TZP, and found a correlation between substructure thickness and color difference.[13] For 0.04-mm substructure thickness, the polished group presented higher mean values of color difference than the glazed group. However, for a substructure thickness of 0.08 mm, the glazed group demonstrated higher mean values than

the polished group. In the present study, specimens with the same thickness were used for all groups, and the glazed group presented the highest mean values of color difference. The thickness of the specimens (1.5 mm) is clinically representative because it is similar to the thickness of the occlusal surface of inlays, onlays and crowns recommended by the manufacturer.

There is no consensus regarding the correlation between color differences and SR. Some authors affirmed that color differences can be affected by SR,[19] while others argued that there was no correlation between the two.[25] In this study, the highest color difference was observed in the glazed group, suggesting that rougher surfaces are related to high mean values of color differences. In addition, the rough surface can lead to plaque accumulation, dental caries, gingival inflammation, and antagonist wear,[19,21] resulting in a decrease in the clinical aesthetic outcome of the prosthesis.[17] Sehovic et al. reported that toothbrushing with a conventional dentifrice can lead to an increase in SR of extrinsically stained 3Y-TZP.[31] Lee et al. found that extrinsically stained 5Y-TZP showed a decrease in SR after 50,000 toothbrushing cycles, while this study found no differences in SR at baseline and after toothbrushing for glazed and polished multilayer 5Y-TZP for the same number of toothbrushing cycles.[32] However, comparisons among these studies are limited because of the different protocols for finishing procedures and toothbrushing, such as the type of toothbrush, machines and load applied.[30]

Moreover, SR has been demonstrated to affect light reflection.[40] After toothbrushing, the polished and glazed groups exhibited different behaviors for RT, but no differences were found in SR. The relative translucency increased in the G group after toothbrushing, while a decrease was observed in the P group. These outcomes may be associated with alterations in the grain size of 5Y-TZP. The grain size had no influence on the SR of 3Y-TZP; however, it could have an influence on RT.[41] In addition, Lee et al. investigated the effects of the thickness of extrinsic stain and glazed layers on RT of 5Y-TZP using the CIEDE2000, and concluded that the thickness of extrinsic stain affected RT, but these phenomena were not observed in the glazed group.[42]

Heintze et al. evaluated the changes in gloss and SR after toothbrushing of restorative materials and found a strong correlation between surface gloss and roughness.[43] This study demonstrated that the use of finishing procedures (polishing or glazing) did not influence surface gloss and roughness after toothbrushing using a conventional dentifrice. In contrast, other studies have reported that toothbrushing can lead to a decrease in gloss, depending on the type of dentifrice used.[26,27]

The present study demonstrated that finishing procedures influenced SR and RT, and toothbrushing using a conventional dentifrice did not influence SR and gloss, but affected RT and color of polished and glazed multilayer 5Y-TZP. Considering that a finishing procedure should be chosen for monolithic zirconia, the findings of this study suggest that polishing results in a smoother surface and lower mean values of color difference compared to glazing.

Limitations

The limitations of this in vitro study included the fact that toothbrushing cannot simulate the dynamic oral environment, such as pH and masticatory forces. The toothbrushing environment lacked natural compounds found in saliva or chemical insults associated with the intake of meals and beverages. Additionally, dentifrices with different compositions were not investigated. Direct comparisons with other studies are precluded by the presence of different toothbrushing protocols. Our results were limited to polychromic multilayer 5Y-TZP. However, the observed outcomes are relevant because the surface of restorative materials is subjected to toothbrushing with a dentifrice, and the effects of this procedure need to be known.

Further research is necessary to investigate different types of dentifrice, different brands of polychromic multilayer 5Y-TZP and hybrid multilayer zirconia. Additionally, the effect of finishing procedures and toothbrushing on the microstructure and grain size of multilayer zirconia should be investigated. Research efforts should prioritize the development of more stable zirconia to avoid color and translucency variations, considering that these changes have an influence on the success and longevity of dental prosthesis treatment.

Conclusions

Based on the results and within the limitations of this in vitro study, it can be concluded that the application of glazing increased SR and decreased RT, while polishing did not influence SR but increased RT of multilayer 5Y-TZP. The use of a conventional dentifrice during toothbrushing did not influence SR and gloss; however, it led to clinically observable color differences and affected RT of the tested material.

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.

Use of AI and AI-assisted technologies

Not applicable.

ORCID iDs

Lívia Fiorin https://orcid.org/0000-0001-5109-4033
Stephanie Francoi Poole https://orcid.org/0000-0002-8810-3009
Alia Oka Al Houch https://orcid.org/0000-0001-8085-8784
Adriana Cláudia Lapria Faria https://orcid.org/0000-0001-6353-1480
Ricardo Faria Ribeiro https://orcid.org/0000-0003-4211-0542
Renata Cristina Silveira Rodrigues https://orcid.org/0000-0003-4140-4143

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Original paper

Potential inhibition of *Porphyromonas gingivalis* Lys-gingipain by 4-caffeoylquinic acid of *Moringa oleifera* extract: In silico docking and dynamic simulation

Lekha Alanija Devarajan[1,B,D,E], Shanmugapriya Ramamurthy[1,A,D,F], Pradeep Yadalam[2,A], Arunmozhi Ulaganathan[1,E], Raaja Sreepathy Chandran Selvaraj[1,B,C], Reshma Rajendran[1,C], Meenakshi Adhappan[1,E]

[1] Department of Periodontics, Sri Venkateswara Dental College and Hospital, Chennai, India
[2] Department of Periodontics, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), 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

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Address for correspondence
Shanmugapriya Ramamurthy
E-mail: drshanpriya@gmail.com

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Abstract

Background. Periodontitis is an inflammatory disease of the oral cavity that affects the soft and hard tissues of the periodontium due to dysbiosis by *Porphyromonas gingivalis*. The bacterium establishes its pathogenicity through its virulence factors, such as fimbriae, and by the releasing proteases like gingipains. The lysine-specific gingipain K (Kgp) is characterized by the presence of a hemagglutinin (HA)–adhesin domain, which provides the micronutrients for the survival of the microbe. 4-Caffeoylquinic acid (4-CQA) is classified as a phenylpropanoid. It exhibits a variety of bioactivities, including anti-inflammatory, antimicrobial, antihistaminic, and antioxidant properties. *Moringa oleifera* has multiple therapeutic benefits and is used in the treatment of cancer, infections, diabetes, and arthritis. 4-Caffeoylquinic acid was identified among the phenolic phytocomponents present in *M. oleifera*.

Objectives. The aim of the present study was to use in silico docking and a dynamic model to evaluate the potential inhibition of Lys-gingipain of *P. gingivalis* by 4-CQA of *M. oleifera*.

Material and methods. Molecular docking and dynamic simulations of the Lys-gingipain protein and 4-CQA ligands were performed using the Desmond software. The protein structure of Lys-gingipain was downloaded from the Protein Data Bank (PDB) and preprocessed using the optimized potentials for liquid simulations (OPLS 2005) force field.

Results. During the course of the dynamic simulation, the trajectories were saved for the analysis every 100 ns. The stability of the complex was confirmed by a root mean square deviation (RMSD) plot. In the context of molecular docking, the protein (Lys-gingipain) and the ligand (4-CQA) were found to have a potential binding site with the use of hydrogen bonds. The compound had a docking score of −6.6 kcal/mol. According to the results of the dynamic study, as depicted in the RMSD plot, the compound demonstrated stability within the range of 1.0–3.0 Å.

Conclusions. The inhibition of Lys-gingipain by 4-CQA is a promising avenue for further investigation, whether in vitro or in vivo.

Keywords: *Moringa oleifera*, chronic periodontitis, molecular docking, gingipains, *Porphyromonas gingivalis*

Highlights

- *Porphyromonas gingivalis*, a keystone pathogen in chronic periodontitis, expresses Lys-gingipain protease, which contributes to host tissue destruction and dysbiosis.
- 4-Caffeoylquinic acid (4-CQA), a phenolic compound derived from *Moringa oleifera*, demonstrated strong binding affinity (−6.6 kcal/mol) to Lys-gingipain via multiple hydrogen and π−π interactions in molecular docking.
- Molecular dynamics simulation confirmed the stability of the 4-CQA–Lys-gingipain complex up to 100 ns, supporting its inhibitory potential.
- 4-CQA may function as a natural gingipain inhibitor, offering a promising adjunctive or preventive approach for the management of periodontitis.
- Further in vitro and in vivo studies are needed to confirm the bioactivity and translational potential of *M. oleifera*-derived phytocompounds in periodontal therapy.

Introduction

Periodontitis is a common oral condition prevalent in individuals with poor oral hygiene and affecting both the hard and soft supporting structures of the teeth. Additional factors that could influence the course of the disease include various systemic conditions, stress, malocclusion, orthodontic therapy, ill-fitting dentures, and genetics.[1] Population-based studies have concluded that untreated periodontitis results in compromised quality of life due to functional, aesthetic and social disabilities.[2] Periodontitis is caused by dysbiosis of oral microbial flora colonizing the supra- and subgingival regions of the teeth.[3] Regarding the polymicrobial etiology, the complex red organisms are commonly associated with periodontitis, including *Porphyromonas gingivalis*, *Treponema denticola* and *Tannerella forsythia*.[3,4] Of *P. gingivalis*, a Gram-negative anaerobic rod exhibits a more significant effect on the oral microbiome and disrupts host–microbe hemostasis.[5] As *P. gingivalis* initiates the dysbiosis of oral flora, it is called the keystone pathogen. It establishes and propagates periodontal disease through a virulence factor called gingipain. Gingipains are a group of cysteine proteases that are found in the outer membrane or the vesicles of the bacteria.[6] They play a crucial part in maintaining the pathogenic functions of the bacterium in the host. Gingipains aid in colonization and adherence of the pathogen to epithelial cells, as well as cause the breakdown of erythrocytes, resulting in hemolysis. The generated heme provides an additional nutritional source for further multiplication and colonization of the bacteria. Subsequently, *P. gingivalis* modulates the host inflammatory response, leading to further progression and tissue destruction.[7]

Based on its amino acid composition, a gingipain can be classified as[8]:

- arginine-specific gingipain A (RgpA);
- arginine-specific gingipain B (RgpB);
- lysine-specific gingipain K (Kgp).

Lysine-specific gingipain K has a maximum of 3–5 hemagglutinin (HA)/adhesin domains in its genomic protein. In contrast, there are only 4 HA/adhesin domains in RgpA and no such domains in RgpB. As the number of domains increases, there is a concomitant increase in the effectiveness of host cell adhesion and heme acquisition.[9] The distribution of domains in Kgp is a critical virulent factor of *P. gingivalis*.[8] The analysis of the pathogens involved in the development of this pathology is a recurrent theme in the literature, which underscores its importance as a public health problem. Precisely for this purpose, the ability to identify targeted treatments against selected bacterial species is of current and future interest.[10]

Scientific evidence has suggested that since *P. gingivalis* is the keystone pathogen of periodontitis, treatment strategies directed towards it may prevent disharmony of the oral microbiome and inhibit disease progression. There are several treatment modalities for periodontitis, including non-surgical therapy involving scaling and root planning with or without antimicrobial therapy to decrease the microbial load, host modulation therapy, and surgical therapy for repairing or regenerating the lost periodontium.[11] Advanced treatment strategies that could inhibit gingipain functions may have an indirect biological effect on *P. gingivalis*. They could suppress the availability of micronutrients for the growth of the bacterium and make it vulnerable to the defensive actions of immune cells. Apart from chemotherapeutic agents, natural remedies such as tulsi, aloe vera, neem, propolis, tea tree oil,[12] and tropical fruits like mangosteen[13] have been studied for their antimicrobial effect in the non-surgical treatment of periodontitis. The extensive research on herbal remedies is attributable to their anti-inflammatory, antioxidant and antimicrobial properties, along with a reduced incidence of side effects.[14]

Moringa oleifera, also known as the miracle tree, is one of the most commonly cultivated trees in tropical and subtropical regions. It is a rich source of phytonutrients, vitamins, minerals, and essential amino acids. *Moringa oleifera* also provides a rare combination of zeatin, quercetin, sitosterol, caffeoylquinic acid, and kaempferol.[15] Various in vitro studies have concluded that many parts of *M. oleifera,* like leaves, pods, barks, nuts, flowers, and tubers, possess

significant health benefits.[16] 4-Caffeoylquinic acid (4-CQA) is a phenylpropanoid of *M. oleifera*, the bioactivities of which include antioxidant, antibacterial, antidiabetic, anticancer, and antihistaminic effects.[17] The antibacterial activity of phenolic compounds has been attributed to the loss of cellular integrity, leading to increased membrane permeability, subsequent disturbance of the cellular membrane, and ultimately, cell death.[18]

Molecular docking is a computer-assisted tool that is used to identify the complete binding site between the target protein and the drug in a three-dimensional assembly.[19] This identification is a preliminary step in drug design that is performed before conducting in vitro and in vivo research. Hence, the present study aimed to assess the potential inhibition of *P. gingivalis* Lys-gingipain by 4-CQA of *M. oleifera* by means of in silico docking and dynamic simulations.

Material and methods

Molecular docking and dynamic simulations of the Lys-gingipain protein and 4-CQA ligands[20] were performed using the Desmond software (Schrödinger, New York, USA).

The 3D protein structure of Lys-gingipain was downloaded from the Protein Data Bank (PDB) database (https://www.rcsb.org/structure/3M1H). The protein structure was processed using the Maestro platform (https://www.schrodinger.com/platform/products/maestro; Schrödinger). The Protein Preparation Wizard (Maestro; Schrödinger) was used to preprocess the receptor–ligand complex. In general, water molecules present in the protein can be easily displaced by the ligand or be a hindrance to the binding pocket. Therefore, the preprocessing steps in the protein preparation removed all heteroatoms and loosely bound water molecules by the addition of hydrogen ions. The selected protein structure was then examined for gaps and built further to fill the loops. After optimization, it was minimized using the optimized potentials for liquid simulations (OPLS 2005) force field. Conformers for each compound were obtained using force field estimates by the OPLS 2005 between atoms within and between the molecules.

The ligand was prepared after downloading the chemical structure of 4-CQA from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/#query=9798666). Then, the ligand was subjected to energy minimization using the OPLS 2005 force field to achieve correct bond length, order and angle with minimal energy. This grid-based ligand docking method was used to evaluate the interaction between the protein and the ligand. A grid box was utilized to describe the protein's binding site with the following dimensions: center X – 9.599; center Y – 3.6929; center Z – 29.3808; size of X – 50.2525510788; size of Y – 33.8531600094; and size of Z – 34.8833085537.

Molecular dynamic simulations

The System Builder tool was used in the preparation of each system. The tool is designed to simulate the protein and the ligand. The default solvent water model TIP3P (3 points of transferable intermolecular interaction potential) with an orthorhombic box was selected to perform the dynamic simulation. Since there is a water-mediated interaction between the protein and the ligand in this TIP3 model, water must be included during the docking calculation. The OPLS 2005 force field was utilized in the simulation to generate the essential topology records.

The addition of counterions served to neutralize the models. To simulate the physiological condition, 0.15 M of sodium chloride (NaCl) was added. The isothermal–isobaric (NPT) ensemble was used, maintaining a temperature of 300 K and 1-Atm pressure throughout the simulation. The models were loosened before the simulation. Every 100 ns, the trajectories were saved for the analysis. The stability of the simulation was confirmed by contrasting the root mean square deviation (RMSD) of the protein and ligand over time.

Results

The three-dimensional docking model of the ligand–protein complex and the protein–ligand complex of Lys-gingipain and 4-CQA are depicted in Fig. 1 and Fig. 2, respectively.

The interaction between the binding site residues of Lys-gingipain protein and the 4-CQA ligand are depicted two-dimensionally in Fig. 3 and three-dimensionally in Fig. 4.

The docking scores or binding affinities provide an estimation of the strength of the interaction between the ligand and the receptor. Lower scores or more negative binding energies generally indicate stronger binding.

Fig. 1. Three-dimensional docking model of the protein–ligand interaction of Lys-gingipain and 4-caffeoylquinic acid (4-CQA)

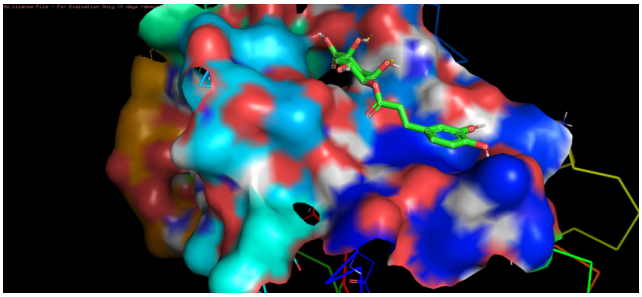

Fig. 2. Three-dimensional docking model of the ligand–protein interaction of Lys-gingipain and 4-CQA

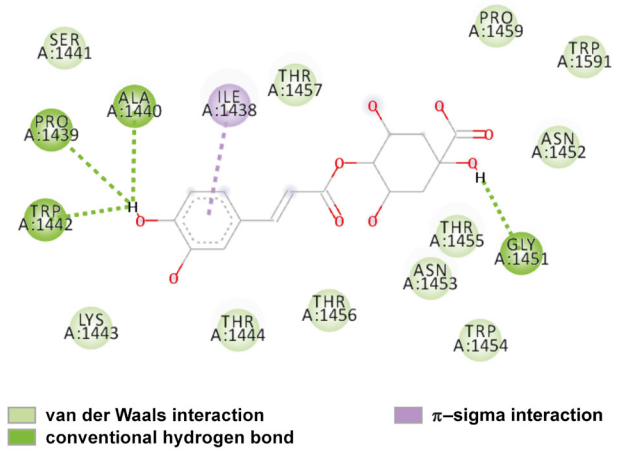

Fig. 3. Two-dimensional model of interactions between the binding site residues of the protein and the ligand

The dotted green and purple lines represent hydrogen bonding and π–π interactions, respectively. SER – serine; ALA – alanine; ILE – isoleucine; PRO – proline; THR – threonine; ASN – asparagine; GLY – glysine; TRP – tryptophan; LYS – lysine.

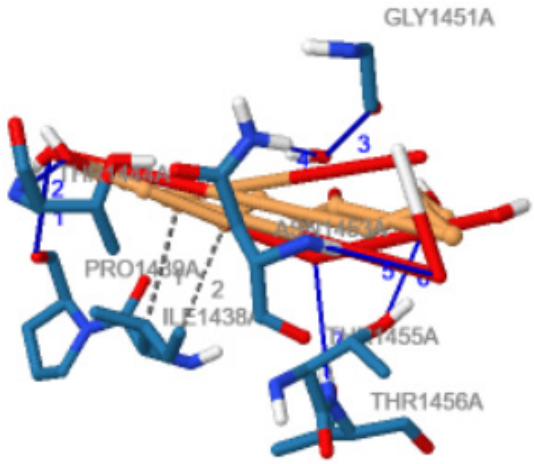

Fig. 4. Three-dimensional model of interactions between the binding site residues of the protein and the ligand

The grey lines represent unknown interactions between the protein and the ligand, which may also play a major role in their binding.

However, it is essential to validate these outcomes with experimental data or alternative methods to assess their reliability. Docking studies are a method of predicting the binding mode of a ligand within a receptor's active site. An examination of the specific interactions, such as hydrogen bonding, hydrophobic contacts and electrostatic interactions, can help understand the key molecular interactions responsible for ligand binding. In the present study, the compound demonstrated a docking score of –6.6 kcal/mol, therefore substantiating strong binding between the ligand and the protein.

These results indicate that the 4-CQA ligand demonstrates the most favorable affinity for binding with the Lys-gingipain protein by forming strong hydrogen bond interactions with amino acids such as ALA 1440, PRO 1439, TRP 1442, and GLY 1451. The compound also forms a π–π interaction with ILE 1438.

Figure 5 presents the RMSD of the ligand molecule docked on the protein. According to the complex protein–ligand RMSD plot, the complex reached stability at 60 ns. Following this, the RMSD values for the protein remained within the 1.0–2.0 Å range throughout the simulation, while the ligand RMSD fluctuated within the 1.0–3.0 Å range. The RMSD figure demonstrated that the proteins within the complex reached stability at 60 ns. Following that, fluctuations in the RMSD values for the protein remained within the 1.0–2.0 Å range throughout the simulation. In contrast, the ligand RMSD values varied more widely, within the 8.0–36.0 Å range up to 100 ns, indicating higher conformational flexibility of the ligand within the binding pocket.

Table 1 presents the hydrophobic interactions between the amino acid residues of the protein and the ligand. Table 2 shows the hydrogen bonds formed between the amino acid residues of the protein and the ligand. These parameters include the interaction distances and the donor angles that characterize the bonding between the protein and the ligand.

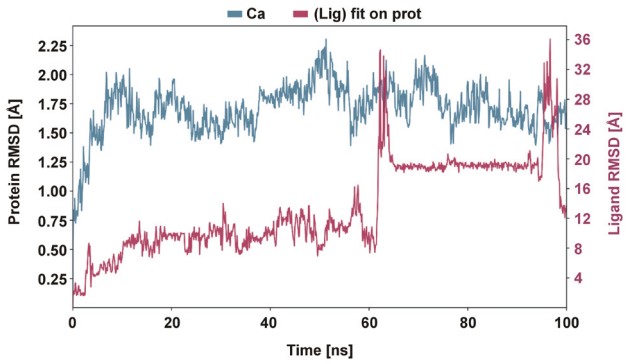

Fig. 5. Root mean square deviation (RMSD) plot of the protein and the ligand

Ca – calcium ion; (Lig) fit on prot – ligand fitted onto the protein. The Ca serves as a cofactor in the protein's active site.

Table 1. Hydrophobic interactions between the amino acid residues of the protein and the ligand

Index	Residue	Amino acid	Distance [pm]	Ligand atom	Protein atom
1	1438A	ILE	3.77	1608	108
2	1438A	ILE	3.58	1607	111

ILE – isoleucine.

Table 2. Hydrogen bonding between the amino acid residues of the protein and the ligand

Index	Residue	Amino acid	H–A distance [pm]	D–A distance [pm]	Donor angle	Protein donor	Side chain	Donor atom	Acceptor atom
1	1439A	PRO	2.88	3.62	134.07	no	no	1618	1161 (O_2)
2	1444A	THR	2.10	2.92	137.58	yes	no	163	1617 (O_2)
3	1453A	ASN	2.31	3.16	142.28	yes	yes	241	1620 (O_3)
4	1453A	ASN	2.89	3.80	151.84	yes	no	235	1622 (O_3)
5	1455A	THR	3.05	3.75	127.62	yes	yes	268	1619 (O_3)
6	1456A	THR	3.18	4.09	152.24	yes	no	271	1616 (O_3)

PRO – proline; THR – threonine; ASN – asparagine; H–A distance – distance between the hydrogen bond and the acceptor atom; D–A distance – distance between the donor and the acceptor atom.

Discussion

Periodontitis is caused by microbial pathogens present in biofilm complexes.[21] These pathogens have a strong affinity to enter the bloodstream and rupture atherosclerotic plaque, causing fatality like stroke.[22] Among the pathogens, *P. gingivalis* in the red complex group significantly contributes to chronic periodontitis.[23] Though there are many periopathogens, *P. gingivalis* is the one that is commonly isolated from subgingival plaque samples.[24] It has various virulence factors like fimbriae, capsules, vesicles, and proteases in the outer membrane.[25–27] The virulence factors help colonize and multiply the bacteria by evading the host defense mechanism. Notably, heme is required for the growth of *P. gingivalis*, which, in turn, is acquired by the bacteria itself through the process of hemolysis of the host erythrocytes. The process of hemolysis is facilitated by the gingipain protease of *P. gingivalis*.[28] Gingipains are a group of cysteine proteinases that are commonly found in the outer membrane of bacteria. Apart from degrading the proteins for their nutrition, they also compromise the host immune response and cause destruction of the periodontal soft and hard tissues.[26] A periodontal treatment that targets the HA domain of gingipain has the potential to inhibit the colonization of *P. gingivalis* on the root surface.[29] Cysteine peptidases, such as Kgp, RgpA and RgpB, which account for 85% of the pathogen's extracellular proteolytic activity, are good candidates for inactivation. FA-70C1 is a strong *P. gingivalis* gingipain inhibitor derived from Streptomyces FA-70 culture supernatant.[30] Previous research has reported the high-resolution (1.20) complex structure of Kgp with KYT-36, a peptide-derived, effective, bioavailable, and highly selective inhibitor.[31]

Phytocomponents are a rich source of protease inhibitors. In this regard, the extract of rice grain of *Oryza sativa* exhibited a significant gingipain inhibitory effect on Kgp and Rgps.[30] These rice proteins were denoted as 16 unassigned peptidase inhibitor homologues in the database.[30] Canavanine present in sword bean (*Canavalia gladiata*), when administered orally, decreased the alveolar bone loss in *P. gingivalis*-induced periodontitis in rats.[32] Polyphenols present in cranberry significantly reduced biofilm formation by *P. gingivalis* and *Fusobacterium nucleatum*. Catechin, a polyphenol present in green tea, reduced the inflammatory reaction through its inhibitory effect on Rgp gingipain.[33] *Moringa oleifera* contains phytocompounds, including flavonoids and phenolic acids. Phenolic acids are secondary plant metabolites that contain one or more hydroxyl groups connected to aromatic rings and act as scavengers.[34] Among the phenolic compounds present in *M. oleifera*, CQA is also isolated from extracts of *M. oleifera*.[35] Caffeoylquinic acid has shown antioxidant, antibacterial and anti-inflammatory properties.[36]

Caffeoylquinic acid is otherwise called chlorogenic acid (CA).[37] A study was conducted to assess the effect of coffee on periodontitis, given the presence of caffeine and CA in the beverage.[38] The study concluded that CA demonstrated significant anti-inflammatory activity because it inhibited nuclear factor kappa B (NF-κB) and resulted in substantial radical oxygen scavenging. The anti-inflammatory effect of CQA is enhanced in the presence of other phytocomponents, such as flavonoids.[38]

In another study, the antibacterial effect of CA was tested against *P. gingivalis*. The results demonstrated that CQA exhibited substantial anti-proteinase activity and had a prolonged inhibitory effect on *P. gingivalis*.[39]

The effect of 4-CQA of *M. oleifera* on Lys-gingipain has not been elucidated. The current study focused on the impact of CQA in *M. oleifera* on proteinase Lys-gingipain of *P. gingivalis* by in silico docking and dynamic simulation study. Based on the present findings, 4-CQA, a phenolic extract of *M. oleifera*, has been demonstrated to be a potent inhibitor of Lys-gingipain from *P. gingivalis.* The protein and the ligand exhibited a strong binding affinity for each other, as well as for amino acid residues like ALA 1440, PRO 1439, TRP 1442, and GLY 1451 by forming a hydrogen bond with the hydroxyl groups of the aromatic rings. The compound exhibited a docking score of –6.6 kcal/mol. According to the results of the RMSD plot, the compound demonstrated stability at 60 ns within the 1.0–3.0 Å range.

In subsequent in vivo studies, *M. oleifera* extracts can be applied topically to the periodontal pockets in the form of fibers, thermo-reversible gels, chips, or nanoparticle mouthwashes[40] to test their efficacy against periodontal pathogens.

Limitations

The present study was able to assess the interaction between the protein and the ligand in an in silico molecular docking model. However, further research is necessary to confirm the bioactivity between *P. gingivalis* and 4-CQA in an in vitro design. Additionally, given that the blind docking technique was employed in this stage, future studies may utilize active site docking to evaluate the specific protein binding efficiency.

Conclusions

In recent years, ethnomedicine has emerged as a novel approach to prevent multi-drug resistance while treating various diseases, including periodontitis. This study concludes that 4-CQA, a phenolic extract acquired from the leaves of *M. oleifera*, could be used as a potent, novel and natural inhibitor of Lys-gingipain to prevent the progression of periodontitis.

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.

Use of AI and AI-assisted technologies

Not applicable.

ORCID iDs

Lekha Alanija Devarajan https://orcid.org/0000-0002-4852-3156
Shanmugapriya Ramamurthy https://orcid.org/0000-0003-2121-4932
Pradeep Yadalam https://orcid.org/0000-0002-6653-4123
Arunmozhi Ulaganathan https://orcid.org/0000-0002-6061-6654
Raaja Sreepathy Chandran Selvaraj https://orcid.org/0000-0002-0145-857X
Reshma Rajendran https://orcid.org/0000-0001-6415-6918
Meenakshi Adhappan https://orcid.org/0009-0006-3118-0448

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Original paper

Collaborative role of calcitriol with buparlisib in the tongue squamous cell carcinoma cell line by modulating the *Casp3* and *Akt1* gene expression

Karma Nabeel Shamaa[A–D,F], Basma Abdelrahman Ahmed[A,C,E,F], Iman Mohamed Helmy[A,C,E,F]

Department of Oral Pathology, Faculty of Dentistry, Ain Shams University, Cairo, Egypt

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

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Address for correspondence
Karma Nabeel Shamaa
E-mail: karmah.shamaa.std@dent.asu.edu.eg

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Abstract

Background. Oral squamous cell carcinoma (OSCC) is a devastating disease with an increasing incidence. Among the commonly dysregulated pathways in oncogenesis are the phosphatidylinositol 3-kinase / protein kinase-B/ mammalian target of rapamycin (PI3K/AKT/mTOR) and apoptotic pathways. Buparlisib, a pan-class I PI3K inhibitor, has antineoplastic effects, but its associated toxicities hinder its beneficial role in patients. Calcitriol, active vitamin D (Vit D), possesses anticancer functions by targeting both pathways. Therefore, Vit D could help achieve low buparlisib doses and boost its effects.

Objectives. The present study aimed to determine the effects of buparlisib and Vit D, separately and in co-administration, on cell viability, as well as the apoptotic and PI3K pathways in the human tongue squamous cell carcinoma (TSSC) HNO97 cell line.

Material and methods. The MTT assay was used to estimate the IC50 and the IC70 buparlisib doses, which were then co-administrated with 100 nmol and 1,000 nmol Vit D. The quantitative real-time polymerase chain reaction (qRT-PCR) analysis was performed to evaluate the altered caspase-3 (*Casp3*) and *Akt1* gene expressions after 48-hour treatment.

Results. The co-administration of either 100 nmol or 1,000 nmol Vit D lowered the IC50 and the IC70 buparlisib doses. The qRT-PCR showed that for *Casp3* expression, the 4 combination groups differed significantly from the IC50 and IC70 buparlisib doses. For *Akt1* expression, the IC70 co-administration dose of buparlisib with 100 nmol Vit D, the IC50 co-administration dose of buparlisib with 1,000 nmol Vit D and the IC70 co-administration dose of buparlisib with 1,000 nmol Vit D were significantly different from the IC50 buparlisib dose. The IC70 buparlisib dose showed no significant alteration from the 4 combination groups.

Conclusions. Vitamin D represents an efficient anticancer adjuvant that permits a novel therapeutic strategy for cancer patients.

Keywords: caspase-3, vitamin D, AKT1 protein, BKM120, oral tongue squamous cell carcinoma

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Highlights

- Co-administering vitamin D with buparlisib could enhance cancer therapy by promoting apoptosis and inhibiting the PI3K/AKT/mTOR pathway.
- The combination of vitamin D and buparlisib offers a synergistic effect, potentially improving therapeutic outcomes in cancer treatment.
- Vitamin D may lower the required dosage of buparlisib while maintaining its potent antineoplastic effects, improving patient safety and treatment efficacy.

Introduction

Oral squamous cell carcinoma (OSCC) comprises more than 90% of oral cancers, which persist as one of the leading causes of mortality worldwide. Regardless of improved remedies, the 5-year survival rate of head and neck squamous cell carcinoma (HNSCC) remains close to 50%.[1–4]

Neoplastic cells may dysregulate the apoptotic pathway by inducing caspase-3 (*Casp3*) aberrant gene expression. Therefore, anticancer medications should redirect cancer cells toward apoptosis to optimize their effects.[5] The phosphatidylinositol 3-kinase / protein kinase-B / mammalian target of rapamycin (PI3K/AKT/mTOR) pathway is frequently aberrated in human cancer. This critical pathway mediates various oncogenic events, such as cell growth, survival and proliferation.[6]

Buparlisib, a pan-PI3K inhibitor, impedes the phosphorylation of the 4 class I PI3K isomers with the subsequent inhibition of the PI3K/AKT/mTOR pathway. Buparlisib was applied in several clinical trials as a single agent or combined with other drugs. Nevertheless, several toxicities developed, hindering its use.[7,8] Calcitriol is the active form of the lipid-soluble hormone vitamin D (Vit D). Prior studies recommended assessing the impact of Vit D on carcinogenesis and cancer progression.[9,10] One of the mechanisms by which Vit D mediates its anticancer functions is modulating the PI3K/AKT/mTOR and apoptotic pathways.[11]

Accordingly, if Vit D could enhance the anticancer functions of buparlisib, it might be possible to seize lower effective doses of buparlisib. Achieving low buparlisib doses expands its therapeutic application in cancer patients with relatively fewer side effects. Therefore, it could pave the way to approve buparlisib as a safe anticancer agent. The importance of Vit D in head and neck cancer and the potential benefits of its combination therapies are not yet fully understood.[9,10] The purpose of this study was to assess the impact of buparlisib and Vit D separately, as well as their co-administration, on cell viability, and the apoptotic and PI3K pathways of the human tongue squamous cell carcinoma (TSCC) HNO97 cell line.

Material and methods

Cell culture

The human TSCC cell line (HNO97) was acquired from Cell Lines Service (CLS, Eppelheim, Germany). Buparlisib (BKM120) (ab273384) and calcitriol, Vit D receptor (VDR) agonist (ab141456), were obtained from Abcam (Cambridge, UK). The stock solutions for each agent were adjusted by dilution in dimethyl sulfoxide (DMSO) and kept at –20°C until further use. All cell culture reagents were purchased from Gibco (Thermo Fisher Scientific, Dreieich, Germany). They included Dulbecco's Modified Eagle's Medium (DMEM), fetal bovine serum (FBS), 1% penicillin G sodium (10.000 IU), streptomycin (10 mg), phosphate-buffered saline (PBS), and 0.25% trypsin-EDTA solution.

The cells were maintained in F-12K containing DMEM, supplemented by 1% penicillin/streptomycin in 10% FBS. The flask was incubated at 37°C in a 5% CO_2 humidified atmosphere. The medium was replenished 2–3 times per week. After reaching 70–80% confluence, trypsin was added, followed by a PBS wash to allow subculturing.

MTT assay and the determination of the IC50 and IC70 buparlisib doses

For determining the IC50 and IC70 buparlisib doses, the cells were inserted into 96-well plates, with approx. 1×10^4 cells in 200 µL of the medium per well. Serial dilutions of buparlisib were prepared, with or without Vit D (100 nmol or 1,000 nmol), to achieve final concentrations of 0.195, 0.39, 0.78, 1.56, 3.125, 6.25, 12.5, 25, 50, and 100 µg/mL, and were added to the cells.

The study design comprised several groups: a control group of untreated cells (Con); IC50 buparlisib (B1); IC70 buparlisib (B2); the optimum dose of Vit D (100 nmol) (D1); a higher Vit D dose of 1,000 nmol (D2); IC50 co-administration dose of buparlisib with 100 nmol Vit D (C1); IC70 co-administration dose of buparlisib with 100 nmol Vit D (C2); IC50 co-administration dose of buparlisib with 1,000 nmol Vit D (C3); and IC70 co-administration dose of buparlisib with 1,000 nmol Vit D (C4).

The MTT assay was conducted following 48 h treatment to assess cell viability, using the Vybrant® MTT cell proliferation assay kit (catalog No. M6494; Thermo Fisher Scientific). Each well received 20 µL of the 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution (1 mg/mL) (Invitrogen, Thermo Fisher Scientific) and incubated at 37°C for 4 h. The MTT was replaced by 100 µL of sodium dodecyl sulfate with hydrochloric acid to dissolve the precipitated crystals. The absorbance was estimated using a spectrophotometer at wavelength 570 nm (ELx 800; Bio-Tek Instruments Inc., Winooski, USA).

Microscopic imaging

The LC-6 USB3.0 colorful CMOS digital camera (5MP) (Labomed Inc., Los Angeles, USA) was used to photograph the plates after 48-hour treatment at ×20 original magnification.

Quantitative real-time polymerase chain reaction (qRT-PCR)

A 12-well plate containing 100 µL of cell suspension and 1 mL of the medium with 1% penicillin-streptomycin, an antimycotic agent and 1% FBS was used for the PCR analysis. The selected primers for this study were obtained from Qiagen (Hilden, Germany). The primers ID for *Casp3*, *Akt1* and *β-actin* were QT00023947, QT00085379 and QT00095431, respectively.

The RNeasy Mini Kit (catalog No. 74004; Qiagen) was utilized for extracting and purifying RNA from the treated and untreated cells. The procedures were carried out following the manufacturer's instructions. The isolated RNA was reverse-transcribed into cDNA, using the QuantiTect Reverse Transcription Kit (catalog No. 205310; Qiagen).

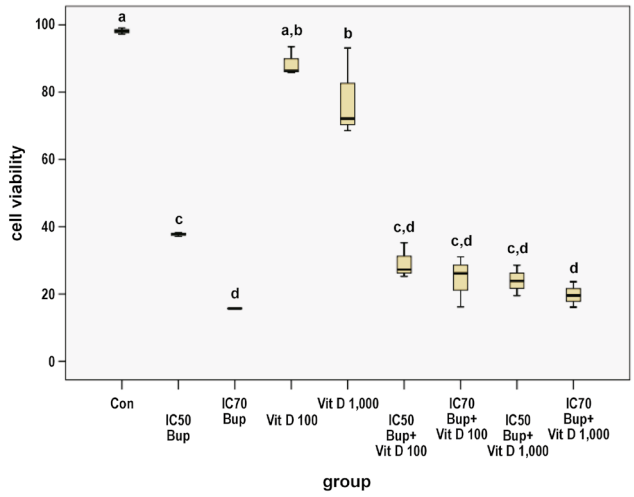

Fig. 1. Box plot demonstrating the alterations of the mean cell viability among different study groups after 48-hour treatment

Con – control group; Bup – buparlisib, Vit D – vitamin D. Different letters exhibit a statistically significant difference between the groups ($p < 0.05$).

The 2 genes of interest, *Casp3* and *Akt1*, along with the housekeeping gene, *β-actin*, were amplified from cDNA, using the QuantiTect SYBR Green PCR kit (catalog No. 204141; Qiagen) on a Rotor-Gene 5-plex PCR analyzer (Qiagen). The corresponding fold changes in the gene expressions were estimated using the $2^{-\Delta\Delta Ct}$ method.[12]

Statistical analysis

All experiments were performed in triplicate. Statistical analysis was conducted using the IBM SPSS Statistics for Windows, v. 22.0 (IBM Corp., Armonk, USA). The one-way analysis of variance (ANOVA) was applied, followed by post hoc Tukey's test, to detect the presence of a statistically significant difference among the study groups. The results were presented as mean and standard deviation ($M \pm SD$). The level of significance was set at $p < 0.05$.

Results

Determination of the IC50 and IC70 buparlisib doses, and the cytotoxic effects of different kinds of treatment

The IC50 dose of buparlisib (B1) was 19.13 µg/mL, and the IC70 dose (B2) was 44.80 µg/mL. After applying 100 nmol Vit D, the IC50 and IC70 doses of buparlisib were decreased respectively to 3.26 µg/mL (C1 – the IC50 co-administration dose of buparlisib with 100 nmol Vit D) and 7.46 µg/mL (C2 – the IC70 co-administration dose of buparlisib with 100 nmol Vit D). In the presence of 1,000 nmol Vit D in the medium, the IC50 and IC70 doses of buparlisib were further reduced to 1.84 µg/mL (C3 – the IC50 co-administration dose of buparlisib with 1,000 nmol Vit D) and 4.27 µg/mL (C4 – the IC70 co-administration dose of buparlisib with 1,000 nmol Vit D), respectively.

The 48-hour treatment of the HNO97 cells lowered the mean cell viability in all the treated groups in comparison with the control group (Con) (Fig. 1, Table 1). The viability of the control cells was significantly higher as compared to all treated groups except for the D1 group ($p = 0.571$). The 2 separate buparlisib doses (B1 and B2) showed a statistically significant difference between each other, with a p-value of 0.006. The cells treated with the IC50 buparlisib (B1) varied significantly from those from the D1 ($p = 0.000$), D2 ($p = 0.000$) and C4 ($p = 0.033$) groups. The B2 group showed a significant difference from Vit D doses D1 and D2 ($p = 0.000$). Regarding Vit D as a single agent, the D1 and D2 groups did not differ statistically ($p = 0.449$). However, the D1 and D2 groups differed statistically from the 4 combination groups, with a p-value of 0.000. Meanwhile, the 4 combination groups demonstrated a non-significant difference when compared to each other (Fig. 1, Table 1).

Table 1. Comparison of cell viability between all groups

Group	vs. group	*p*-value (post hoc Tukey's test)	*p*-value (ANOVA)
Con 98.15 ±0.95	B1	0.000*	0.000*
	B2	0.000*	
	D1	0.571	
	D2	0.013*	
	C1	0.000*	
	C2	0.000*	
	C3	0.000*	
	C4	0.000*	
B1 37.74 ±0.58	B2	0.006*	
	D1	0.000*	
	D2	0.000*	
	C1	0.708	
	C2	0.200	
	C3	0.169	
	C4	0.033*	
B2 15.69 ±0.04	D1	0.000*	
	D2	0.000*	
	C1	0.181	
	C2	0.673	
	C3	0.731	
	C4	0.993	
D1 88.54 ±4.31	D2	0.449	
	C1	0.000*	
	C2	0.000*	
	C3	0.000*	
	C4	0.000*	
D2 77.93 ±13.27	C1	0.000*	
	C2	0.000*	
	C3	0.000*	
	C4	0.000*	
C1 29.25 ±5.27	C2	0.982	
	C3	0.969	
	C4	0.587	
C2 24.47 ±7.59	C3	1.000	
	C4	0.984	
C3 23.99 ±4.54	C4	0.992	
C4 19.78 ±3.78	–	–	–

Data presented as mean ± standard deviation (*M* ±*SD*).
Groups: Con – control (untreated cells); B1 – IC50 buparlisib; B2 – IC70 buparlisib; D1 – 100 nmol Vit D; D2 – 1,000 nmol Vit D; C1 – IC50 buparlisib and 100 nmol Vit D; C2 – IC70 buparlisib and 100 nmol Vit D; C3 – IC50 buparlisib and 1,000 nmol Vit D; C4 – IC70 buparlisib and 1,000 nmol Vit D.
* statistically significant.

Microscopic changes

The decrease in the cell viability of the treated groups was consistent with the microscopic images, which displayed reduced cell confluence and morphological changes in response to the applied doses as compared to the control cells (Fig. 2). Accordingly, the cells became smaller and rounder, indicating apoptotic changes.

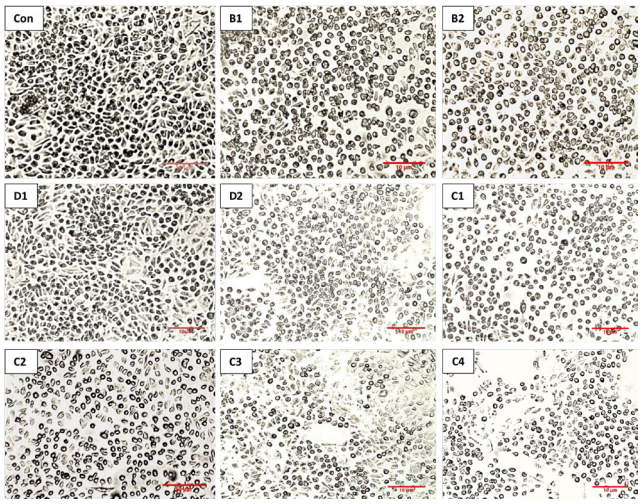

Fig. 2. Photomicrographs of the HNO97 cells in different study groups, showing a decrease in cell size and confluence after 48-hour treatment

Casp3 gene expression

The *Casp3* gene expression was positively regulated by treating the HNO97 cells for 48 h in the study groups relative to the control group, as shown in Table 2 and Fig. 3A. The gene expression of the control group was statistically different as compared to the B2 (p = 0.039), C1, C2, C3, and C4 groups (p = 0.000). The individual treatment with buparlisib of either dose did not differ statistically from each other or the 2 Vit D doses. However, the B1 group varied significantly from the 4 combination groups (p = 0.000), and the B2 group displayed a similar alteration relative to the C1 (p = 0.002), C2, C3, and C4 groups (p = 0.000).

The Vit D groups (D1 and D2) were significantly different from all the combination groups, with a p-value of 0.000, without displaying a statistical difference between each other. Although adding Vit D to buparlisib improved its up-regulatory effect on the *Casp3* gene expression with a significant difference relative to the other treated groups, the combination groups differed statistically from one another in a variable manner. A significant difference was observed when comparing the C1 group to the C3 (p = 0.001) and C4 groups (p = 0.000). The C2 group differed statistically from the C4 group only (p = 0.000). Meanwhile, the C4 group exhibited a significant upregulation with regard to the C3 group (p = 0.000).

Akt1 gene expression

After treating the cells, a down-regulatory effect on the *Akt1* gene expression was observed among different study groups in comparison with the control group (Fig. 3B).

Multiple comparisons between the study groups revealed variable statistically significant results, as displayed in Table 3. The control group varied significantly only from the B2 ($p = 0.001$), D2 ($p = 0.005$), C1 ($p = 0.001$), C2, C3, and C4 groups ($p = 0.000$). Even though the B1 group differed statistically from the C2 ($p = 0.040$), C3 ($p = 0.004$) and C4 ($p = 0.001$) groups only, the B2 group differed statistically from the D1 group ($p = 0.015$). The D1 and D2 groups displayed a non-considerable variation relative to each other. The former group was statistically different from C1 ($p = 0.016$), C2 ($p = 0.001$), C3, and C4 groups ($p = 0.000$). Contrastingly, the D2 group was statistically different from the C4 group only ($p = 0.013$). A non-significant difference was evident among the 4 combination groups.

Table 2. *Casp3* mRNA expression in the study groups after 48-hour treatment

Group	vs. group	*p*-value (post hoc Tukey's test)	*p*-value (ANOVA)
Con 1.015 ±0.223	B1	0.727	0.000*
	B2	0.039*	
	D1	1.000	
	D2	0.599	
	C1	0.000*	
	C2	0.000*	
	C3	0.000*	
	C4	0.000*	
B1 3.811 ±0.581	B2	0.613	
	D1	0.945	
	D2	1.000	
	C1	0.000*	
	C2	0.000*	
	C3	0.000*	
	C4	0.000*	
B2 6.921 ±0.414	D1	0.103	
	D2	0.741	
	C1	0.002*	
	C2	0.000*	
	C3	0.000*	
	C4	0.000*	
D1 1.851 ±0.255	D2	0.874	
	C1	0.000*	
	C2	0.000*	
	C3	0.000*	
	C4	0.000*	
D2 4.164 ±0.962	C1	0.000*	
	C2	0.000*	
	C3	0.000*	
	C4	0.000*	
C1 15.036 ±1.913	C2	0.212	
	C3	0.001*	
	C4	0.000*	
C2 19.437 ±1.057	C3	0.185	
	C4	0.000*	
C3 23.969 ±3.586	C4	0.000*	
C4 35.813 ±4.054	–	–	–

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

Table 3. *Akt1* mRNA expression in the study groups after 48-hour treatment

Group	vs. group	*p*-value (post hoc Tukey's test)	*p*-value (ANOVA)
Con 1.011 ±0.158	B1	0.088	0.000*
	B2	0.001*	
	D1	0.806	
	D2	0.005*	
	C1	0.001*	
	C2	0.000*	
	C3	0.000*	
	C4	0.000*	
B1 0.691 ±0.085	B2	0.307	
	D1	0.777	
	D2	0.883	
	C1	0.318	
	C2	0.040*	
	C3	0.004*	
	C4	0.001*	
B2 0.445 ±0.100	D1	0.015*	
	D2	0.972	
	C1	1.000	
	C2	0.959	
	C3	0.392	
	C4	0.104	
D1 0.854 ±0.121	D2	0.121	
	C1	0.016*	
	C2	0.001*	
	C3	0.000*	
	C4	0.000*	
D2 0.552 ±0.210	C1	0.975	
	C2	0.437	
	C3	0.065	
	C4	0.013*	
C1 0.447 ±0.157	C2	0.954	
	C3	0.380	
	C4	0.099	
C2 0.331 ±0.063	C3	0.957	
	C4	0.576	
C3 0.216 ±0.065	C4	0.994	
C4 0.134 ±0.016	–	–	–

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

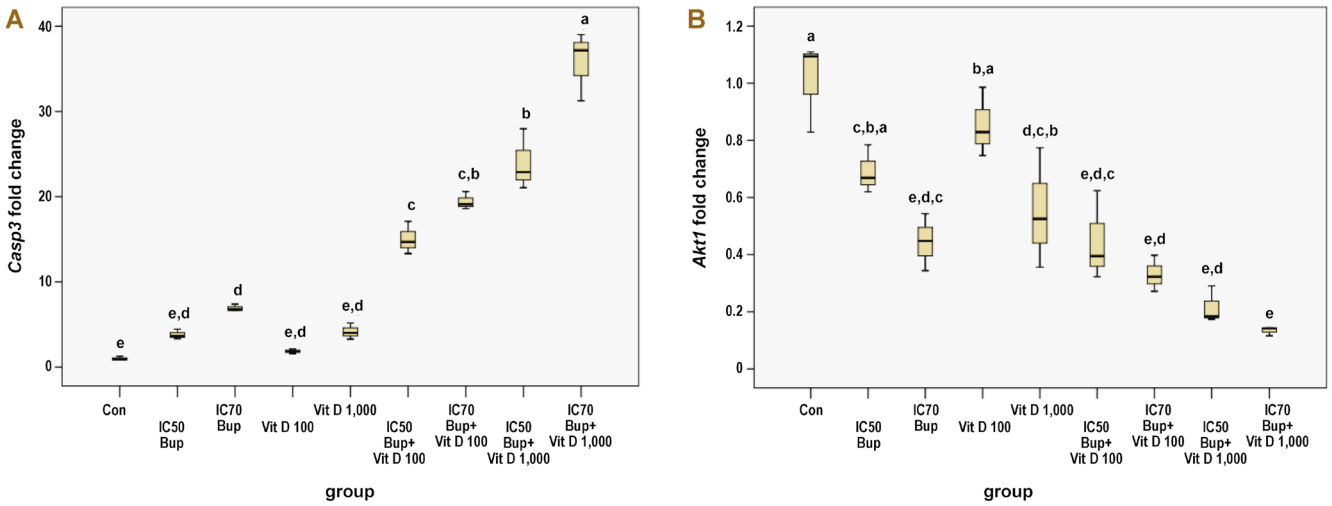

Fig. 3. Box plots of the PCR results, demonstrating the alterations of the mean gene expression among different study groups after 48-hour treatment
A – *Casp3* gene mean fold changes; B – *Akt1* gene mean fold changes. Different letters exhibit a statistically significant difference between the groups ($p < 0.05$).

Discussion

The associated toxicities of buparlisib were inevitable upon its use in different clinical trials.[7,8] Since Vit D is a possible option for ameliorating HNSCC,[11] the combined therapy of Vit D with buparlisib could provide a clue to reduce the associated buparlisib toxicities.

To the best of our knowledge, the influence of the single use of buparlisib or Vit D and their co-administration on the HNO97 cell line was not investigated in any prior study. We used the MTT viability assay to determine the effects of different kinds of treatment on the metabolic activity of the HNO97 cells. Since the ideal anticancer therapies include translational approaches that specifically eradicate cancer cells by amplifying or suppressing the relevant genes,[13] we used qRT-PCR to monitor the altered gene expression after 48-hour treatment.

Two doses of Vit D were utilized – the optimum dose of 100 nmol, as mentioned in the literature by Cataldi et al.,[14] and a higher dose of 1,000 nmol.[15] Our findings showed that the dual application of Vit D with buparlisib reduced the initial IC50 and IC70 buparlisib doses, suggesting the capability of Vit D to enhance the effect of buparlisib.

Treating the HNO97 cells for 48 h with buparlisib induced a significant, dose-dependent cytotoxic effect as compared to the control cells. The dose-dependent inhibitory effect of buparlisib was reported previously in different cell lines, such as OSCC, radioresistant OSCC and pancreatic ductal adenocarcinoma (PDAC) cell lines.[16,17] Applying the 2 Vit D doses on the HNO97 cells slightly reduced cell viability, and 1,000 nmol Vit D showed a bit more inhibitory effect than 100 nmol Vit D. In lung cancer cell lines, Vit D mediated a similar reduction, directly related to the elevated doses and durations.[18] On the other hand, other studies stated that Vit D lacked an inhibitory effect on cell viability.[19,20]

The 2 doses of buparlisib suppressed cell viability superior to the single doses of Vit D, consistently with the higher effect of an AKT inhibitor compared to 100 nmol Vit D in rat glioma cells.[21] The 4 combination groups decreased cell viability in the sequential order of the C1, C2, C3, and C4 groups, without any significant differences between the groups. Despite the least cell viability found in the B2 group, it did not vary prominently from any other combination group.

All study groups maintained an up-regulatory effect on the *Casp3* gene expression to a variable extent, indicating a pro-apoptotic response corresponding to 48-hour treatment. Some studies agree with our findings. Zhao et al. found that buparlisib generated elevated protein levels of CASP3 and poly(ADP-ribose) polymerase (PARP), and high numbers of apoptotic cells with the Caspase-3/7 reagent in fluorescent images.[22] Pereira et al. clarified that buparlisib promoted intrinsic and extrinsic apoptotic pathways by elevating the BAX, BCL2 and FAS levels, and activating procaspase-3, 8 and 9 in acute lymphoblastic leukemia (ALL) cell lines.[23] Contrarily, other studies denied the pro-apoptotic effect of buparlisib.[24,25] Oliveira et al. reported that buparlisib reduced cell viability by increasing the P27 levels and arresting the cell cycle at the G0–G1 phase.[25] In HNSCC cell lines, buparlisib failed to induce apoptosis separately, unlike its combination with erlotinib, which banned the translation of BCL2 through the eukaryotic translation initiation factor 4E-binding protein 1 (4E-BP1) axis, leading to apoptosis through the mTOR pathway.[24]

Vitamin D 1,000 nmol increased the CASP3 levels significantly more than 100 nmol. The increased *Casp3* gene expression caused by calcitriol was validated by the increase of the cleaved CASP3 and other pro-apoptotic proteins, beside lowering the level of the anti-apoptotic BCL2 protein in kidney cancer cells.[26] Vitamin D was also reported to increase the activity of CASP3 in non-small

cell lung cancer (NSCLC) cell lines[18] and melanoma cell lines.[27] Opposingly, Kucukhuseyin et al. showed that calcitriol, depending on the caspase pathway, mediates anti-apoptotic rather than pro-apoptotic effects.[21]

Despite the higher *Casp3* gene expression of B2 as compared to B1, D1 and D2, the groups did not vary significantly. Similar to our outcome, 24-hour Vit D treatment revealed a comparable apoptotic effect to AKT inhibitor XI,[27] and a similar apoptotic effect to PI3K, AKT and mTOR inhibitors individually.[18]

Vitamin D and buparlisib cooperatively accentuated their pro-apoptotic activity by increasing the *Casp3* gene expression among the 4 combination groups. This synergistic effect was significantly higher than in the other treated groups. In agreement with this result, Vit D combined with an AKT inhibitor efficiently raised the *Casp3* gene expression, to a greater extent than either agent alone.[27] Another study clarified that the synergistic effect of Vit D combined with an AKT inhibitor consisted in cell cycle arrest rather than apoptosis, as observed by high levels of the cyclin-dependent kinase inhibitor p21.[28]

Buparlisib treatment abolished the PI3K pathway dose-dependently by downregulating the *Akt1* gene expression. The reduced *Akt* gene expression was elaborated previously in HNSCC cell lines at the protein level.[29,30] A similar effect was also obtained in different cell line types.[25,31]

In our study, calcitriol mediated an inhibitory effect on the PI3K pathway by lowering the *Akt1* gene expression. The *Akt1* gene expression was reduced more by increasing the applied dose. Lee and Park clarified that silencing forkhead box O3 (FOXO3) suppressed the expression of BCL2-interacting mediator of cell death (BIM) and the degradation of PARP1 prominently.[26] Therefore, Vit D plays a crucial role in stimulating apoptosis by hindering AKT phosphorylation, leading to the subsequent activation of FOXO3.

Additionally, the Vit D treatment of melanoma cell lines decreased p-AKT and p-mTORC1 (mTORC1 – mechanistic target of rapamycin complex 1) by triggering phosphatase and tensin homolog deleted on chromosome 10 (PTEN).[27] A considerable increase in the *PTEN* expression, and the inhibition of the PI3K, AKT1 and mTOR genes and proteins resulted from applying Vit D solely on colon cancer cell lines.[19]

The co-administration of buparlisib and Vit D synergistically influenced the *Akt1* gene expression, which was lower than with the use of either agent separately. A similar synergistic effect was shown by a decreased p-AKT level when combining Vit D with an AKT inhibitor in prostate cancer cells, regardless of the functional status of PTEN.[28] Shariev et al. also obtained a synergistic effect upon combining Vit D with another AKT inhibitor.[27]

The dual use of calcitriol with PI3K, AKT or mTOR inhibitors declined the levels of phosphorylated PI3K, AKT and mTOR proteins more than the single use of any of the inhibitors.[18] This finding implies that the co-administration of Vit D with the inhibitors efficiently hinders the pathway at several points. Also, combining Vit D with cisplatin enhanced its anticancer effects by significantly lowering p-AKT and p-mTOR to levels not reached by cisplatin alone.[20] Based on these findings, the authors recommended that future research investigate the potential benefits of combining vitamin D with AKT and mTOR inhibitors.[20]

On the other hand, the combined therapy with calcitriol and an AKT inhibitor lowered the expression of the *PI3K* and *mTOR* genes to levels comparable to those obtained with the inhibitor alone in rat glioma cells.[21]

The current study showed that the co-administration of Vit D with buparlisib had a synergistic effect, which could be explored in future studies for determining other mechanisms and examining their effects on resistant TSCC cell lines.

Limitations

In our study, the experiment was conducted on a single cell line, with one treatment duration. We assessed the apoptotic effect by monitoring the *Casp3* gene expression without quantifying the induced apoptosis in the study groups, e.g. through using immunofluorescence staining for CASP3. Also, we did not examine the apoptotic effect on the level of CASP3 or other apoptotic proteins, such as BAX and BCL2. We recommend that future studies take these factors into account to better generalize the synergistic effects of buparlisib and vitamin D on cancer cell lines. Furthermore, it would be valuable to correlate the PCR-detected downregulation of AKT with the markers of the cell cycle.

Conclusions

The co-administration of Vit D with buparlisib significantly reduced the effective dose of buparlisib through a synergistic interaction, enhanced apoptosis and inhibited the PI3K/AKT/mTOR pathway. These findings suggest that Vit D may serve as an effective adjuvant to buparlisib, enabling lower therapeutic doses while maintaining strong antineoplastic efficacy and minimizing potential toxicities in cancer treatment.

Ethics approval and consent to participate

This study received an exemption from review by the Scientific Research Ethics Committee of Ain Shams University, Cairo, Egypt (No. of approval: FDASU-Rec EM112102).

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.

Use of AI and AI-assisted technologies

Not applicable.

ORCID iDs

Karma Nabeel Shamaa https://orcid.org/0000-0001-5812-2519
Basma Abdelrahman Ahmed https://orcid.org/0000-0001-9216-3146
Iman Mohamed Helmy https://orcid.org/0000-0001-7945-5822

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Original paper

Chronological age estimation by measuring pulp chamber volume in teeth with open apices: A CBCT analysis

Kevser Kolcakoglu[1,A–F], Mehmet Amuk[2,A–C,E,F], Esra Kizilci[1,A,D–F], Gamze Sirin Sarıbal[3,B–D,F], Özlem Askaroglu[4,B,C,F], Damla İzel Korkut[5,B,C,F]

[1] Department of Pediatric Dentistry, Faculty of Dentistry, Erciyes University, Kayseri, Turkey
[2] Dream Smile Dental Clinic, Samsun, Turkey
[3] Department of Oral and Maxillofacial Radiology, Faculty of Dentistry, Pamukkale University, Denizli, Turkey
[4] Nimet Bayraktar Oral and Dental Health Hospital, Kayseri, Turkey
[5] Denta Forum Oral and Dental Health Polyclinic, Kayseri, 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

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Address for correspondence
Esra Kizilci
E-mail: esra_ayhan85@hotmail.com

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Abstract

Background. In the domain of forensic medicine, the estimation of age is a critical aspect of human identification, including that of adolescents.

Objectives. The study aimed to examine the relationship between the pulp chamber volume of teeth with open apices and the chronological age of adolescents from the Turkish population.

Material and methods. The study was conducted by examining cone beam computed tomography (CBCT) images of 51 pediatric patients who visited the Department of Pedodontics for routine dental examinations. All CBCT images were captured using a NewTom 5G unit (QR, Verona, Italy). The measurements were recorded in DICOM format using the SimPlant Pro 16 software (Materialise NV, Leuven, Belgium).

Results. A moderate and positive significant correlation was observed between the age of the premolar group patients and impacted pulp volume (IMPV). The increase in volume measurements of the patients in the premolar group indicates that their age is significantly higher ($r = 0.561$, $p = 0.030$). A moderate and negative significant relationship was identified between the age of the premolar group patients and erupted pulp volume (EPV). The increase in the EPV measurements of the patients in the premolar group indicates that their age is significantly lower ($r = –0.491$, $p = 0.041$).

Conclusions. The pulp chamber volume of premolars, which narrows due to secondary dentin deposition, served as the physical marker for chronological age estimation of adolescents in the Turkish population. The measurement of the mature and immature premolar pulp volume could be used for age estimation, particularly in the context of CBCT analysis.

Keywords: cone beam computed tomography, dentin, bicuspid, forensic dentistry, chronological age

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Highlights

- Premolar teeth are key indicators in the estimation of chronological age in adolescents.
- Three-dimensional imaging methods support accurate age estimation in teenagers with teeth displaying open apices.
- Pulp volume can be used for age estimation.

Introduction

The estimation of age is a critical aspect of human identification for forensic purposes. When a professional medical opinion is required to determine an individual's status regarding, for example, adoption or punitive offenses or whether the individual is a juvenile or adult, the estimation of their age can be critical. Due to its frequent application in legal contexts, the determination of age has become increasingly significant in the field of forensic medicine, including adolescent cases.[1,2] However, age estimation can be influenced by developmental changes, as biological age and chronological age are not necessarily aligned.[3,4]

The scientific literature has described different methods for assessing age indicators related to skeletal changes.[5] In addition, many techniques have been investigated for age-related changes in dental structure, which are less influenced by nutritional and endocrine factors.[5,6] Given that environmental and pathological aspects have a smaller influence on tooth development than skeletal factors,[1] particularly during the preadult stage, tooth development is frequently used to determine the age of individuals. This method has been found to offer more accurate results than conventional methods used for adult age estimation.[6]

Dental age can be determined by radiological, morphophysiological and biochemical methods.[7] Radiological techniques have become popular due to their simplicity and reproducibility in applications on both living and deceased individuals.[7,8] Periapical and panoramic radiography along with advanced digital technologies for imaging lateral oblique and cephalometric views, are particularly effective in age identification.[8] In general, radiological methods of age estimation are based on the evaluation of properties such as the development and resemblance of tooth germs, the earliest detectable sign or beginning of mineralization, the degree of crown completion, the degree of root completion of erupted or unerupted teeth, the degree of resorption of primary teeth, the measurement of open apices in teeth, the eruption of crowns into the oral cavity, the volume of the pulp chamber, root canals, and the formation of physiological secondary teeth.[2,7,8] Based on these factors, the estimated age of patients can serve as a crucial aspect of the planning of interceptive orthodontic treatment for children.[9]

Due to secondary dentin deposition in the wall of the pulp cavity, odontoblasts gradually shrink the coronal pulp chamber and root volume throughout an individual's life.[5,10] The majority of dental publications have correlated age with the volume of the dental pulp chamber and secondary dentin apposition.[2,10,11] The volume of the dental pulp chamber can be measured by using radiographic methods, including 2D or 3D radiography.[12] Two-dimensional radiographs are used to estimate age; however, they provide a limited visualization of the dental structure owing to superposition and distortion. This type of radiographs represents linear measurements, which are not sufficient to reveal changes in pulp volume related to secondary dentin apposition in 3 dimensions.[13] Even so, studies aimed at determining the age of children with open apices in their teeth have involved 2D radiography.[9,11,14]

Dental modeling, developed with 3D cone beam computed tomography (CBCT), is based on the geometric approximation of the various components of a tooth, including the root, the pulp and the crown. Dental modeling with CBCT is a well-established method that produces reliable results and enables the rapid assessment of the pulp volumes of teeth,[2] namely by calculating pulp volume using commercial licensed software.[6] However, in studies focused on the correlation between chronological age and the pulp–tooth volume ratio, samples have represented all age groups, from infants to older adults, instead of specific ones. Additionally, few studies have employed the pulp–tooth volume ratio in conjunction with CBCT values to determine the dental age of adolescents.[15,16] Against that background, the aim of this study was to propose a CBCT-based method for age estimation that utilizes the pulp chamber volume in adolescents.

Material and methods

This retrospective study was approved by Erciyes University Non-Invasive Clinical Practices Ethics Committee (decision No. 2022/113). The study was conducted in the Department of Oral and Maxillofacial Radiology and the Department of Pediatric Dentistry of the Faculty of Dentistry at Erciyes University, Kayseri, Turkey. Throughout the study, adherence to the principles of the Declaration of Helsinki was maintained. Informed consent forms were obtained from the parents of all patients.

Inclusion and exclusion criteria

Two radiologists recorded each patient's sex and chronological age in years and months, while a technician was responsible for taking all images. Firstly, panoramic radiographs were obtained from patients who had applied to our clinic for dental treatment. The CBCT images were taken if necessary, in the presence of impacted teeth or due to other dental reasons (1,598 patients). In order to be included in the study, patients had to be between the ages of 9 and 14 years, as determined by CBCT and panoramic images from 2014–2020, and must have had permanent dentition in the maxillary region. A total of 51 individuals were included in the study. The patients were not requested to undergo radiographic imaging for the study. Panoramic radiography results are depicted in Fig. 1.

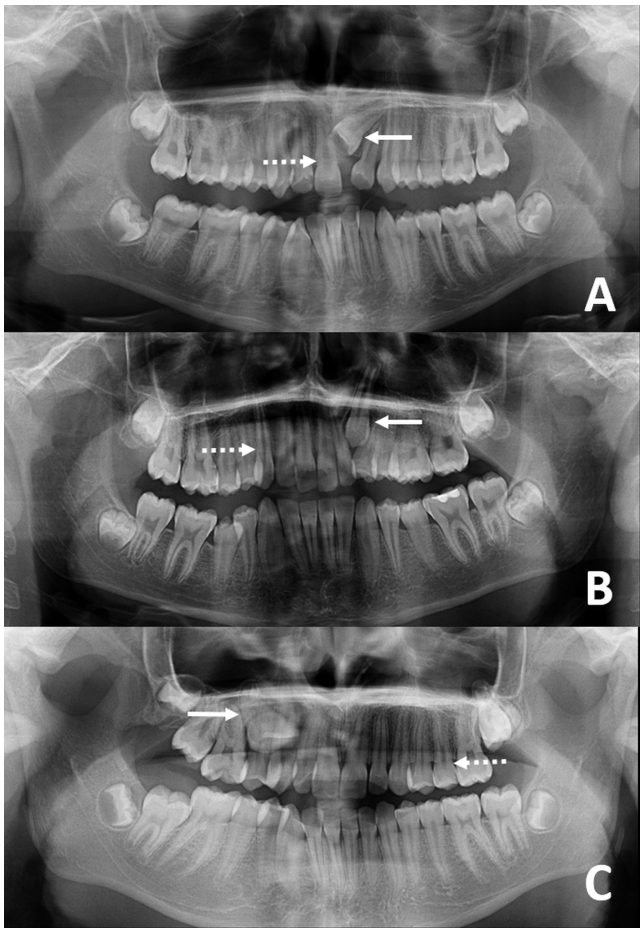

Fig. 1. Results of panoramic radiography

A. Impacted left maxillary incisor that has completed at least ⅔ of its root development (arrow), and a right maxillary incisor in an erupted condition, which is symmetrical to the impacted tooth (striped arrow); B. Impacted left maxillary canine that has completed at least ⅔ of its root development (arrow), and a right maxillary canine in an erupted condition, which is symmetrical to the impacted tooth (striped arrow); C. Impacted right maxillary premolar that has completed at least ⅔ of its root development (arrow), and a left maxillary premolar in an erupted condition, which is symmetrical to the impacted tooth (striped arrow).

Patients with caries-affected teeth, teeth with pathological wear, coronal fracture, external and internal resorption, or teeth that had received general dental treatment for odontoma were excluded from the study. Patients with teeth exhibiting abnormal dental anatomy, which complicates measurement, were also excluded.

Pulp volume measurement

All CBCT images were acquired using a NewTom 5G unit (QR, Verona, Italy) in a standard mode, with a field of view of 12 cm × 8 cm and a voxel size of 250 µm. The kVp and mA values were derived from images obtained through the use of a preview mode. The CBCT images were also examined in terms of the established criteria (Fig. 2). Subsequently, a study group was formed.

Radiographs with poor image quality were excluded from the study. The images were analyzed using the Dell Precision T1500 WorkStation (Dell D02M; Dell Technologies, Warsaw, Poland) and a 19-inch 1920 × 1080 pixel resolution Dell monitor (Dell E190S; Dell Technologies, Beijing, China). The reported measurements were recorded in DICOM format using the NNT software, v. 9.01 (NewTom, Verona, Italy) and the NewTom 5G CBCT device, and reconstructed in the SimPlant Pro 16 software (Materialise NV, Leuven, Belgium).

To measure pulp volume from the CBCT data, a threshold for soft tissue value was set, and the mask creation and segmentation technique was used to determine the contour of the pulp and pinpoint the value of the volume. First, a mask was created to form the pulp chamber and hard tissue segments of each involved tooth. Second, the optimal separation grayscale threshold was selected, which exhibited the pulp chamber within the tooth in all sections and planes (i.e., axial, coronal and sagittal). Third,

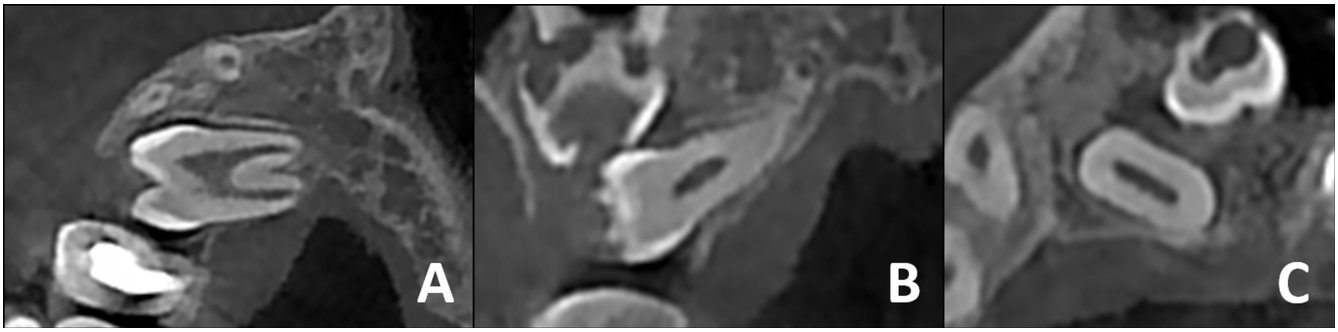

Fig. 2. Impacted left maxillary premolar of the same patient in the corrected coronal (A), sagittal (B) and axial (C) planes

for the 3D calculation, regions that did not correspond to the pulp cavity were approximately identified and deleted. Fourth, manual erasing and corrected drawing were performed to remove bone fragments at the root and in surrounding teeth at the crown level. Standardization was achieved using fixed threshold values in all teeth. Lastly, with the mask representing the pulp chamber in all planes and sections, an image was generated to calculate the pulp volume. The image value was automatically measured by the software, and the pulp volume was obtained (Fig. 3).

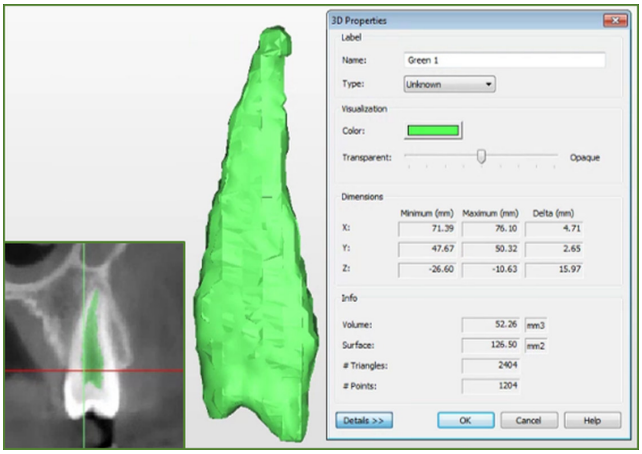

Fig. 3. Measurement of the pulp volume (SimPlant Pro 16 software)

Statistical analysis

The normality of the data was determined using Q–Q plots, a histogram and the Shapiro–Wilk test. Levene's test was performed to assess the homogeneity of variance, and a set of descriptive statistics, including frequency, percentage, mean (*M*), and standard deviation (*SD*) values, was calculated. In all patients and groups, the relationship between pulp volumes and age was examined using Pearson's correlation analysis. The multiple regression analysis was utilized to model and clarify the association between age and pulp volume. The analyses were conducted using the IBM SPSS Statistics for Windows software, v. 20.0 (IBM Corp., Armonk, USA). Statistically significant results were defined as $p < 0.05$.

Results

Of the patients included in the sample, 51% (n = 26) were male, and 49% (n = 25) were female. The patient groups consisted of the incisal group (31.4%), the canine group (37.3%) and the premolar group (31.4%). The mean age of the patients was 145.90 ±18.15 months. The mean impacted dental pulp volume (IMPV) was 67.34 ±21.45 mm^3, while the mean erupted pulp volume (EPV) was 56.44 ±18.36 mm^3 (Table 1).

Table 1. Characteristics of the study participants (N = 51)

Variable		Result
Sex, *n* (%)	male	26 (51.0)
	female	25 (49.0)
Group, *n* (%)	incisal	16 (31.4)
	canine	19 (37.3)
	premolar	16 (31.4)
Age [months] *M* ±*SD* (min–max)		145.90 ±18.15 (110.00–170.20)
IMPV [mm^3] *M* ±*SD* (min–max)		67.34 ±21.45 (30.67–129.53)
EPV [mm^3] *M* ±*SD* (min–max)		56.44 ±18.36 (21.67–99.35)

IMPV – impacted pulp volume; EPV – erupted pulp volume; *M* – mean; *SD* – standard deviation.

No statistically significant difference (p = 0.932, range = 0.889–0.987) was observed between radiologists in pulp measurements. A weak positive correlation was identified between IMPV and age (r = 0.393, p = 0.011), and a weak negative correlation was observed between EPV and age (r = –0.271, p = 0.041) (Table 2).

Table 2. Correlation between impacted pulp volume, erupted pulp volume and age in all study groups

Variable		Age
IMPV	*r*	0.393
	p-value	0.010*
EPV	*r*	–0.271
	p-value	0.040*

* statistically significant ($p < 0.05$, Pearson's correlation).

In the incisal group, no significant correlation was identified between age and IMPV (r = 0.072, p = 0.810), or between age and EPV (r = 0.150, p = 0.594). Likewise, in the canine group, no significant correlation was observed between age and IMPV (r = 0.010, p = 0.993), or between age and EPV (r = –0.162, p = 0.522). However, in the premolar group, there was a moderate positive significant correlation between age and IMPV (r = 0.561, p = 0.032). This finding suggests that as IMPV values increase, the age of the participant is found to be significantly higher. In the same group, there was a moderate negative significant correlation between age and EPV (r = –0.492, p = 0.041), and an elevated EPV value indicated that a subject's age was significantly lower (Table 3).

In addition, there was a significant correlation between pulp volume and age in the premolar group (p = 0.011). The multiple regression formula for this relationship was calculated as follows (Equation 1):

$$\text{Age} = 110.25 + 0.88 \times \text{IMPV} - 0.43 \times \text{EPV} \quad (1)$$

The model determined that IMPV and EPV in the premolar group affected the age significantly (Table 4).

Table 3. Relationship between impacted pulp volume, erupted pulp volume and age among the study groups

Variable		Age		
		incisal group	canine group	premolar group
IMPV	*r*	0.072	0.010	0.561
	p-value	0.810	0.993	0.032*
EPV	*r*	0.150	–0.162	–0.492
	p-value	0.594	0.522	0.041*

* statistically significant ($p < 0.05$, Pearson's correlation).

Table 4. Modelling the relationship between impacted pulp volume and erupted pulp volume in the premolar group of patients

Dependent variable		Independent variables		F_{Model}	R^2
		IMPV	EPV		
Age	*β*	0.880	–0.430	$F = 18.963$	0.311
	t	5.421	–4.821	$p = 0.010$*	
	p-value	0.010*	0.011*		

* statistically significant ($p < 0.05$, Pearson's correlation).

Discussion

There are distinct differences between the teeth and the palate.[17] A digital model of the palate is an excellent candidate for disaster victim identification because it changes only 3 µm per year throughout a person's life.[18] Contrarily, teeth undergo continuous modification. Although teeth are subject to plenty of age-related changes during life, all anatomical dental characteristics are unique, which has made teeth a useful option for comparative identification for centuries.[19] However, our results prove that teeth could also be used for age estimation in adolescents. According to the literature, the constriction of pulp dimensions due to secondary deposition can serve as a useful indicator of chronological age.[5,10,12,20]

Secondary dentin deposition can be assessed using the pulp–tooth volume ratio with 2D dental radiographic methods. However, the primary disadvantage of conventional radiographic techniques is that they are subject to substantial errors in magnification and distortion. Therefore, concurrent evaluation of mesiodistal and buccolingual dimensions of teeth is recommended.[19–21] By contrast, CBCT provides an excellent means to collect high-quality 3D tooth radiographs in dental practice and is more appealing than CT and micro-CT for determining age.[22]

During the developmental process of a tooth, the diameter of the apical foramen decreases, and the crown develops with enamel production. Cameriere et al. determined the maturity of teeth under these conditions by counting the number of teeth with an entirely closed apical foramen and measuring the distance between the length of the inner point in the apical foramen and the length of the teeth.[23] They also quantified the dental age of children by measuring the width of the apical foramen's opening.[23] In most studies, teeth with open apices have been evaluated using 2D radiographic techniques.[1,11,14,23,24] However, with the advancement of medical technology, CBCT has become a valuable tool in dentistry clinics, offering a higher metric resolution and isotropic voxel resolution than conventional medical computed tomography.[25] Cone beam computed tomography image analysis using the segmentation function can help to design the scanned structure's 3D model and ascertain its volume and superficial area.[26] Once the pulp–tooth ratio is measured, mature teeth can be evaluated with CBCT.[21,27–30] In the present study, the authors used CBCT to evaluate both mature and immature teeth.

Researchers have traditionally used incisors, canines and premolars to estimate age, either in isolation or together.[15,21,22,27,29–31] For example, Star et al. analyzed mono-radicular incisal, canine and premolar teeth in subjects from Belgium aged 8–19 years.[27] The authors have used the SimPlant Pro software to calculate pulp volume from 111 CBCT images of teeth. As a result, no statistically significant relationship has been identified between pulp volume and age across different tooth types ($p = 0.15$), and pulp volume–tooth ratios ranged between 0.002 and 0.091 (0.027 ±0.020). Upon calculating regression formulas for each tooth, it was observed that the relationship between the pulp volume ratio of incisors and age was stronger for females than for males. However, the calculated difference was not statistically significant ($p = 0.86$), and no significant interaction between types of teeth and sex was observed ($p = 0.50$).[27] Many previous studies have also shown that no significant relationship exists between pulp volume and sex,[21,22,27,32,33] as confirmed by the findings of the present study.

Gulsahi et al. performed a CBCT analysis on a sample of 655 maxillary central incisors, lateral canines, mandibular canines, and first and second premolars in Turkey.[21] Following the implementation of a simple linear regression analysis and the utilization of the 3D-DOCTOR software for tissue segmentation, the regression analysis showed that 53.2% of the variance in maxillary central incisors could be explained, along with 21.7%, 21.0%, 20.7%, and 15.3% explanations for the variance observed in mandibular second premolars, mandibular canines, mandibular first premolars, and maxillary canines, respectively.[21] In another study, Aboshi et al. investigated the relationship between age and different levels of pulp volume in lower premolars.[12] This tooth type was selected due to its superior resistance to decay compared to incisors and canines, and its more straightforward and stable root shape compared to molars.[12] The study findings revealed that pulp volume in lower premolars diminished progressively over time, with the largest decline occurring at ages of 20 and 50, and the most substantial loss observed at the coronal third of the root. In addition, the study's accuracy in estimating age using the model for lower second premolars

(R^2 = 0.685) exhibited a marginal improvement over that for lower first premolars (R^2 = 0.617).[12] By comparison, Tardivo et al. examined 210 CT scans from individuals aged 15–85 years with 4 healthy canines.[19] The samples composed of 840 canines were modeled using the Mimics® 10.01 software. The authors formulated 7 regression models and determined the most powerful one, which took maxillary canines into consideration.[19] The decision to study canines was made due to their relatively simple anatomy and large dimensions of their various volumes.[19]

This study was performed using the SimPlant Pro 16 software (Materialise NV) with CBCT, and included participants aged 9–14 years. The authors evaluated incisors, canines and premolars collectively to examine the relationship between each tooth and age. A weak positive significant relationship was observed between the age of the patients and IMPV. Therefore, we propose that the rise in IMPV values may signify a considerably higher age of the patients (r = 0.393, p = 0.010). As IMPV volume increases, chronological age increases. Meanwhile, taking into consideration the weak negative significant relationship between the age of the patients and EPV, the increase in EPV values may be indicative of the patients' significantly lower age (r = –0.271, p = 0.040).

In other comparable studies, Yang et al. examined left maxillary incisors and canines in a sample of individuals aged 8–19 years from China.[15] They reported means and standard deviations of the pulp–tooth volume ratio to be 0.053 ±0.024 for male left maxillary incisors, 0.049 ±0.069 for female left maxillary incisors, 0.080 ±0.030 for male left maxillary canines, and 0.020 ±0.022 for female left maxillary canines. The researchers also determined that the correlation coefficients were –0.70 (male), –0.63 (female) and –0.67 (total) for central incisors, and –0.88 (male), –0.81 (female) and –0.83 (total) for canines.[15] Meanwhile, Pinchi et al. examined left maxillary central incisors in a sample of Italian individuals aged 10–80 years.[22] The authors found that pulp volume was a statistically significant predictor of age ($p < 0.001$) and calculated the linear regression formula (–64.14 – 32.00 × pulp volume (mm^3)) as a reliable parameter for determining age in adults.[22] However, there is a considerable discrepancy between our result, where the slope is –0.43 × pulp volume, and the result of Pinchi et al.,[22] where the slope is 32 × pulp volume. This difference is thought to be due to the small sample size of adolescent participants. In addition, Manjrekar et al. examined a sample of youth aged 4–15 years in western India, specifically focusing on 7 left permanent mandibular teeth with open apices.[14] The analysis was conducted using panoramic radiographs. Based on the regression equation for the Western Indian population, the authors found no statistically significant difference between the estimated and chronological ages of children aged 4–13 years.[14] Likewise, Guo et al. examined 785 healthy children (397 females and 388 males) aged 5–15 years from China.[24] This study employed panoramic radiographs. In the analysis of the 7 left permanent mandibular teeth with the Cameriere's method,[23] the regression models were calculated using the following formula (Equation 2):

$$\text{Age} = 10.202 + 0.826\,g - 4.068\,X_3 - 1.536\,X_4 - 1.959\,X_7 + 0.536\,N_0 - 0.219\,s \times N_0 \quad (2)$$

where:
g – variable of gender (i.e., 1 for male and 0 for female);
s – sum of the normalized open apices;
N_0 – number of teeth with complete root development;
X_3 – canine teeth;
X_4 – first premolars;
X_7 – second molars.

The results explained 91.2% (R^2 = 0.912) of the total variance.[24] Next, Kazmi et al. examined 521 left maxillary and 681 left mandibular canines from 368 females and 349 males, aged 15–65 years, of Pakistani ancestry.[30] Their findings indicated that sex and the volume of the mandibular canine pulp had the most significant predictive effect (R^2 = 0.33). The regression analysis was calculated using the following formula (Equation 3):

$$\text{Estimated age} = 60.370 + \text{lower pulp volume} \times (-715.260) + \text{sex} \times 8.791 \quad (3)$$

The equation added 8.791 to a person's estimated age when they were males. The researchers reported that the most accurate results could be achieved by estimating chronological age using sex and the pulp volume of mandibular canines.[30] Finally, Rosset et al. used CBCT with sound upper canines from 91 individuals aged 17–80 years and analyzed pulp volume using the OsiriX open-source software.[34] As a result, De Angelis et al. found that the regression model was formulated to exhibit enhanced compatibility for females (R^2 = 0.485).[6] In the current study, the regression model of 110.25 + 0.88 × IMPV – 0.43 × EPV was calculated to generate an age estimation, which was more accurate (R^2 = 0.31) in the premolar group. A comparison of our results with those from other studies revealed that our findings had a lower R^2 index, likely due to the inclusion criteria employed.

Conclusions

Pulp volume, which undergoes a decrease when secondary dentin deposition occurs, serves as the physical marker for estimating the age of teenagers. In the estimation of chronological age in adolescents, the focus should be on the evaluation of premolar teeth rather than incisor and canine teeth.

Three-dimensional imaging methods can be used for teeth with an open apex to estimate chronological age. The pulp volume of mature and immature teeth should

be formulated together in the chronological age estimation of teenagers. Future research may employ additional dental features to enhance the performance of regression algorithms in a large group.

Ethics approval and consent to participate

This retrospective study was approved by Erciyes University Non-Invasive Clinical Practices Ethics Committee, Kayseri, Turkey (decision No. 2022/113). Throughout the study, adherence to the principles of the Declaration of Helsinki was maintained. Informed consent forms were obtained from the parents of all patients.

Data availability

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

Consent for publication

Not applicable.

Use of AI and AI-assisted technologies

Not applicable.

ORCID iDs

Kevser Kolcakoglu https://orcid.org/0000-0003-2596-8678
Mehmet Amuk https://orcid.org/0000-0001-6390-7169
Esra Kizilci https://orcid.org/0000-0003-0746-7087
Gamze Sirin Sarıbal https://orcid.org/0000-0002-5191-377X
Özlem Askaroglu https://orcid.org/0000-0001-7480-8262
Damla İzel Korkut https://orcid.org/0000-0002-7396-915X

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Original paper

New effect size and sample size guidelines in dentistry

Grzegorz Zieliński[1,A–F], Mieszko Więckiewicz[2,E,F]

[1] Department of Sports Medicine, Medical University of Lublin, Poland
[2] Department of Experimental Dentistry, 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

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Address for correspondence
Grzegorz Zieliński
E-mail: grzegorz.zielinski@umlub.pl

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Abstract

Background. Cohen has emphasized that the recommended thresholds for effect sizes should only be used in the absence of detailed information about effect size distributions within specific fields.

Objectives. The study aimed to establish updated effect size thresholds (Cohen's *d*, Hedges' *g* and Pearson's *r*) tailored for research in dentistry.

Material and methods. Following methodologies from prior research on effect sizes, the data was extracted from meta-analyses published in the top 10 ranked dentistry journals. The 25th, 50th and 75th percentiles were calculated for Pearson's *r* values, as well as for Cohen's *d* or Hedges' *g*. A total of 4,250 studies were analyzed, with statistical analyses conducted using the R programming language.

Results. The 25th, 50th and 75th percentiles for Pearson's *r* in individual differences research were 0.16, 0.40 and 0.67, respectively. For Hedges' *g*, the percentiles corresponding to small, medium and large effect sizes were 0.10, 0.35 and 0.86, respectively.

Conclusions. In light of these findings, researchers in the field of dentistry are encouraged to adopt the following thresholds: for Pearson's *r*, 0.20 for small effects, 0.40 for medium effects and 0.70 for large effects; and for Cohen's *d* or Hedges' *g*, 0.10 for small effects, 0.40 for medium effects and 0.90 for large effects. These updated thresholds can improve the rigor and quality of dental research, ultimately benefiting patients through enhanced diagnostics and treatment strategies.

Keywords: dentistry, sample size, effect size, stomatology, statistical power

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Highlights

- Recommended effect size thresholds for dental research are: Pearson's r = 0.20 (small), 0.40 (medium) and 0.70 (large); and Hedges' g = 0.10 (small), 0.40 (medium) and 0.90 (large).
- Adoption of these thresholds may improve methodological rigor, enhance research quality, and support more accurate diagnostics and treatment in dentistry.
- The study provides guidance on determining appropriate sample sizes in dental research based on desired statistical power and effect size.

Introduction

Scientific research has a tangible impact on health of the population. Over the years, an increase in the number of studies has been observed, and a systematic rise is predicted.[1] Alongside the growth in research quantity, quality should also improve. One factor determining the quality of research is the rigor of the statistical analysis.[2] Contemporary research focuses primarily on reporting p-values for statistical significance, often neglecting the value of the effect size.[2,3]

The significance of research findings is not always adequately represented by statistical significance.[4–7] Results that achieve the predetermined significance level may not be clinically significant, and vice versa.[5] For example, in very large samples, statistical significance is almost always achieved, which may be misinterpreted (without analyzing the effect size) as sample variability.[7] Therefore, regardless of statistical significance, researchers must assess whether the results are clinically meaningful and relevant to their scientific field.[5]

Recommendations for reporting effect sizes are systematically published to enhance the quality of scientific research, thereby improving decision-making in patient treatment.[7,8] Cohen is the most prominent researcher who provided guidelines for effect size analysis. He defined the following thresholds for Cohen's d and Hedges' g: 0.20 (small effect); 0.50 (medium effect); and 0.80 (large effect). For Pearson's r, the established thresholds are 0.10 (small effect), 0.30 (medium effect) and 0.50 (large effect).[9] However, it has been observed that effect sizes may vary across research fields.[10]

For instance, different thresholds have been developed for rehabilitation.[11] To further refine the statistical framework, specific guidelines have been established for physiotherapy.[12] In addition, thresholds have been formulated for gerontology,[6] hearing research,[5] and exercise-based treatments for tendinopathy,[13] as well as for research related to the temporomandibular joint (TMJ) and masticatory muscles.[14]

To date, no guidelines for Cohen's d, Hedges' g or Pearson's r specific to dentistry have been identified. Dentistry, as a branch of medicine, differs significantly from other medical fields. These differences are evident from the outset, including preclinical and clinical education for dentistry students compared to medical students.[15–21] Further distinctions emerge in professional practice, with unique methods of treatment and patient care.[20,22–26] The analysis of the function, pathologies, and treatment of teeth, periodontium, tongue, oral mucosa, and surrounding tissues, as well as TMJ, sets dentistry apart from other medical fields.[14,27–29] Based on these differences, it is rational to investigate whether distinct effect size thresholds exist in dentistry, as observed in other medical fields.[5,6,30]

This issue is of particular concern in the context of public health. The World Health Organization (WHO) has noted a strong relationship between socioeconomic status and the prevalence and severity of oral diseases. This connection has been observed across various populations, ranging from childhood to advanced age.[31]

Dental diseases affect a significant proportion of the population. The global prevalence of dental caries in primary teeth among children is 46%, while the prevalence of caries in permanent teeth among children reaches 54%.[32] Periodontal disease in adults is estimated to impact around 62% of the population, with severe periodontitis occurring in 24%.[33] Approximately 22% of individuals experience edentulism.[34] Sleep bruxism is present in 21% of the population, while daytime bruxism afflicts 23%.[35] Temporomandibular disorders affect 34% of the population, and it is projected that by 2050 this figure will rise to 44%.[36]

Cleft palate has been diagnosed in 33% and cleft lip in 30% of cases involving cleft conditions, with cleft lip and palate occurring approximately once in every 1,000 live births.[37] Cancers of the lip, oral cavity, and pharynx account for about 4% of all cancer cases and 4% of all cancer-related deaths worldwide.[38] In the past decade, noma has been diagnosed in at least 23 countries.[39] Oro-dental trauma affects about 20% of children.[31] These are just a few examples of conditions and disorders associated with dentistry. This highlights the importance of improving research methods, including statistical approaches, within this field.

Considering the abovementioned information, a study was conducted to establish novel effect size thresholds (Cohen's d, Hedges' g and Pearson's r) for research in dentistry.

Material and methods

The project was initially registered with the Open Science Framework (OSF).[40]

The search procedure was replicated in accordance with the methodology outlined by Brydges.[6] Ten journals were searched: Journal of Dental Research (ISSN 0022-0345); Journal of Endodontics (ISSN 0099-2399); Dental Materials (ISSN 0109-5641); International Endodontic Journal (ISSN 0143-2885); Journal of Dentistry (ISSN 0300-5712); Oral Surgery, Oral Medicine, Oral Pathology and Oral Radiology (ISSN 2212-4403); Journal of the American Dental Association (ISSN 0002-8177); Community Dentistry and Oral Epidemiology (ISSN 0301-5661); Caries Research (ISSN 0008-6568); and Journal of Oral Rehabilitation (ISSN 0305-182X). The identification of these journals was conducted using the Scimago Journal & Country Rank database,[41] with a selection of the "dentistry (miscellaneous)" category and a sorting method based on the highest H-index over the entire period.[2,6,12,14,42] The list of journals was created at the beginning of the project on August 12, 2024.[40]

Considering the continuous development of dentistry, the search period was constrained to the last 20 years, a decision informed by prior studies.[12,14,35,43,44] Articles published between December 31, 2003, and December 31, 2023, were reviewed. The search focused on studies containing the term "meta" in the title during the specified timeframe. The following types of articles were excluded from the analysis: editorials; corrections; correspondence; short communications; conference abstracts; and reviews that did not involve meta-analyses, such as systematic reviews, narrative reviews and scoping reviews. Subsequently, full-text articles were analyzed.

A database similar to the one created by Brydges[6] was constructed, containing the Digital Object Identifier (DOI) numbers of the meta-analyses, along with information on study category, authors, publication year, sample size, and effect size. A total of 4,250 records were screened, and 567 meta-analyses were included for full-text analysis. In 326 publications, none of the studied effect sizes (Cohen's *d*, Hedges' *g*, or Pearson's *r*) were reported. Individual effect sizes were not specified in 89 studies. In 17 studies, data was missing (e.g., sample size explicitly tied to the effect size was not available). Ultimately, 135 meta-analyses were included in the analysis. Comprehensive details regarding the studies, the reasons for exclusion, and the number of included studies per journal are provided in the supplementary materials.

Statistical analysis

In the current study, 2 types of analyses were conducted: studies estimating effects within a group over time (test–retest); and studies evaluating differences between 2 groups. For the within-group analyses, the effect size was measured using the Pearson's *r* correlation coefficient, while for the between-group analyses, the effect size was quantified using Hedges' *g*.

The evaluation of effect sizes was based on Cohen's convention for small, medium and large effects. For the calculation of correlation coefficients, the thresholds were set at 0.10, 0.30 and 0.50, respectively. For between-group differences, the corresponding thresholds were 0.20, 0.50 and 0.80.[9]

The distribution of effect sizes was made by calculating a range of percentiles for both Pearson's *r* and Hedges' *g*. In line with previous literature,[6,30,45] the 25th, 50th (median) and 75th percentiles were interpreted as approximate indicators of small, medium and large effects according to Cohen's guidelines.[9,46] It should be noted, however, that this comparison is conceptual and does not assume that the underlying distribution of effect sizes perfectly aligns with Cohen's benchmarks. This convention does not imply that the distribution of effect sizes in the current data was symmetric.

Additionally, percentiles were determined for 2 subsamples of Hedges' *g* effect sizes, with studies classified into biopsychosocial, diagnosis, health promotion and prevention, and treatment categories according to the research focus of the meta-analysis. Furthermore, to account for the specificity of dental research, an additional division into 7 descriptive subgroups was made: cariology; periodontology; fixed and removable prosthodontics; oral surgery; orthodontics; endodontics; and conservative dentistry. These subgroup analyses were exploratory in nature and aimed to provide a descriptive overview of effect size distributions across research domains. No inferential statistical comparisons were performed between the subgroups; hence, no adjustments were applied for multiple comparisons.

To assess potential inflation bias, one-directional contour-enhanced funnel plots were generated. In these plots, effect sizes are plotted against their corresponding standard errors, with added contour regions representing key levels of statistical significance. Specifically, the orange-shaded region corresponds to the range of $0.10 > p > 0.05$, while the red-shaded region corresponds to $0.05 > p > 0.01$.[6,12,14] An excessive proportion of studies falling within these contours may indicate the presence of inflation bias, suggesting that the reported effect sizes could be overestimates of the true effect sizes. Such inflation may result from factors such as sampling error, publication bias or *p*-hacking. These funnel plots serve as a diagnostic tool to identify potential biases in the reported data.

A series of a priori power analyses were conducted to determine the sample sizes required for future research to achieve various levels of statistical power for both within-group and between-group differences, including biomedical and psychosocial subsamples. For within-group differences, calculations were based on

correlation analyses, while for between-group differences, calculations assumed a two-sample comparison with equal group sizes.

All analyses utilized a two-tailed alpha level of 0.05 and estimated the sample sizes necessary to achieve power levels of 60%, 70%, 80%, and 90% for small, medium and large effect sizes, corresponding to the 25th, 50th and 75th percentiles of the observed effect size distributions, respectively. These calculations provide critical benchmarks for designing adequately powered future studies.[6,12,14]

The analyses were conducted using the R programming language (v. 4.3.3; R Foundation for Statistical Computing, Vienna, Austria) on a Windows 11 Pro 64-bit operating system (build 22631; Microsoft Corporation, Redmond, USA). A comprehensive description of the statistical analysis, including the use of packages in the R language, the estimation of effect sizes for individual studies with group differences, the estimation of variance for Hedges' *g*, and the random-effects model, is provided in the supplementary material 2.

Results

Characteristics of the sample

The analysis encompassed a total of 4,250 dentistry studies, which were categorized into 4 research domains: biopsychosocial (n = 127, 2.99%); diagnosis (n = 796, 18.73%); health promotion and prevention (n = 271, 6.38%); and treatment (n = 3,056, 71.91%). Two types of effect sizes were utilized in the studies: those measuring between-group effects (Hedges' g, n = 4,038, 95.01%), and those measuring within-group effects (Pearson's r, n = 212, 4.99%). The median group sizes ranged from 20 to 24, with an interquartile range of 12–45. The complete database of publications used in the analyses is available in the supplementary material 3.

Within-group differences

The first (25%), second (50%) and third (75%) percentiles for within-group differences research corresponded to Pearson's r values of 0.16, 0.40 and 0.67, respectively (Table 1,2). This finding indicates that, in dentistry research focused on individual differences, the median effect size is Pearson's r = 0.40.

The observed effect sizes were noticeably higher than those in Cohen's guidelines for small, medium and large effects, with differences ranging from 0.06 for small effects to 0.17 for effects classified as large (Table 2). Compared to Cohen's benchmarks, only 64.2% of the observed correlations would qualify as medium effects or stronger ($r \geq 0.30$), and just 34.0% would be classified as strong effects ($r \geq 0.50$).

Table 1. Percentiles associated with observed within-group correlations (Pearson's r) and between-group differences (Hedges' g)

Percentile	Pearson's r	Hedges' g
5th	0.02	0.01
10th	0.05	0.03
15th	0.08	0.05
20th	0.12	0.08
25th	0.16	0.10
30th	0.21	0.14
35th	0.28	0.20
40th	0.33	0.25
45th	0.35	0.32
50th	0.40	0.35
55th	0.44	0.48
60th	0.46	0.58
65th	0.50	0.69
70th	0.53	0.84
75th	0.67	0.86
80th	0.83	1.35
85th	0.89	1.80
90th	0.92	2.64
95th	0.95	4.36

The distributions of effect sizes for within-group and between-group differences in Fig. 1A and Fig. 1B are reported with 25th, 50th and 75th percentiles corresponding to small (r = 0.16, g = 0.10), medium (r = 0.40, g = 0.35) and large (r = 0.67, g = 0.86) effects, respectively. This indicates that the majority of observed relationships in dentistry research on individual differences are of medium to large size, suggesting clinically meaningful associations in this domain. Additionally, it is important to emphasize the differences observed in the domains of dentistry: in oral surgery, the small effect was 0.08, the medium effect was 0.27 and the large effect was 0.66; in orthodontics, the respective values were 0.40, 0.93 and 1.87; in periodontology, the values were 0.11, 0.29 and 0.63; in cariology – 0.10, 0.40 and 1.01; in conservative dentistry – 0.10,

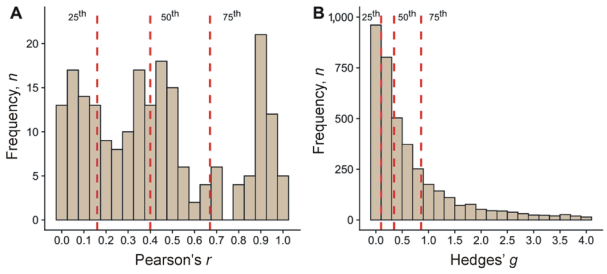

Fig. 1. Distribution of Pearson's r (A) and Hedges' g (B) effect sizes for within-group and between-group differences

Dashed red lines represent the 25th, 50th and 75th percentiles corresponding to small (r = 0.16, g = 0.10), medium (r = 0.40, g = 0.35) and large (r = 0.67, g = 0.86) effect sizes.

Table 2. Comparison of Cohen's guidelines with quantitatively derived estimates of effect sizes

Characteristic			Studies, *n*	Effect size		
				small	medium	large
Individual differences (Pearson's *r*)	Cohen[9]		–	0.10	0.30	0.50
	current study	obtained values	212	0.16	0.40	0.67
		rounded values		0.20	0.40	0.70
	category	diagnosis	44	0.29	0.50	0.85
		health promotion and prevention	87	0.06	0.17	0.41
		treatment	81	0.35	0.47	0.89
		oral surgery	81	0.35	0.47	0.89
		cariology	87	0.06	0.17	0.41
		conservative dentistry	31	0.23	0.40	0.52
Group differences (Hedges' *g*)	Cohen[9]		–	0.20	0.50	0.80
	current study	obtained values	4,038	0.10	0.35	0.86
		rounded values		0.10	0.40	0.90
	category	biopsychosocial	127	0.05	0.14	0.36
		diagnosis	752	0.05	0.18	0.51
		health promotion and prevention	184	0.09	0.27	0.80
		treatment	2975	0.15	0.50	1.29
		oral surgery	1274	0.08	0.27	0.66
		orthodontics	199	0.40	0.93	1.87
		periodontology	474	0.11	0.29	0.63
		cariology	480	0.10	0.40	1.01
		conservative dentistry	282	0.10	0.31	0.73
		endodontics	176	0.04	0.19	0.77
		fixed and removable prosthodontics	517	0.56	1.54	3.35
		temporomandibular joint and masticatory muscle research*	456	0.10	0.30	0.70

* data obtained from the study by Zieliński and Gawda.[14]

0.31 and 0.73; in endodontics – 0.04, 0.19 and 0.77; and in fixed and removable prosthodontics – 0.56, 1.54 and 3.35 (Table 2). It should be noted that most of the effects for between-group differences were below the thresholds recommended by Cohen.

The median sample size for within-group differences was 117 participants. This sample size is large enough to detect a medium (r = 0.40; power = 1.00) or large effect (r = 0.67; power = 1.00), but not to detect a small effect (r = 0.16; power = 0.41).

A visual assessment of the distribution of effect sizes was prepared to evaluate potential publication bias and the symmetry of the effect size distributions within each domain. Contour lines indicate the regions of statistical significance (supplementary material 2: Fig. 2).

A total of 70.95% of the studies demonstrated sufficient power to detect a medium effect, as indicated by their distribution within the gray region of the contour-enhanced funnel plot (supplementary material 2: Fig. 2A,3) (Table 3), corresponding to $p < 0.01$. Furthermore, the funnel plot did not exhibit an overrepresentation of just-significant results (p-values: 0.05–0.01, represented by the red region) or marginally significant results (p-values: 0.10–0.05, represented by the orange region). This pattern indicates that inflation bias, including potential concerns such as publication bias or p-hacking, is unlikely to pose a significant issue in dentistry studies investigating individual differences.

The sample size calculations presented in Table 4 provide critical benchmarks for designing future studies in individual differences research. Achieving adequate statistical power necessitates the determination of the required sample size, which varies substantially depending on the effect size and the desired power level. For small effects (r = 0.16), achieving 80% power requires a sample size of 304, which increases to 406 for 90% power, indicating the need for larger samples to reliably detect subtle effects. For medium effects (r = 0.40), a sample size of 46 is sufficient for 80% power, while for large effects (r = 0.67), only 15 participants are needed to achieve the same power level.

Only 62% of the analyzed studies were adequately powered to detect a medium effect, and nearly 90% were powered to identify a large effect.

Table 3. Distribution of studies across funnel plot color regions based on the research domain and type of comparison

Comparison	Category	Color region [%]			
		white ($p > 0.10$)	orange ($0.10 \geq p > 0.05$)	red ($0.05 \geq p > 0.01$)	gray ($p \leq 0.01$)
Within-group differences	overall	18.09	3.33	7.62	70.95
	diagnosis	4.65	0.00	9.30	86.00
	health promotion and prevention	18.60	3.49	3.49	74.42
	treatment	24.69	4.94	11.11	59.26
	oral surgery	24.69	4.94	11.11	59.26
	cariology	18.60	3.49	3.49	74.42
	conservative dentistry	6.67	0.00	13.33	80.00
Between-group differences	overall	56.71	5.19	8.00	30.09
	treatment	50.45	4.90	7.61	37.04
	health promotion and prevention	55.49	6.59	6.59	31.32
	diagnosis	61.82	4.62	7.34	26.22
	biopsychosocial	68.03	3.28	8.20	20.49
	oral surgery	28.08	4.93	11.33	55.66
	orthodontics	64.76	5.01	7.83	22.39
	periodontology	62.74	5.51	4.94	26.81
	cariology	46.77	4.92	8.00	40.31
	conservative dentistry	61.64	6.51	6.16	25.69
	endodontics	62.23	3.12	12.45	22.23
	fixed and removable prosthodontics	28.33	3.87	6.19	61.61

Between-group differences

In the between-group differences sample, the 25th, 50th and 75th percentiles corresponded to Hedges' g values of 0.10, 0.35 and 0.86, respectively (Table 1,2). For small and medium effects, these values are lower than Cohen's benchmarks of 0.20 and 0.50,[9,46] while for large effects, the 75th percentile exceeds Cohen's guideline of 0.80. A comparison of these results with Cohen's recommendations reveals that only 40.4% of the observed effect sizes in this sample would qualify as medium or stronger effects ($g \geq 0.50$), and just 27% would be considered large ($g \geq 0.80$). This finding indicates that a substantial proportion of the observed group differences reflects smaller-than-expected effects, based on established guidelines.

An examination of specific research domains revealed significant variability. In biopsychosocial studies, the derived thresholds for small ($g = 0.05$), medium ($g = 0.14$) and large ($g = 0.36$) effects are substantially smaller than those reported in Cohen's guidelines, indicating that even the modest effects within this domain hold practical significance. Similarly, diagnostic studies show lower thresholds for small ($g = 0.05$) and medium ($g = 0.18$) effects, with large effects ($g = 0.51$) aligning more closely with Cohen's recommendations.

Health promotion and prevention studies have demonstrated thresholds for small, medium and large effects

Table 4. Distribution of sample sizes required to achieve various levels of statistical power in research on within-group differences

Category	Effect size	Statistical power			
		60%	70%	80%	90%
All studies ($N = 212$)	small ($r = 0.16$)	191	240	304	406
	medium ($r = 0.40$)	30	37	46	61
	large ($r = 0.67$)	10	12	15	19
Diagnosis ($n = 44$)	small ($r = 0.29$)	56	70	89	118
	medium ($r = 0.50$)	18	22	27	36
	large ($r = 0.85$)	6	6	7	9
Health promotion and prevention ($n = 87$)	small ($r = 0.06$)	1,360	1,712	2,177	2,914
	medium ($r = 0.17$)	168	212	269	359
	large ($r = 0.41$)	28	35	44	58
Treatment ($n = 81$)	small ($r = 0.35$)	39	48	61	81
	medium ($r = 0.47$)	21	26	32	42
	large ($r = 0.89$)	5	6	7	8
Oral surgery ($n = 81$)	small ($r = 0.35$)	39	48	61	81
	medium ($r = 0.47$)	21	26	32	42
	large ($r = 0.89$)	5	6	7	8
Cariology ($n = 87$)	small ($r = 0.06$)	1,360	1,712	2,177	2,914
	medium ($r = 0.17$)	168	212	269	359
	large ($r = 0.41$)	28	35	44	58
Conservative dentistry ($n = 31$)	small ($r = 0.23$)	88	110	139	185
	medium ($r = 0.40$)	30	37	46	61
	large ($r = 0.52$)	17	21	26	34

Table 5. Distribution of sample sizes required to achieve various levels of statistical power in research on between-group differences

Category	Effect size	Statistical power			
		60%	70%	80%	90%
All studies (N = 4,038)	small (g = 0.10)	1,017	1,290	1,628	2,178
	medium (g = 0.35)	84	105	133	177
	large (g = 0.86)	15	18	23	30
Biopsychosocial (n = 752)	small (g = 0.05)	3,927	4,947	6,292	8,422
	medium (g = 0.14)	507	638	811	1,086
	large (g = 0.36)	79	99	126	168
Diagnosis (n = 184)	small (g = 0.05)	3,927	4,947	6,292	8,422
	medium (g = 0.18)	294	370	471	629
	large (g = 0.51)	39	49	61	82
Health promotion and prevention (n = 184)	small (g = 0.09)	1,354	1,705	2,168	2,902
	medium (g = 0.27)	139	175	222	297
	large (g = 0.80)	17	21	26	34
Treatment (n = 2795)	small (g = 0.15)	454	572	727	973
	medium (g = 0.50)	41	51	64	85
	large (g = 1.29)	7	9	11	14
Oral surgery (n = 1274)	small (g = 0.08)	62	78	99	133
	medium (g = 0.27)	12	15	19	25
	large (g = 0.66)	4	5	6	7
Orthodontics (n = 199)	small (g = 0.40)	1,655	2,085	2,652	3,550
	medium (g = 0.93)	138	173	220	294
	large (g = 1.87)	24	30	37	50
Periodontology (n = 474)	small (g = 0.11)	824	1,039	1,320	1,767
	medium (g = 0.93)	117	147	187	250
	large (g = 0.63)	26	32	41	55
Cariology (n = 480)	small (g = 0.10)	904	1,139	1,448	1,938
	medium (g = 0.40)	62	78	98	131
	large (g = 1.01)	11	13	16	22
Conservative dentistry (n = 282)	small (g = 0.10)	965	1,215	1,545	2,068
	medium (g = 0.31)	106	133	169	226
	large (g = 0.73)	20	25	31	41
Endodontics (n = 176)	small (g = 0.04)	7,151	9,013	11,462	15,345
	medium (g = 0.19)	275	346	439	589
	large (g = 0.77)	18	22	28	37
Fixed and removable prosthodontics (n = 517)	small (g = 0.56)	23	40	51	68
	medium (g = 1.54)	5	6	8	9
	large (g = 3.35)	2	3	3	3
Temporomandibular joint and masticatory muscle research* (n = 456)	small (g = 0.10)	1,020	1,280	1,630	2,180
	medium (g = 0.30)	80	100	130	180
	large (g = 0.70)	14	17	20	30

* data obtained from the study by Zieliński and Gawda.[14]

(g = 0.09, g = 0.27 and g = 0.80, respectively) that are closer to Cohen's benchmarks, particularly for large effects. Treatment studies have revealed thresholds (g = 0.15, g = 0.50 and g = 1.29, respectively) that closely align with or exceed Cohen's benchmarks, especially for large effects.

A visual representation of the variation in effect sizes within each category highlighted the differences in the distribution of study outcomes (supplementary material 2: Fig. 4,5). The treatment category demonstrates a wide distribution of effect sizes, with a peak around moderate values of Hedges' g and a noticeable tail extending into higher effect sizes. The health promotion and prevention category shows a narrower distribution, with the majority of effect sizes clustering around smaller to moderate values. The diagnosis domain exhibits a sharply peaked distribution, concentrated around smaller effect sizes, with a steep decline as the values increase. The biopsychosocial category has a similarly narrow distribution, with most studies reporting smaller effect sizes and a small proportion extending to moderate values.

The median sample sizes for the case and control groups were 24 and 20 participants, respectively. These sample sizes are insufficient to reliably detect a large (g = 0.86; power = 0.79), medium (g = 0.35; power = 0.20), or small effect (g = 0.10; power = 0.06). Notably, only 6% of the studies included in the analysis were adequately powered to detect a medium effect (g = 0.35) with a statistical power of 0.80. This finding highlights a critical limitation in the statistical power of most studies, emphasizing the need for larger sample sizes in future research to ensure the robustness and reliability of findings.

The data presented in Table 3 further supports the conclusion that inflation bias is unlikely to have a significant impact on dentistry studies that investigate group differences. Across all studies, only 5.19% of results fall within the orange region (marginally significant results: $0.10 \geq p > 0.05$), and 8.00% fall within the red region (just-significant results: $0.05 \geq p > 0.01$). A similar pattern is observed across specific research categories. Treatment studies indicated 4.90% of results in the orange region and 7.61% in the red region. Health promotion and prevention studies showed 6.59% in both regions. Diagnosis studies demonstrated 4.62% in the orange region and 7.34% in the red region. Finally, biopsychosocial studies indicated 3.28% in the orange region and 8.20% in the red region. The relatively low proportion of results in these regions, combined with the high percentage of robustly significant findings in the gray region ($p < 0.01$), suggests that inflation bias, including publication bias or p-hacking, is unlikely to be a major concern in these studies.

The sample size requirements presented in Table 5 provide insights into the feasibility of achieving adequate statistical power in between-group differences research across various dentistry domains.

For all studies combined, detecting small effects (g = 0.10) with 80% power requires substantial sample sizes (n = 1,628), while medium (g = 0.35) and large (g = 0.86) effects require significantly fewer participants (n = 133 and n = 23, respectively). This underscores the challenge of reliably detecting small effects, which necessitate much larger sample sizes compared to medium or large effects.

When examining specific dentistry domains, considerable variability in sample size requirements is evident. In the context of biopsychosocial studies, detecting small effects (g = 0.05) with 80% power demands an extremely large sample size (n = 6,292), while medium (g = 0.14) and large (g = 0.36) effects require 811 and 126 participants, respectively. Similarly, diagnosis studies require large samples to detect small effects (g = 0.05; n = 6,292), with moderate reductions for medium (g = 0.18; n = 471) and large effects (g = 0.51; n = 61). These results highlight the difficulty of achieving sufficient power in studies that target small effects within these domains.

In health promotion and prevention studies, the sample size requirements are comparatively moderate. The detection of small effects (g = 0.09) necessitates a sample size of 2,168 individuals to achieve 80% power, while medium (g = 0.27; n = 222) and large (g = 0.80; n = 26) effects are more easily achievable. Treatment studies, in contrast, have demonstrated the most favorable sample size requirements. For small effects (g = 0.15), a sample of 727 participants is required to attain 80% power, while medium (g = 0.50; n = 64) and large (g = 1.29; n = 11) effects require considerably smaller samples.

Additionally, it is important to observe how sample size requirements vary across different categories of dentistry. For example, to detect small effects with 60% power (g = 0.08) in oral surgery, a sample size of 62 participants is needed. Within the same category, detecting large effects (g = 0.66) would require only 6 individuals. However, under the same assumptions (small effect and 60% power), cariology would require a sample of 904 patients, while detecting large effects in this category would necessitate a study sample of 11 participants. In each of the presented categories of dentistry, the results highlight the difficulty of achieving sufficient statistical power in studies targeting small effects in these areas (Table 5).

For large effects with 90% power, a distinct picture emerges. In oral surgery (g = 0.66), a sample size of 7 individuals is needed; in orthodontics (g = 1.87) – 50; in periodontology (g = 0.63) – 55; in cariology (g = 1.01) – 22; in conservative dentistry (g = 0.73) – 41; in endodontics (g = 0.77) – 37; and in fixed and removable prosthodontics (g = 3.35) – 3.

These findings demonstrate that conducting studies focused on detecting large effects is highly feasible for researchers within each category of dentistry.

Discussion

The growing significance of dental diseases and the increasing proportion of affected individuals is evident. Beyond the percentage-based data, this trend is also reflected in the rising number of scientific publications focused on dental research, as well as in the specific nature of the discipline itself.

The aim of the study was to establish novel, data-driven thresholds for effect sizes (Cohen's d, Hedges' g and Pearson's r) relevant to dental research, rather than relying on general, arbitrary benchmarks that may not adequately reflect the specific characteristics of the field. Additionally, the study offers guidance on the minimum required sample size, contingent upon statistical power. The inclusion of information regarding sample size and effect size calculations in standardized sections of research papers constitutes a key component of transparent reporting.[2]

It is important to acknowledge that, while Cohen's benchmarks serve as a useful comparative tool, their application should not be done without careful consideration of the clinical context.[12,47,48] Cohen's thresholds are arbitrary and fail to account for clinical relevance, domain-specific nuances or individual patient needs.

For this reason, researchers are encouraged to explore alternative approaches and to consider effect size as part of a broader clinical evaluation process, rather than as a definitive indicator of an intervention's value. The clinical significance of a change is not solely determined by its effect size. As Sullivan and Feinn have observed, p-values indicate statistical significance, whereas effect sizes convey the magnitude of the difference.[7] However, it is only within a clinical context that one can assess whether a change holds real value for the patient.[7]

Therefore, when interpreting results, it is essential to consider p-values, effect sizes, patient-reported outcomes, functional performance, and clinical judgment collectively. It is crucial not to rely solely on numerical indicators. Clinical relevance should emerge from a comprehensive analysis that accounts for individual needs, therapeutic decisions, treatment conditions, and the patient's quality of life. From this perspective, the new effect size thresholds do not replace clinical judgment but are intended to serve as a tool to facilitate the interpretation of findings.[7,12,47,48]

The results of this analysis indicate that the majority of observed effect sizes in dental research deviate substantially from the thresholds proposed by Cohen. In particular, the majority of the effects were smaller than Cohen's benchmarks, which calls into question the validity of using Cohen's thresholds as reference points in the field of dentistry.

In the present study, it was observed that for Pearson's r, values of 0.16 (≈ 0.20) represented small effects, 0.40 indicated medium effects and 0.67 (≈ 0.70) corresponded to large effects. For Hedges' g, the established thresholds were 0.10, 0.35 (≈ 0.40) and 0.86 (≈ 0.90). Calculations were also performed separately for individual domains within dentistry, such as oral surgery, orthodontics, periodontology, cariology, conservative dentistry, endodontics, and both fixed and removable prosthodontics (Table 3).

With regard to within-group differences (Pearson's r), Cohen's original thresholds are inadequate for research in dentistry. Our results also exceed those reported by

Gignac and Szodorai for psychological studies,[49] Brydges' estimates in gerontology research[6] and Zieliński for physiotherapy.[12] When comparing the effect sizes obtained in the present study for Hedges' *g* (0.10, 0.40 and 0.90), it can be observed that the thresholds for small effects are consistent with those established for TMJ and masticatory muscle research.[14] However, a discrepancy in the values for medium and large effects is evident. In the broader field of dentistry, medium and large effect size thresholds are elevated by 0.10 and 0.20, respectively. This highlights the specificity of the discipline under investigation.

A significant observation presented in Tables 4 and 5 highlights their value as a framework for planning future studies in individual differences research. The minimum sample size requirements to ensure adequate statistical power vary considerably depending on effect size and the desired power levels. For small effects (r = 0.16), achieving 80% power requires a sample size of 304, increasing to 406 for 90% power. This underscores the need for larger samples to ensure reliable detection of small effects. In contrast, medium effects (r = 0.40) require 46 participants for 80% power, while large effects (r = 0.67) require just 15 participants to achieve the same power level. Tables 4 and 5 provide practical guidelines on the appropriate sample size needed for dental studies across the aforementioned fields of dentistry, based on specific assumptions regarding statistical power and effect size.

Limitations

This study has several limitations that should be acknowledged. First, the investigation was restricted to meta-analyses that were published over a 20-year period. Although this temporal constraint may limit the study's scope, it aligns with the dynamic nature of dental and medical research and reflects current developments in the field.[12,14,49] A key limitation is the potential for systematic biases, such as publication bias, sampling error, and questionable research practices (e.g., *p*-hacking), which may distort the distribution and interpretation of effect sizes.[6,50,51] These risks have been extensively documented in meta-research and are acknowledged in similar studies.[5,6,12,14,49] The study relied solely on published data, assuming that the original authors applied appropriate statistical methods. While this is considered standard practice, there is a risk that the included studies may have failed to meet methodological standards.[5,6,12,14,49] On the other hand, the relatively large sample size strengthens the robustness and generalizability of the findings in comparison to prior studies.[6,14]

In conclusion, the present study proposes updated, empirically-based effect size thresholds for dental research, grounded in discipline-specific data rather than arbitrary general values. These thresholds are not intended to replace clinical evaluation; rather, they are designed to serve as a tool that enhances the interpretation of the results, reporting transparency, and the planning of future studies. The clinical relevance of findings should be assessed by integrating statistical data with patient impact, expert judgment, and the broader healthcare context.

Conclusions

Based on these findings, researchers in the field of dentistry are encouraged to adopt the following thresholds: for Pearson's r, 0.20 for small effects, 0.40 for medium effects and 0.70 for large effects; and for Cohen's d or Hedges' g, 0.10 for small effects, 0.40 for medium effects and 0.90 for large effects. These updated thresholds have the potential to improve the rigor and quality of dental research, ultimately benefiting patients through enhanced diagnostics and treatment strategies.

Ethics approval and consent to participate

Not applicable.

Data availability

The data related to this article, including supplementary materials, can be accessed in the Open Science Framework (OSF) database via the following link: https://osf.io/9fghx/files. The script used in the analysis is available from the corresponding author upon reasonable request.

Consent for publication

Not applicable.

Use of AI and AI-assisted technologies

Not applicable.

ORCID iDs

Grzegorz Zieliński https://orcid.org/0000-0002-2849-0641
Mieszko Więckiewicz https://orcid.org/0000-0003-4953-7143

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Original paper

Transforming TMJ pain relief: Hyaluronic acid's efficacy in focus – a comprehensive systematic review and meta-analysis of randomized controlled trials

Javier Flores-Fraile[1,A,F], Giuseppe Minervini[2,E], Carmen García-Ortega[1,A,B], Jorge Cortés-Bretón-Brinkmann[3,B,C], Santiago Bazal-Bonelli[3,C], Pedro Campos Lopes[4,C], Daniel Sarmiento Govea[5,B], Juan-Antonio Santos-Marino[1,D,E]

[1] Department of Surgery, Faculty of Medicine, University of Salamanca, Spain
[2] Multidisciplinary Department of Medical-Surgical and Dental Specialties, University of Campania Luigi Vanvitelli, Naples, Italy
[3] Department of Dental Clinical Specialties, Faculty of Dentistry, Complutense University of Madrid, Spain
[4] Centre for Interdisciplinary Research in Health, Faculty of Dental Medicine, Universidade Católica Portuguesa, Lisbon, Portugal
[5] Department of Dentistry, Fernando Pessoa Canarias University, Las Palmas, 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

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Address for correspondence
Javier Flores-Fraile
E-mail: j.flores@usal.es

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Abstract

Background. Hyaluronic acid (HA) is a naturally occurring linear polymer with a large molecular size and a simple structure. It is classified as a glycosaminoglycan (GAG), which is a critical element of the extracellular matrix (ECM). Notably hydrophilic, HA has unique qualities such as viscoelasticity, biodegradability and biocompatibility. Its molecular weight (MW) has an influence on its activity, resulting in a wide spectrum of potential effects. Hyaluronic acid and its derivatives are biomaterials with great potential for usage in the medical, dental, pharmaceutical, and cosmetic industries.

Objectives. The aim of the study was to assess the impact of HA on the stomatognathic function of the temporomandibular joint (TMJ).

Material and methods. A meta-analysis was conducted, contrasting HA with alternative TMJ injectable materials, and a review of the literature based on PubMed® publications was carried out.

Results. Hyaluronic acid is considered a safe and effective injectable material for the treatment of TMJ disorders. While HA has shown positive results in clinical applications, it is important to note that other injectable materials may prove equally or more effective, depending on the specific condition and the patient's needs. These alternative materials are being explored to identify the most suitable treatment option for TMJ disorders.

Conclusions. In individuals with TMJ pain and dysfunction, HA has shown safety and effectiveness in reducing pain and enhancing the maximum mouth opening (MMO). However, when compared to platelet-rich plasma (PRP), HA has demonstrated superior long-term results.

Keywords: temporomandibular joint, filling material, hyaluronic acid

Highlights

- Hyaluronic acid (HA) significantly reduces pain in temporomandibular joint (TMJ) disorders while improving the maximum mouth opening (MMO) and overall stomatognathic function.
- Hyaluronic acid is a safe and effective intra-articular material with minimal adverse effects.
- Compared with platelet-rich plasma (PRP), HA shows superior long-term outcomes.
- Current evidence supports HA as a reliable and minimally invasive treatment for TMJ dysfunction.

Introduction

Hyaluronic acid (HA) is a natural and unbranched member of the glycosaminoglycan (GAG) group, which is mostly composed of extracellular matrix (ECM) components. Hyaluronic acid is distinguished from other GAGs due to its large molecular size and a simple structure.[1]

The essential structural elements common to all GAGs are the disaccharide units of an amino sugar and a uronic sugar. However, HA is the only GAG that is not bonded to a core protein and does not undergo postsynthetic modifications. Additionally, it is not produced by Golgi enzymes or sulfated.[2] The main structure of HA is composed of a naturally occurring linear polymer with miles of repeating disaccharide units. Each disaccharide is made up of d-glucuronic acid and N-acetyl-D-glucosamine joined by ß-1,4- and ß-1,3-glycosidic linkages in alternating sequences.[1–3]

Certain enzymes, known as HA synthetases (HAS), are responsible for the synthesis of HA on the inner surface of cell membranes.[3] The human body breaks down HA through various mechanisms. Hyaluronic acid found in blood is broken down systemically in the liver and lymph nodes, while HA present in tissues is broken down extracellularly by reactive oxygen species (ROS) and hyaluronidase enzymes. The molecule regenerates quickly, and its half-life ranges from 12 h to 24 h in the epidermis to a few minutes in the bloodstream. This observation suggests a continuous cycle of synthesis and degradation of this polymer.[3,4]

Hyaluronan with a high molecular weight (HMW-HA) is the original long polymer form of HA. It can be broken down into low molecular weight hyaluronan (LMW-HA) components.[1] Both HMW-HA and LMW-HA can exhibit opposing biological actions.[2]

While LMW-HA is a strong pro-inflammatory molecule that promotes angiogenesis and tissue remodeling throughout the healing process, as well as demonstrates antiapoptotic and immunostimulating activities, HMW-HA has been observed to possess immunosuppressive, antiangiogenic and anti-inflammatory properties.[1,5]

The hydrophilic groups on the HA molecule interact with water molecules as well as one another to form hydrogen bonds, contributing to the high solubility and hydrophilicity of HA.[6] Hyaluronic acid is negatively charged, extremely hydrophilic, and produces a red viscose at HMW due to the carboxyl groups present in the molecule.[4] This network is reliant on molecular weight (MW) and HA concentration, since HA networks become stronger and HA solutions exhibit gradually increased viscosity and viscoelasticity with increasing MW and HA concentration. Hyaluronic acid is negatively charged in an aqueous solution and forms highly hydrophilic cells known as hyaluronan or hyaluronate.[2] Due to its viscoelastic properties, HA can enter tissues with ease and occupy significant amounts of extracellular space. It is a remarkable biomaterial filler, exhibiting a high degree of malleability. The hallmarks of HA solutions include shear thinning, viscoelastic behavior and non-Newtonian behavior. Moreover, HA solutions are not thixotropic; rather, they return to their original viscosity and structure once the shear rate ends. Hyaluronan's distinct rheological behavior is uncommon and crucial since it affects numerous physiological processes as well as the drug, food, medicine, and cosmetic uses of the substance.[2]

Owing to its physicochemical characteristics, HA controls tissue homeostasis, hydration of ECM, and resistance to compression pressures. Numerous proteoglycans interact with HA to produce chemical compounds that stabilize the structure of ECM and maintain the matrix's gel state.[4]

Consequently, these polymers can serve as shock absorbers by withstanding compressive pressures on the cartilage and lubricating the synovial fluid in the joints.[3] Moreover, HA surrounds the majority of cells in a pericellular layer where it operates as a signaling molecule, regulating cell adhesion, motility and proliferation through interactions with binding proteins. During the processes of tissue repair and regeneration, it is present in high concentrations. Consequently, HA plays a crucial part in numerous physiological and pathological circumstances.

Application of HA and its derivatives

Native HA and its derivatives represent intriguing biomaterials for a range of medical applications due to their distinctive biological and physicochemical properties (e.g., biodegradability, biocompatibility, viscoelasticity), as well as their safety profile. Additionally, they can

undergo numerous chemical modifications. While some HA products are being studied for their effectiveness, others are already commercially available and/or in clinical use.[2] The hydrodynamic qualities of HA are crucial in tissue hydration and physical characteristics. Hyaluronic acid is essential to the formation and maintenance of tissue architecture and its mechanical characteristics, largely due to its interactions with ECM proteins.[3] Treatment with HA has been shown to exert a range of positive effects, including immunosuppressive, antiaging, anti-inflammatory, healing, and antiangiogenic properties.[7]

In light of the current state of knowledge, the primary goal of the study was to assess whether HA injections into the temporomandibular joint (TMJ) are beneficial in enhancing the stomatognathic function.

Material and methods

Study design

This systematic review was registered in the International Prospective Register of Systematic Reviews (PROSPERO; registration No. CRD42022321304) and adhered to the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA)[8] standards. The review was designed using the following PICOS (Population, Intervention, Comparison, Outcome, Study design) model:

– Population – adult patients with TMJ pain or dysfunction;
– Intervention – injection of HA into the TMJ;
– Comparison – other injection materials or no intervention;
– Outcome – analgesic effect, improvement of temporomandibular function and prevention of complications;
– Study design – randomized controlled trials (RCTs).

The following PICO question was formulated: "In adult patients diagnosed with TMJ pain and dysfunction (P), what is the efficacy of infiltrating HA (I) in reducing pain, improving temporomandibular function and preventing complications (O) in comparison to other substances or no intervention (C)?"

Inclusion criteria

The present study incorporated RCTs written in English or Spanish that evaluated patients who were administered HA injections into the TMJ, with a minimum follow-up duration of half a year. In the included studies, in addition to HA, another material or technique was used in the control group to improve pain in the TMJ.

Exclusion criteria

Non-randomized prospective or retrospective studies without a control group, studies that did not include individuals of different age groups or sexes, and studies that did not achieve the established objectives were excluded from the analysis.

Data collection

The primary outcome used to assess the effectiveness of HA in TMJ disorders was the degree of joint pain relief. Secondary outcomes included the increase in the maximum mouth opening (MMO), chewing efficiency and quality of life of the patients.

Search strategy

The search was conducted using 4 online databases: Web of Science; Cochrane Library; MEDLINE; and PubMed®. The same search strategy was implemented for all databases. The search terms included "temporomandibular joint" AND "hyaluronic acid", with a time span covering the period from May 1, 2014 to May 1, 2023. The languages considered were English and Spanish. The search was conducted without the application of any filters. In addition, a manual search was carried out using the reference lists of papers found during the database search and of publications concerning the TMJ that were indexed in PubMed®.

Screening and selection procedures

The papers identified through database search and manual search were chosen by 2 reviewers (CGO and SBB) who separately screened the abstracts and titles. The same reviewers examined the full texts of articles that met the inclusion criteria, as well as those that lacked adequate information in the abstract and title to support a conclusion. Disagreements among the reviewers were resolved through consultation with the third reviewer (JCBB). The inter-reviewer reliability in full-text selection was determined by calculating the percentage of agreement and the kappa correlation coefficient. The studies with the longest duration of follow-up were chosen when multiple trials involved the same patient group.

Extraction of clinical data

The data was extracted in triplicate by 2 reviewers (CGO and SBB) independently. The authors were contacted when there were gaps or missing data, and they were asked to provide the missing information. Consequently, the data was not included only when it was unavailable.

The following clinical information was extracted: MMO before and after intervention; pain measured using the visual analogue scale (VAS); authors; year and journal of publication; study design; number of patients; follow-up duration; injection site; type of material injected into each joint; and clinical data.

Risk of bias

The risk of bias in each study was evaluated using the Cochrane method that is aimed at RCTs.

The quality of the evidence was assessed using the GRADE (Grading of Recommendations, Assessment, Development and Evaluation) classification system as follows:

1. High quality of evidence: the true effect is expected to be close to the estimate;
2. Moderate quality of evidence: moderate confidence in the estimated effect. The true effect is considered to be close to the estimate, but there is a possibility that it is different;
3. Low quality of evidence: the estimated effect and the true effect may be different;
4. Very low quality of evidence: very little confidence in the estimated effect. The true effect is likely to be substantially different from the estimated effect.

The adequate level of evidence was assigned to the studies with the use of the GRADEpro 3.2 software (Evidence Prime Inc., Hamilton, USA).

Statistical analysis

The meta-analysis was performed using the Review Manager (RevMan) v. 5.3 software (The Cochrane Collaboration, Copenhagen, Denmark).

The odds ratio (*OR*) was used for the presentation of the dichotomous variables (complications and duration of HA filling). The difference of means (*MD*) with the standard deviation (*SD*) was utilized for the continuous variables (effectiveness of other materials), and the 95% confidence intervals (95% *CI*s) were used for both dichotomous and continuous variables. The differences were deemed significant for $p < 0.05$. To ascertain whether the outcomes had changed, a series of meta-analyses was carried out, with each analysis excluding a single study.

The heterogeneity was determined by examining the overlapping *CI*s in the forest plot and estimating I^2 and χ^2 values.

The χ^2 statistic evaluates the homogeneity of the studies. If the *p*-value is low, the null hypothesis is rejected, and it can be concluded that heterogeneity exists. The I^2 value measures the degree to which the studies agree with each other, and serves as an indicator of any inconsistencies present. Values close to 0% signify little or no heterogeneity, while values exceeding 75% indicate high heterogeneity. There are no universal guidelines for interpreting intermediate values. Generally, when I^2 falls below 30–40%, the heterogeneity is regarded as low. Values ranging from 30% to 60% are classified as moderate, while those between 50% and 90% are considered significant. The test's reliability is compromised due to its level of uncertainty. Higher *p*-values for χ^2 and lower I^2 values are indicative of greater consistency between studies.

Results

Study selection

The initial database search yielded 2 titles in the Cochrane Library and 80 articles in the MEDLINE/PubMed® databases. Two additional documents were discovered during the manual search. Fourteen articles were identified as duplicates and eliminated. The full texts of 21 publications were examined following an initial screening to identify articles that did not align with the PICO criteria. This was followed by screening of titles and abstracts. Ultimately, after the extraction and analysis of the data, a total of 14 papers were chosen for the analysis (Table 1). The steps involved in the selection process are depicted in the flow diagram presented in Fig. 1.

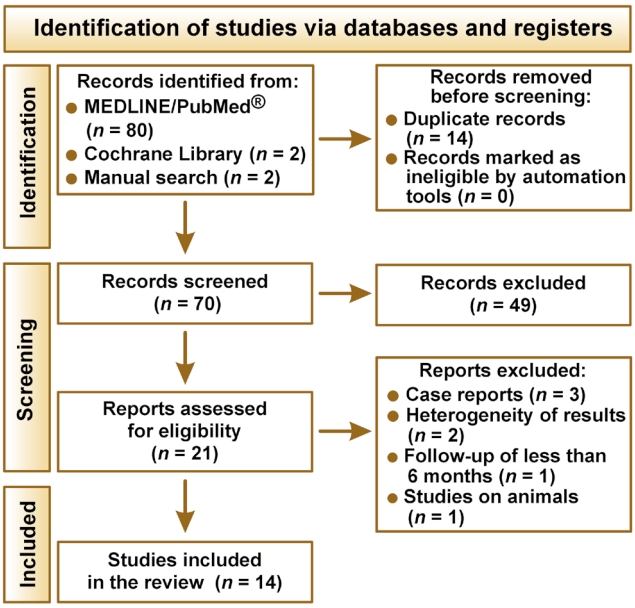

Fig. 1. Flowchart of the study

Synthesis of results

In addition to examining the features of each study and the quantity of events (patients treated with HA and patients treated with other treatment modalities), a comparison was made between the efficacy of HA and other treatments in improving the stomatognathic function of the TMJ.

The results of the meta-analysis are displayed in Fig. 2. A meta-analysis of fixed effects with relative risk has been conducted. The studies depicted on the left side of the forest plot suggest that HA treatment is more successful, while those on the right indicate that alternative therapies yield better outcomes and provide greater support for the stomatognathic function. The overall effect is expressed as a risk ratio (*RR*) of 1.01, with 95% *CI*s of 0.92–1.11 and a *p*-value of 0.81.

Table 1. Studies included in the meta-analysis

Study	Title	Journal	Study design	Treatment area	Conclusions
Yuce and Komerik 2020[10]	Comparison of the efficacy of intra-articular injection of liquid platelet-rich fibrin and hyaluronic acid after in conjunction with arthrocentesis for the treatment of internal temporomandibular joint derangements	Journal of Craniofacial Surgery	RCT	TMJ	All conventional treatment procedures improve MMO and reduce pain. However, arthrocentesis + L-PRF provided superior outcomes.
Marzook et al. 2020[9]	Intra-articular injection of a mixture of hyaluronic acid and corticosteroid versus arthrocentesis in TMJ internal derangement	Journal of Stomatology Oral and Maxillofacial Surgery	RCT	TMJ	The study found improvements in all measured outcomes with no notable variations observed across the groups. Due to its simplicity, intra-articular injection of HA and CS is the treatment of choice.
De Sousa et al. 2020[19]	Different treatments in patients with temporomandibular joint disorders: A comparative randomized study	Medicina (Kaunas)	RCT	TMJ	Long-term success was attained by combining the PRP injection with the splint.
Bergstrand et al. 2019[14]	Long-term effectiveness of arthrocentesis with and without hyaluronic acid injection for treatment of temporomandibular joint osteoarthritis	Journal of Oral Science	RCT	TMJ	The application of arthrocentesis with lavage alone and arthrocentesis with lavage + HA resulted in a long-term enhancement in jaw pain and function.
Gokçe Kutuk et al. 2019[20]	Clinical and radiological comparison of effects of platelet-rich plasma, hyaluronic acid, and corticosteroid injections on temporomandibular joint osteoarthritis	Journal of Craniofacial Surgery	RCT	TMJ	Intra-articular PRP injections decreased the pain on palpation of the TMJ more effectively than HA and CS.
Yilmaz et al. 2019[17]	Comparison of treatment efficacy between hyaluronic acid and arthrocentesis plus hyaluronic acid in internal derangements of temporomandibular joint	Journal of Cranio-Maxillofacial Surgery	RCT	TMJ	The combination of arthrocentesis and HA injection yielded better results regarding chewing efficiency and patient quality of life than a single HA injection.
Toameh et al. 2019[11]	Management of patients with disk displacement without reduction of the temporomandibular joint by arthrocentesis alone, plus hyaluronic acid or plus platelet-rich plasma	Dental and Medical Problems	RCT	TMJ	The PRP group demonstrated better results in terms of pain intensity and chewing efficacy. Arthrocentesis + PRP exhibited superior outcomes in comparison to arthrocentesis + HA or arthrocentesis alone.
Sun et al. 2018[22]	Clinical outcome of sodium hyaluronate injection into the superior and inferior joint space for osteoarthritis of the temporomandibular joint evaluated by cone-beam computed tomography: A retrospective study of 51 patients and 56 joints	Medical Science Monitor	RCT	TMJ	Injection of HA into the upper and lower TMJ space alleviated the clinical signs and symptoms of osteoarthritis, but did not reverse or prevent the progression of bone destruction during long-term and short-term follow-up periods.
Batifol et al. 2018[23]	Effect of intra-articular botulinum toxin injections on temporo-mandibular joint pain	Journal of Stomatology Oral and Maxillofacial Surgery	RCT	TMJ	Intra-articular injection of botulinum toxin is a safe and effective treatment for severe and refractory TMJ pain. Furthermore, no complications were reported.
Bouloux et al. 2017[16]	Is hyaluronic acid or corticosteroid superior to lactated ringer solution in the short-term reduction of temporomandibular joint pain after arthrocentesis? Part 1	Journal of Oral and Maxillofacial Surgery	RCT	TMJ	Arthrocentesis with Ringer's solution is as effective as arthrocentesis with HA or CS in reducing TMJ pain. There were no significant differences between the groups.
Fernández-Ferro et al. 2017[21]	Comparison of intra-articular injection of plasma rich in growth factors versus hyaluronic acid following arthroscopy in the treatment of temporomandibular dysfunction: A randomised prospective study	Journal of Cranio-Maxillofacial Surgery	RCT	TMJ	The injection of PRGF after arthroscopy is more effective in mitigating pain compared to the injection of 1% HA post-procedure, particularly in patients with advanced internal TMJ disorder. Regarding MMO, an increase was observed in both groups, with no significant difference between them.
Cömert Kiliç and Güngörmüş 2016[13]	Is arthrocentesis plus platelet-rich plasma superior to arthrocentesis plus hyaluronic acid for the treatment of temporomandibular joint osteoarthritis: A randomized clinical trial	International Journal of Oral and Maxillofacial Surgery	RCT	TMJ	All MMO and VAS metrics revealed significant clinical improvements following both treatment approaches. Patients respond better to the combination of arthrocentesis with HA injection.
Hegab et al. 2015[12]	Platelet-rich plasma injection as an effective treatment for temporomandibular joint osteoarthritis	Journal of Oral and Maxillofacial Surgery	RCT	TMJ	During long-term follow-up, PRP outperformed acid HA in the treatment of TMJ osteoarthritis with respect to pain reduction and increased MMO.
Gencer et al. 2014[18]	A comparative study on the impact of intra-articular injections of hyaluronic acid, tenoxicam and betametazon on the relief of temporomandibular joint disorder complaints	Journal of Cranio-Maxillofacial Surgery	RCT	TMJ	Better pain relief scores were obtained in the HA group compared to the betamethasone or tenoxicam groups. A notable disadvantage of HA is its relatively high cost.

CS – corticosteroid; HA – hyaluronic acid; L-PRF – liquid platelet-rich fibrin; MMO – maximum mouth opening; PRGF – plasma rich in growth factors; PRP – platelet-rich plasma; RCT – randomized controlled trial; TMJ – temporomandibular joint; VAS – visual analogue scale.

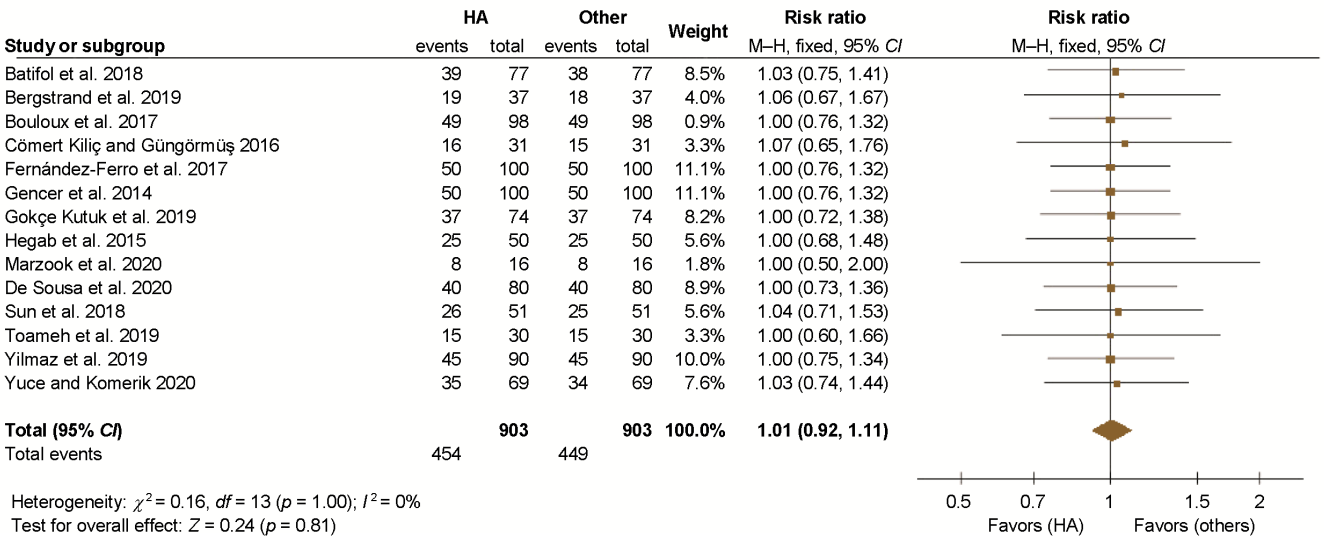

Fig. 2. Results of the meta-analysis evaluating the effectiveness of hyaluronic acid (HA) in improving the stomatognathic function of the temporomandibular joint (TMJ)

CI – confidence interval; *df* – degrees of freedom; M–H – Mantel–Haenszel method.

Studies exhibiting a RR of 1, or, in instances where the RR exceeds 1 but the *CI*s encompass the value of 1, demonstrate that HA and other therapeutic interventions are equally efficacious in enhancing the stomatognathic function of the TMJ. All of the studies demonstrated this outcome.

In general, with a total RR of 1.01 (0.92–1.11), there is no statistical evidence that HA treatment is more effective than another in terms of aesthetics or oral-dentofacial functionality. The sample showed no heterogeneity, with a p-value bigger than 0.05 (p = 1.00) and I^2 of 0%. Therefore, the studies included in the meta-analysis are similar.

Figure 3 presents the funnel plot of the meta-analysis, where the symmetric distribution of the studies is observed. Consequently, the risk of publication bias is low.

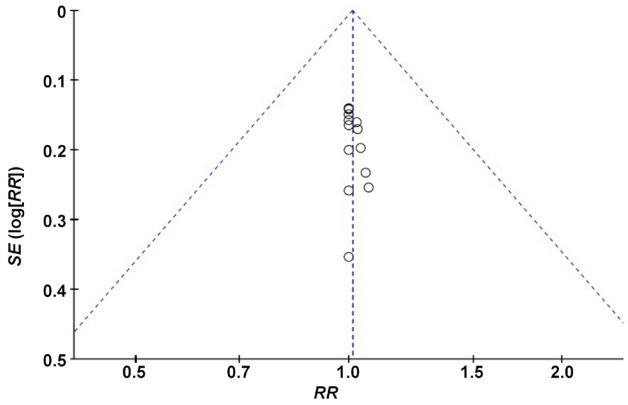

Fig. 3. Funnel plot displaying the heterogeneity between the studies included in the meta-analysis

RR – risk ratio; *SE* – standard error. Heterogeneity: $\chi^2 = 0.16$; $df = 13$ ($p = 1.00$); $I^2 = 0\%$.

Discussion

The use of HA in patients with TMJ pain and dysfunction has been described in numerous studies.

The comparative efficacy of HA and liquid platelet-rich fibrin (L-PRF) injections intraarticularly after arthrocentesis in patients with TMJ pain and dysfunction was assessed in a study by Yuce and Komerik.[10] All techniques have been shown to improve MMO and reduce pain. However, when considering MMO and a consistent improvement in pain reduction, arthrocentesis in conjunction with L-PRF provided superior outcomes.[9,10]

Similar outcomes were observed in the study by Toameh et al., which included individuals with TMJ issues and disc displacement without reduction.[11] When compared to either platelet-rich plasma (PRP) or HA alone, arthrocentesis using Ringer's solution produced a statistically significant improvement in MMO and all other parameters of pain intensity and chewing efficacy.[11]

However, a considerably higher rise in MMO is observed in the PRP and HA groups, and the PRP group demonstrates better results in terms of chewing efficacy and pain intensity. Thus, in the prior study,[11] arthrocentesis + PRP exhibited superior outcomes in comparison to arthrocentesis + HA or arthrocentesis alone. Hegab et al. came to the same conclusions, as they determined that PRP outperformed HA in the long term for the treatment of TMJ osteoarthritis with respect to pain reduction and increased MMO.[12] However, PRP injections should not be considered a first-line therapy option since, according to the experiment conducted by Cömert Kiliç and Güngörmüs, arthrocentesis + PRP injections are not more efficacious than the combination of arthrocentesis with a single HA injection.[13]

Arthrocentesis using Ringer's solution was also implemented in a RCT by Marzook et al., and intra-articular injection was compared to a mixture of 0.5 mL of HA and an equal quantity of corticosteroid (CS).[9] The study

found improvements in all measured outcomes, with no notable variations observed across the groups.[9] Due to its simplicity, intra-articular injection of HA and CS has been the treatment of choice for internal TMJ dysfunction with reduction.[9] In addition, Bergstrand et al. examined the effects of arthrocentesis with lavage alone compared to arthrocentesis with lavage + HA in patients with osteoarthritis.[14] Arthrocentesis of the TMJ increased the stomatognathic function and reduced pain. Although MMO levels increased in all groups, no significant differences were observed in MMO or reported pain levels. No discernible improvement in joint sounds was observed within the groups. The long-term application of both approaches resulted in a significant enhancement in jaw discomfort and function. Compared to arthrocentesis with lavage alone, arthrocentesis with lavage + HA did not demonstrate a significant advantage.[14,15]

In the study by Bouloux et al., the authors noted that arthrocentesis with Ringer's solution is as effective as arthrocentesis with HA or CS in reducing TMJ pain.[16] There were no significant differences between the 3 groups.[16]

Yilmaz et al. posit that arthrocentesis + HA injection and the administration of a single HA injection without arthrocentesis effectively alleviated signs and symptoms in patients with reduced and non-reduced disc displacement-related joint pain, with the exception of joint sounds.[17] However, the combination of arthrocentesis and HA injection yielded better results regarding chewing efficiency and patient quality of life than a single HA injection.[17]

Gencer et al. compared 3 anti-inflammatory agents.[18] Better pain relief scores were obtained in the HA group (intra-articular injection of Hyalgan® 10 mg/mL) compared to the betamethasone or tenoxicam groups. A notable disadvantage of HA is its relatively higher cost in comparison to the other 2 agents. Despite the lower scores of intra-articular tenoxicam or betamethasone, they demonstrated better outcomes than the control group and can be considered more economical alternatives to intra-articular HA injections.[18]

De Sousa et al. examined the impact of various therapies on patients with TMJ arthralgia.[19] Every study participant wore a night bite splint. Some individuals received merely the splint, while others were administered injections of betamethasone (7 mg/mL), PRP or sodium hyaluronate (Hyalart® 10 mg/mL). The administration of each treatment resulted in a decrease in pain and an increase in MMO. By the conclusion of the first week, the patients receiving betamethasone or HA treatments demonstrated the most optimal outcomes. However, a long-term success rate was higher when the splint was used in conjunction with the PRP injection.[19] In the RCT conducted by Gokçe Kutuk et al. in patients with TMJ pain and TMJ osteoarthritis, the authors showed that intra-articular PRP injections decrease pain on palpation more effectively than HA and CS.[20]

Fernández-Ferro et al. noted that the injection of plasma rich in growth factors (PRGF) after arthroscopy is more effective in mitigating pain compared to the injection of 1% HA post-procedure, particularly in patients with advanced internal TMJ disorder.[21] Regarding MMO, an increase was observed in both groups, with no significant difference between them.[21]

Sun et al. evaluated the clinical effects of injecting 20 mg of HA in the upper and lower joint space for the treatment of osteoarthritis.[22] The injection alleviated the clinical signs and symptoms of osteoarthritis but did not reverse or prevent the progression of bone destruction during short- and long-term follow-up periods.[22]

Lastly, Batifol et al. reported a course of treatment for severe and refractory TMJ discomfort that included tongue splints, physical therapy, intramuscular botulinum toxin injections, and HA injections prior to intra-articular Botox® (botulinum toxin type A) injections.[23] According to this study, a botulinum toxin injection is a risk-free, non-surgical treatment for severe and refractory temporal bone pain.[23–30]

Conclusions

Hyaluronic acid improves the stomatognathic function in patients with TMJ dysfunction and associated pain. Additionally, it has been demonstrated to be both effective and safe in reducing pain and increasing MMO. In comparison to PRP, HA has shown superior long-term results.

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.

Use of AI and AI-assisted technologies

Not applicable.

ORCID iDs

Javier Flores-Fraile https://orcid.org/0000-0002-7739-7179
Giuseppe Minervini https://orcid.org/0000-0002-8309-1272
Carmen García-Ortega https://orcid.org/0009-0005-4123-991X
Jorge Cortés-Bretón-Brinkmann https://orcid.org/0000-0003-1701-8867
Santiago Bazal-Bonelli https://orcid.org/0000-0001-8301-0682
Pedro Campos Lopes https://orcid.org/0000-0002-8176-6977
Daniel Sarmiento Govea http://orcid.org/0009-0004-2585-4552
Juan-Antonio Santos-Marino https://orcid.org/0000-0003-4950-5424

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Review

Short-term follow-up complications associated with inferior alveolar nerve block using different anesthetics: A systematic review

Maral Berenova[1,B–D], Ifrah Sarfaraz[1,C,D], Selma Pascoal[2,C–E], João Gião Carvalho[1,C,E], José Paulo Macedo[1,C,E], Jorge Pereira[1,A,C–F]

[1] RISE-Health, FP-I3ID, FP-BHS, School of Dentistry, Faculty of Health Sciences, University Fernando Pessoa, Porto, Portugal
[2] School of Dentistry, UNIPRO – Oral Pathology and Rehabilitation Research Unit, University Institute of Health Sciences-Advanced Polytechnic and University Cooperative (IUCS-CESPU), Gandra, Portugal

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation;
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Address for correspondence
Jorge Pereira
E-mail: jpereira@ufp.edu.pt

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Abstract

Inferior alveolar nerve block (IANB) is considered the most widely used anesthetic technique and the gold standard for blocking the hemimandible. This method is used in routine dental and oral surgical practice. The aim of this systematic review was to analyze reports related to the IANB technique combined with different local anesthetics. The PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines were adopted to identify relevant studies, and the PICO (Patient/Population, Intervention, Comparison, and Outcomes) criteria were used to structure the research question. The literature search was conducted using PubMed/MEDLINE, Cochrane Library and Embase databases. The search was undertaken without temporal constraints. Prospective randomized clinical trials and randomized controlled trials were used as filters. Inclusion and exclusion criteria were chosen to initially select the appropriate articles from the published titles, followed by abstract reading. After evaluating the selected articles, the results of the research indicated that no relevant side effects were noted in any of the groups, irrespective of the anesthetic solution utilized. However, it is important to acknowledge that a follow-up period of 1 day may be too short to observe subsequent complications, evolution, or spontaneous remission of its eventual sequelae. Therefore, future randomized controlled clinical trials with large samples and longer follow-up periods are required to confirm these findings.

Keywords: complications, inferior alveolar nerve block, articaine 4%, lidocaine 2%, mepivacaine 3%

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Highlights

- Inferior alveolar nerve block (IANB) shows short-term safety in dental procedures, regardless of the anesthetic agent used.
- Mild, transient complications such as injection site pain and lip biting occurred in 6.32% of cases.
- Articaine, lidocaine and mepivacaine demonstrated comparable safety; epinephrine did not affect complication rates.
- Inferior alveolar nerve block remains safe and effective in both pediatric and adult participants when supported by careful patient selection and clinical vigilance.

Introduction

Traditionally, inferior alveolar nerve block (IANB) is considered the most widely used anesthetic technique and the gold standard for blocking the hemimandible. This method is employed in routine dental and oral surgical practice. When combined with lingual nerve and long buccal nerve block, it provides adequate anesthesia of a wide anatomical area. This includes one side of the mandibular teeth and gingivae, the body and inferior ramus of the mandible, the anterior two-thirds of the tongue and the floor of the mouth.[1–3]

Many surgical procedures on the mandible can benefit from IANB, such as tooth extraction, surgical reconstruction, root canal treatment, periodontal treatment, and stabilization in cases of traumatic injury and fracture.[4–6]

The identification of anatomical landmarks is of the utmost importance.[7] To improve upon the conventional IANB technique, microprocessor-aided electronic devices with digital controls can be used to facilitate aspiration and continuous delivery of local anesthetic solution. This approach is assumed to be less threatening and less painful.[8,9]

However, the use of this technique has been previously associated with risks and complications, and the precise mechanism of nerve injury is still discussed. The potential consequences of this procedure may manifest as direct trauma or be caused by the neurotoxicity of the local anesthetic solution chosen.[10,11]

As a result of direct trauma, the potential sources of injury include the injection needle, which can cause neural or vascular injury (with the facial nerve being the most frequently affected when the anesthetic solution is applied inside the parotid gland). Other possible causes of injury include hematoma and associated trismus, intravascular injection, mucosal and muscular injury, needle fracture, and post-injection infection related to its contamination.[3,12,13]

The occurrence of adverse effects has been associated with the neurotoxicity of the local anesthetic solution. Allergic reactions have been observed in association with amide local anesthetics. Furthermore, the presence of high concentrations of any local anesthetic in the bloodstream has been documented in cases of multiples injections, excessive doses of the anesthetic solution, or intravascular injection. Also, methemoglobinemia is a reported side effect resulting from an accumulation of metabolites from the anesthetic solution.[3,14,15]

Local anesthetics are differentiated based on their chemical structure, specifically the linkage (the amide linkage vs. the ester linkage) between the elements of the compound. Articaine, lidocaine and mepivacaine are the most commonly used local anesthetic agents in clinical dentistry. Lidocaine and mepivacaine are classified as amide-type local anesthetics. However, articaine, another amide-type anesthetic agent, contains an additional ester linkage. While both types of local anesthetics share the same mechanism of action, they differ slightly in their metabolic processes, binding to cellular sodium channels, and inhibiting the influx of sodium into the cell. This inhibition prevents cell depolarization and subsequent transmission of the previously propagating action potential.[8,16]

The selection of an appropriate local anesthetic for a patient necessitates the consideration of several factors, such as surgical time extension, the possibility of self-mutilation in the postoperative period, the necessity for hemostasis, the potential need for post-treatment pain control, and the presence of any relative or absolute contraindications to the local anesthetic solution selected for administration.[15]

As adverse events can occur due to trauma or the anesthetic solution, it is important to carefully select the injection method and solution. These factors are essential for a successful and secure procedure.

Thus, the objective of the present study was to extract and analyze available data on the IANB technique combined with different local anesthetics (2% lidocaine with 1:80,000 epinephrine, 2% lidocaine with 1:100,000 epinephrine, 3% plain mepivacaine, 4% plain articaine, 4% articaine with 1:100,000 epinephrine, 4% articaine with 1:200,000 epinephrine, and 2% articaine with 1:200,000 epinephrine) in pediatric and adult patients. The study aimed to provide valid evidence for comparing results concerning possible complications.

Material and methods

Methodology

The PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines were adopted for the current review.[17,18]

Formulation of research question and keyword selection

The PICO (Patient/Population, Intervention, Comparison, and Outcomes) approach was used to structure and respond to the research question. It was found that higher precision and improved relevance of search results can be achieved through the use of PICO templates.[19]

The research question was formulated using the PICO criteria, as follows: "Are there different complications (O) reported by patients (P) who underwent IANB (I) with different anesthetics (C)?". The following keywords and Medical Subject Headings (MeSH) were used for the search according to the research question: ("complications" OR "side-effects" OR "adverse reaction") AND ("IANB" OR "inferior alveolar nerve block") AND ("anesthetics" OR "articaine 4%" OR "mepivacaine 3%" OR "lidocaine 2%"). The applied filters included clinical trials and randomized controlled trials.

Search strategy

A literature search was performed using the PubMed/MEDLINE, Cochrane Library and Embase databases. Keywords and MeSH terms were searched individually and combined with Boolean operators (AND and OR). No systematic review was found that specifically addressed our research question under the defined criteria, which further justifies our decision to conduct this review. The literature search was carried out from January 24 to February 8, 2022.

Eligibility criteria

The following inclusion criteria were used in the study: articles with patients who underwent IANB; articles published worldwide and written in English with full access; no timeline restrictions; prospective, randomized clinical trials, or randomized controlled trials; articles reporting complications associated with IANB.

Animal studies, books, case–control studies, case reports and case series, cross-sectional studies, cohort studies, commentaries and conference papers, gray literature, meta-analyses, policies and guidelines, unpublished data, and review articles were excluded from the study.

Study selection process

As a result of the systematic literature review, 41 articles were identified: 14 from PubMed/MEDLINE; 24 from Cochrane Library; and 3 from Embase. After removing the duplicates (n = 17), a preliminary screening of titles and abstracts was performed. Ten articles were excluded because they did not meet the eligibility criteria. After revising the full texts of the remaining 14 articles, 8 studies were excluded because they did not meet the inclusion criteria for this systematic review. A total of 6 studies met the inclusion criteria and were selected for analysis and data extraction in accordance with the PRISMA recommendations. A flowchart of the study is presented in Fig. 1.

Quality assessment tool

The Cochrane risk-of-bias (RoB) tool for randomized controlled trials was used to assess the quality of the included studies. If all criteria were met (low risk for every domain), the study was labeled "good". If 1 criterion was not met (high risk in any domain), then the study was considered "fair", and if 2 or more criteria were not met (high risk or unclear risk in more than 2 domains), the study was labeled "poor".[20]

Results

Following a thorough examination of titles, abstracts and full texts of the articles, 6 following studies were identified and included in the systematic review: Elbay et al.[21] (study 1); Kämmerer et al.[22] (study 2); Youssef et al.[23] (study 3); Kämmerer et al.[24] (study 4); Figueiredo et al.[25] (study 5); and Alamoudi et al.[2] (study 6). These studies were categorized as randomized clinical trials. A comprehensive overview of the 6 studies, accompanied by a quality analysis, is presented in Table 1 and Fig. 2–5.

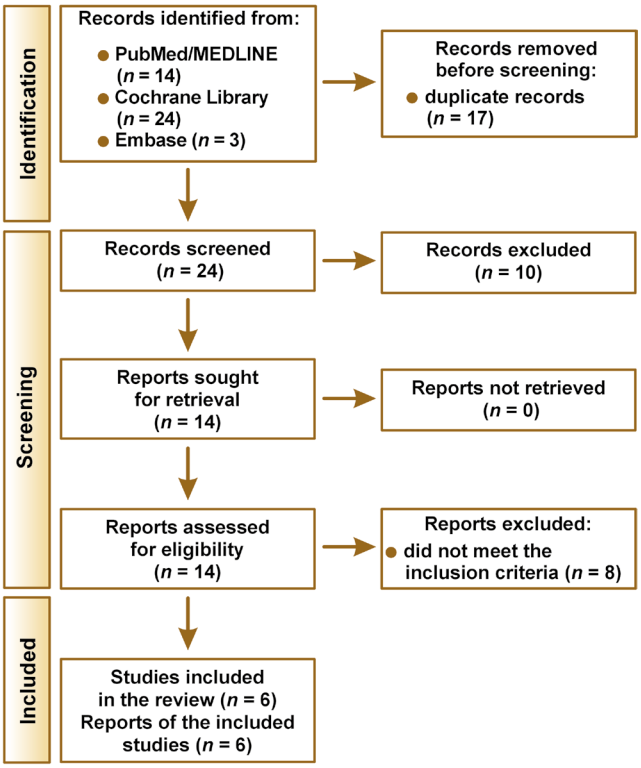

Fig. 1. PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) diagram for the selection of studies to be included in the systematic review

Table 1. Characteristics of the included studies

Study No.	Study	Title	Study design	Patients, *n*	Age [years]	Sex	Anesthetic solution	Technique
1	Elbay et al. 2016[21]	Effects of two different anesthetic solutions on injection pain, efficacy, and duration of soft-tissue anesthesia with inferior alveolar nerve block for primary molars	randomized, controlled crossover, double-blind clinical trial	60	6–12	male and female	3% mepivacaine, 2% lidocaine with 1:80,000 epinephrine	IANB ($n = 120$)
2	Kämmerer et al. 2012[22]	Comparison of 4% articaine with epinephrine (1:100,000) and without epinephrine in inferior alveolar block for tooth extraction: Double-blind randomized clinical trial of anesthetic efficacy	clinical prospective, randomized, double-blind study	88	18–80	male and female	4% articaine with 1:100,000 epinephrine, 4% articaine without epinephrine	IANB ($n = 88$)
3	Youssef et al. 2021[23]	RCT on the effectiveness of the intraligamentary anesthesia and inferior alveolar nerve block on pain during dental treatment	randomized prospective clinical trial	72	18–50	male and female	4% articaine with 1:100,000 epinephrine	IANB ($n = 36$)
4	Kämmerer et al. 2017[24]	Comparison of anesthetic efficacy of 2 and 4% articaine in inferior alveolar nerve block for tooth extraction – a double-blinded randomized clinical trial	clinical prospective, randomized, double-blind trial	95	19–77	male and female	2% articaine with 1:200,000 epinephrine, 4% articaine with 1:200,000 epinephrine	IANB ($n = 95$)
5	Figueiredo et al. 2021[25]	Is it possible to extract lower third molars with infiltration anesthesia techniques using articaine? A double-blind randomized clinical trial	randomized, double-blind clinical trial	118	18–60	male and female	4% articaine with 1:100,000 epinephrine	IANB ($n = 59$)
6	Alamoudi et al. 2016[2]	The effectiveness of computerized anesthesia in primary mandibular molar pulpotomy: A randomized controlled trial	controlled, randomized, double-blind clinical trial	91	5–9	male and female	3% mepivacaine, 2% lidocaine with 1:80,000 epinephrine	IANB ($n = 61$)

IANB – inferior alveolar nerve block; CCDS – computerized controlled delivery system; ILA – intraligamentary anesthesia.

Time of complication report	Complications	Conclusions
no information	None of the patients reported postoperative complications severe enough to require clinical treatment. Two individuals (1.67%) presented with lip biting. None of the patients reported the presence of hematoma, swelling or infection.	Plain mepivacaine and 2% lidocaine with 1:80,000 epinephrine administered by IANB anesthesia via CCDS were equally effective for both primary mandibular molar extraction and pulpotomy. The pain experienced during the injection was more pronounced in the case of 3% mepivacaine compared to 2% lidocaine with 1:80,000 epinephrine. The duration of anesthesia was shorter in the case of mepivacaine compared to lidocaine. Plain mepivacaine and 2% lidocaine with 1:80,000 epinephrine showed similar results in terms of postoperative complications.
24 h	No adverse reactions were reported by the patients or observed by the surgeons during or after the procedure.	Differences between the 2 solutions were observed in terms of the time of onset and the duration of anesthesia. The administration of 4% articaine solution without epinephrine did not influence the clinical efficacy in terms of several anesthetic properties (need of a secondary injection, pain during injection, intra- and postoperative pain). The duration of the local anesthesia without epinephrine was reduced, and postoperative pain remained unchanged. This suggests that the use of local anesthesia without epinephrine could enhance patient comfort after treatment. Therefore, it is possible to successfully use the formulation of 4% articaine without epinephrine for dental extractions in the mandible following IANB.
24 h	A total of 5 (13.89%) patients in the IANB group reported temporary irritations 24 h after the procedure. One individual (2.78%) reported difficulty talking for 1 day after the anesthesia, 3 individuals (8.33%) reported pain at the injection site, and 1 patient (2.78%) experienced pain around the ear after the injection.	ILA has shown to be a safe and reliable method of local anesthesia for the treatment of lower premolars and molars, with a success rate comparable to that of IANB without complications or temporary irritations. Thus, ILA can be considered an effective alternative to IANB for routine dental treatment to reduce known side effects of IANB.
24 h	In the course of the study, as well as in the course of other clinical trials comparing 2% and 4% articaine solutions, no significant side effects were observed in any of the groups.	The local anesthetic effect of the 4% articaine solution does not demontrate a statisticaly significant increase over that of 2% articaine solution for the purpose of mandibular tooth extraction.
no information	No relevant adverse effects, either local complications (local irritation or discomfort) or systemic side effects (palpitations, nausea, vomiting, or dizziness) were reported.	IANB with additional buccal infiltration is more suitable for achieving adequate analgesia in lower third molar extractions than the experimental technique (infiltration in the buccal and lingual areas). Moreover, the standard method is considered safe and provides a shorter onset time and lower initial postoperative pain levels.
24 h	No complications or side effects were immediately observed. After 24 h, 20 patients (32.79%) reported pain at the injection site and 2 individuals (3.28%) presented with lip biting.	IANB and ILA using CCDS were as effective as the gold standard techniques for anesthetizing mandibular second primary molars during all 5 steps of pulpotomy. Therefore, they could be used as an alternative technique. During the pulpotomy procedures, ILA employing CCDS resulted in a delivery of a greater amount of anesthesia to the main nerve supply of the tooth; however, a lower amount of anesthesia was used than that of IANB. This difference was not statistically significant. Postoperative pain exhibited a stronger correlation with intraligamental injection than with both IANB anesthesia techniques, but the difference was not statistically significant.

Characteristics of the included studies

The characteristics of the studies included in this review are summarized in Table 1. Study 1, by Elbay et al., compared the behavior of 3% mepivacaine vs. 2% lidocaine with 1:80,000 epinephrine in computer-assisted IANB.[21] The study by Kämmerer et al. (study 2) compared 4% articaine/1:100,000 epinephrine vs. 4% articaine without epinephrine in IANB.[22] Study 3, by Youssef et al., compared the use of 4% articaine/1:100,000 epinephrine in intraligamentary anesthesia vs. IANB.[23] The study by Kämmerer et al. (study 4) compared 2% vs. 4% articaine/1:200,000 epinephrine in IANB.[24] Figueiredo et al. (study 5) compared infiltrative anesthesia vs. 4% articaine/1:100,000 epinephrine in IANB.[25] The study by Alamoudi et al. (study 6) compared 2% lidocaine/1:100,000 epinephrine in traditional IANB with computer-assisted IANB and computer-assisted intraligamentary anesthesia.[2] Of the 524 randomized subjects, 459 underwent IANB and reported 29 complications (studies 1, 3 and 6), corresponding to 6.32% of all IANB procedures. Telephone calls were made in studies 2, 3, 4, and 6 to assess participants within the first 24 h. Study 1 reported 2 cases of side effects, 1 using 3% mepivacaine and 1 using 2% lidocaine with 1:80,000 epinephrine. Study 3 reported 5 cases using 4% articaine with 1:100,000 epinephrine, while study 6 reported 22 cases using 2% lidocaine with 1:100,000 epinephrine (Fig. 3). The most common side effect was pain at the injection site, corresponding to 5.01% (n = 23) of all cases, followed by lip biting with 0.87% (n = 4), difficulty talking with 0.22% (n = 1), and pain around the ear with 0.22% (n = 1) (Fig. 4). The age range of participants in the 6 included studies was 5–80 years. All of the studies included in this review were published between 2012 and 2021.

Quality assessment of the included studies

The quality assessment of the included studies, along with the most relevant elements of the systematization process, are presented in Fig. 2. The RoB 2 tool was used to evaluate the risk of bias of each study across 5 domains, and to provide an overall evaluation for each trial.[26]

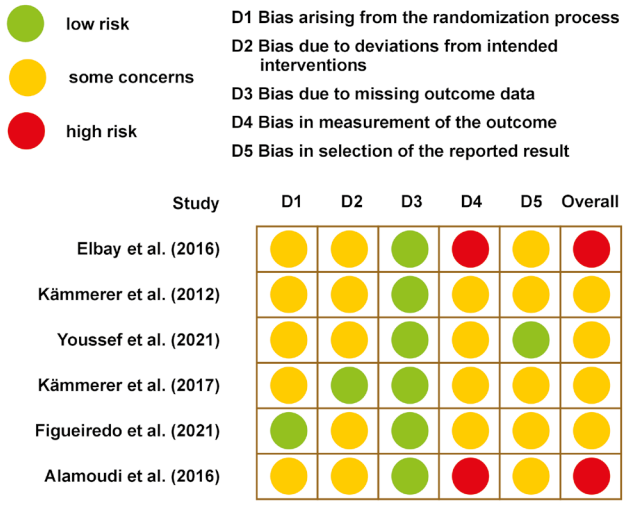

Fig. 2. Assessment of the risk of bias for the included trials (n = 6)

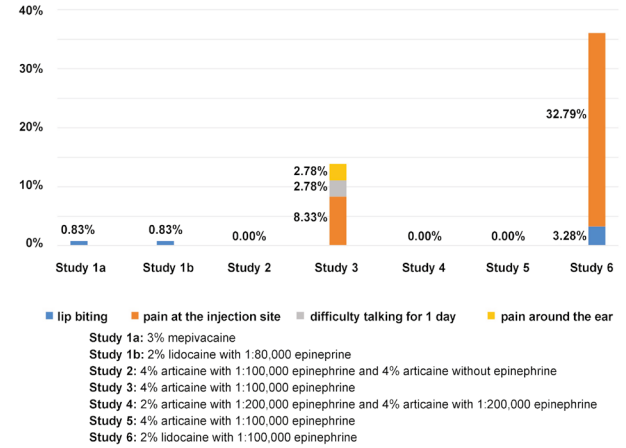

Fig. 3. Distribution of complications reported in the included studies

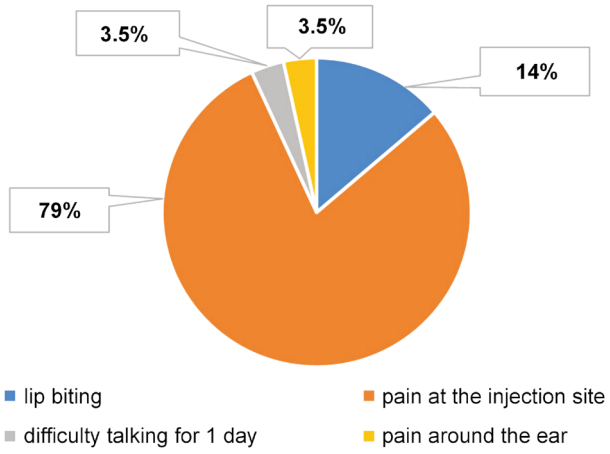

Fig. 4. Distribution of the types of reported complications

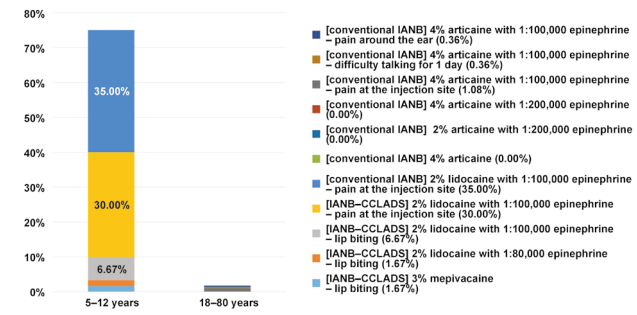

Fig. 5. Distribution of reported complications based on the age of patients and the method of injection

IANB – inferior alveolar nerve block; CCLADS – computer-controlled local anesthetic device.

In study 1, details regarding randomization of allocation are given explicitly, which makes this study free from allocation bias. However, the methodology does not include any information regarding the concealment of allocation. The sample size of the study, the age and sex of the participants, as well as the study source are given. The anesthetic solution was composed of 3% mepivacaine and 2% lidocaine. The double-blindness of the study is mentioned. The study revealed a lack of information concerning patient/caregiver awareness regarding the nature of the intervention being conducted. Additionally, practitioners were blinded to this information. Thus, there is an indication of concerns regarding bias arising from deviations from intended interventions. The outcomes of the randomized participants were documented, and the bias resulting from missing outcome data was low. Parents were informed and advised to call if they observed any postoperative complications. They were also instructed to document the levels (none, mild, moderate) of complications. Parents who are emotionally attached to their child often report complications in excess, resulting in a potential for bias in the measurement of the outcome. Bias in the selection of the reported result shows some concerns. Thus, the overall risk of bias in the study is considered high.

In study 2, the randomization of allocation is described in detail, thereby ensuring that the study is free from allocation bias. The allocation process was performed through the utilization of an online randomization generator, and no information was disclosed regarding the concealment of allocation. The sample size of the study, the age and sex of the participants, and the source of the study are given. The anesthetic solution was composed of 4% articaine and 1:100,000 epinephrine or 4% articaine without epinephrine. The information regarding the blinding of participants and caregivers was not provided. However, the study was double-blind, and the individuals who delivered the intervention were blinded in the study. The absence of information regarding the deviations has given rise to some concerns regarding the possiblity of bias due to deviations from intended interventions. The outcomes of the randomized participants were documented, and the bias resulting from missing outcome data was low. The study did not provide any information regarding outcome measurement. Furthermore, the outcome assessor was blinded. This study did not address the methodology employed to assess complications, which suggests potential concerns regarding the bias in measurement of the outcome. Similarly, concerns have been raised regarding the selection of the reported results. Thus, the overall risk of bias in the study is indicative of some concerns.

In study 3, the randomization of allocation is explicitly delineated, thereby ensuring the study is free from allocation bias. The sample size of the study, as well as the age and sex of the participants, are given. The anesthetic solution was composed of 4% articaine and 1:100,000 epinephrine. The blinding of the participants and caregivers was not mentioned in the study. However, the study was double-blind, and the individuals who delivered the intervention were blinded in the study. The absence of information regarding deviations gives rise to some concerns regarding bias resulting from deviations from intended interventions. The outcomes of the participants who were randomized were disclosed, and the bias resulting from missing outcome data was low. The study does not provide any information regarding the measurement of the outcomes. Furthermore, the individual responsible for assessing the outcomes was blinded. The study did not address the methodology employed to assess complications, which suggests some concerns regarding the bias in measurement of the outcome. The selection of reported results exhibited a low risk of bias. Thus, the overall risk of bias in the study is indicative of some concerns.

In study 4, the details regarding randomization of allocation are provided, which makes the study free from allocation bias. The sample size of the study, as well as the age and sex of the participants, are given. The anesthetic solution was composed of 2% articaine or 4% articaine. The study in question was a double-blind clinical trial. Informed consent was obtained from all eligible participants. However, the study's methodology did not address whether the participants and caregivers were blinded. The individuals responsible for delivering the intervention were blinded, leading to a low bias owing to deviations from intended interventions. The outcomes of the randomized participants were disclosed, and the bias resulting from missing outcome data was low. No information regarding the measurement of outcomes was given. Furthermore, the outcome assessor was blinded. The methodology regarding the assessment of complications was not addressed in the study. Consequently, some concerns regarding bias in the measurement of the outcome were identified. Similarly, the selection of the reported results shows some concerns. Thus, the overall risk of bias in the study is indicative of some concerns.

In study 5, the randomization of allocation is described in detail, thereby ensuring that the study is free from allocation bias. The sample size of the study, as well as the age and sex of the participants are given. The anesthetic solution was composed of 4% articaine and 1:100,000 epinephrine. The study in question was a double-blind clinical trial. Informed consent was obtained from all eligible participants; however, it was not mentioned whether participants and caregivers were blinded or not. The individuals who delivered the intervention were blinded. Therefore, the bias caused by deviations from intended interventions has given rise to some concerns. The outcomes of all randomized participants were documented, and the bias resulting from missing outcome data was low. The study does not provide any information regarding the measurement of outcomes. The individual responsible for

assessing the outcomes was blinded. The study did not address the methodology employed to assess complications, which suggests potential concerns regarding the bias in the measurement of the outcome. Similarly, concerns have been raised regarding the selection of the reported results. Thus, the overall risk of bias in the study is indicative of some concerns.

In study 6, the randomization of allocation was transparent, employing a block randomization technique to assign participants to one of the study groups. However, no information was given regarding the concealment of the allocation sequence until the participants had been enrolled and assigned to interventions. The sample size of the study, as well as the age and sex of the participants, are given. The anesthetic solution was composed of 2% lidocaine and 1:100,000 epinephrine. The study in question was a double-blind clinical trial. Informed consent was obtained from all eligible participants; yet, the study did not mention whether or not the participants and the caregivers were blinded. Nonetheless, the individuals responsible for delivering the intervention were blinded, and no information was disclosed regarding any deviations from intended interventions, resulting in some concerns in this aspect. The outcomes for all randomized participants were documented, and the bias resulting from missing outcome data was low. Potential complications were assessed after 24 h on phone call. The presence of a potential risk of information bias was observed, and a high degree of bias was identified in the measurement of the outcome. Similarly, concerns have been raised regarding the selection of the reported results. Therefore, the overall risk of bias in the study is high.

Overall, 2 trials were identified as being at high risk of bias (33.3%), while 4 trials were evaluated as having some concerns (66.7%). All studies demonstrated a low risk of bias for missing outcome data (100.0%). Five studies indicated some concerns regarding the randomization process and deviations from intended interventions (83.3%), while only 1 study exhibited a low risk of bias for the randomization process (16.7%), and 1 study demonstrated a low risk of bias for deviations from intended interventions (16.7%). Of the 6 trials, 2 showed a high risk of bias for measurement of the outcome (33.3%), and 4 studies demonstrated some concerns (66.7%). One study (16.7%) indicated a low risk of bias due to the selection of the reported result, and 5 studies exhibited some concerns (83.3%) (Fig. 2).

Discussion

Inferior alveolar nerve block is the most frequently used technique for achieving local anesthesia for restorative and surgical procedures, especially in mandibular molars.[1] The main goal of this technique is to effectively anesthetize all teeth in the same mandibular quadrant, as well as the gingival mucosa, the body and inferior ramus of the mandible, the anterior two-thirds of the tongue, and the floor of the mouth. Despite its reputation as a safe technique, there is a degree of risk involved.[27,28]

A total of 524 patients (151 children and 373 adults) were evaluated and 7 different anesthetic solutions were included in this systematic review (2% lidocaine with 1:80,000 epinephrine, 2% lidocaine with 1:100,000 epinephrine, 3% mepivacaine, 4% plain articaine, 4% articaine with 1:100,000 epinephrine, 4% articaine with 1:200,000 epinephrine, and 2% articaine with 1:200,000 epinephrine).

With regard to studies conducted on children's groups, all of them employed the following exclusion criteria: children who were medically compromised (i.e., those with allergies to local anesthetics or sulfites, or a history of significant medical conditions); and children who demonstrated uncooperative behavior.

Elbay et al. conducted a study with 60 children ranging in age from 6 to 12 years.[21] The study compared IANB using 2% lidocaine with 1:80,000 epinephrine and 3% plain mepivacaine. The results indicated that none of the patients reported postoperative complications severe enough to require clinical treatment.[21]

In regard to the experience of pain, the postoperative pain exhibited no significant variation between the 2 anesthetics or the 2 groups observed by Elbay et al., the first group undergoing pulpotomy and the second one under extraction.[21] In contrast, Alamoudi et al. associated 35.5% of postoperative pain after IANB procedure with 2% lidocaine and 1:100,000 epinephrine.[2]

In the study conducted by Alamoudi et al., no complications or side effects were observed immediately after the procedure.[2] After 24 h, all legal guardians of the children were contacted to ascertain whether any postoperative complications had been observed. Two patients (6.66%) in the IANB group reported lip biting.[2]

Elbay et al. found no significant differences between the 2 groups in terms of postoperative complications such as lip or tongue biting, bleeding or hematoma.[21] The occurrence of lip biting was documented in only 1 patient treated with 2% lidocaine and 1:80,000 epinephrine, and 1 patient treated with 3% mepivacaine. With regard to the occurrence of bleeding, no significant difference was observed between the 2 solutions. None of the patients required surgical procedures for hemostasis; however, 5 patients treated with 2% lidocaine and 1:80,000 epinephrine, and 8 patients treated with mepivacaine alone required a change in sponge to achieve hemostasis. All studies conducted on children have shown that patients in all groups have not reported cases of hematoma, swelling or infection.[21]

The 2% lidocaine with 1:80,000 epinephrine was hypothesized to demonstrate reduced bleeding compared to 3% mepivacaine, given that epinephrine is a vasoconstrictor used to minimize blood loss during surgical procedures.

However, no differences were observed related to hemostasis with either anesthetic solution.[21]

Additionally, Elbay et al. found that 2% lidocaine with 1:80,000 epinephrine and 3% mepivacaine administered alone performed similarly when delivered as IANB anesthesia for primary mandibular molars requiring extraction or pulpotomy in children. These results are consistent with the findings of several other studies conducted on adults.[21]

Regarding studies conducted on adult groups, a study by Kämmerer et al. was an exception in that it included patients with healthcare conditions (e.g., hypertension, carcinoma in remission, hepatitis, epilepsy, hypothyroidism, and migraine) in the study group.[22] All other studies included patients with any systemic disease as an exclusion criterion. The studies selected the patients based on diseases requiring special considerations during their dental treatment or patients with contraindications for any of the anesthetic solution, besides patients requiring open surgical extractions or teeth with signs of severe acute infection.

A study by Kämmerer et al. revealed that patients in the higher risk group did not exhibit any adverse reactions during or after the procedure.[22] The article concluded that this finding is in accordance with the established low allergenic and toxic potential of articaine.[22] However, it is important to note that a follow-up period of 1 day may not be sufficient to detect potential complications.

According to the study by Kämmerer et al., none of the groups (2% articaine with 1:200,000 epinephrine or 4% articaine with 1:200,000 epinephrine) exhibited any significant superior side effects.[24] Other clinical trials comparing 2% and 4% articaine solutions yielded similar results.[24] However, this study detected a slightly better anesthetic effect of the solution with the higher concentration, though it was not significant. Additionally, the duration of soft tissue anesthesia was significantly shorter with 2% articaine, consistent with the results of other researchers. Furthermore, the authors reported that no significant neurotoxicity was observed in either articaine group, in accordance with several other studies.[24]

As stated by Youssef et al., 5.4% of patients reported high scores of pain during IANB injection. However, no evidence of detrimental nerve contact or other complications was observed in any patient.[23] Temporary irritations were reported by 5 patients in the IANB group 24 h after the procedure. A single case documented difficulty talking for 1 day after the anesthesia, 3 cases reported pain at the site of injection, and 1 case reported pain around the ear after the injection. With regard to the anesthetic solution, no significant transoperative or postoperative complications were observed in any of the patients who received 4% articaine with 1:100,000 epinephrine.[23]

A comparative analysis of the IANB technique and local infiltration revealed that both methods were found to be safe, with no significant difference in the perception of pain during the injection. Furthermore, no adverse effects were observed, including local complications (local irritation or discomfort) or systemic side effects (palpitations, nausea, vomiting, or dizziness). However, a greater volume of anesthetic solution was used in the local infiltration group, which has been demonstrated to increase the risk of complications.[25]

The current review revealed that a substantial percentage of studies exhibited some concerns regarding the risk of bias. Two studies were identified as being at high risk of bias. The primary cause of high risk in trials is the measurement of the outcome, that is, the method by which complications are assessed. However, a majority of the trials exhibited some concerns regarding the randomization process and deviations from intended interventions. All trials have adequately reported the results, and there was no missing data. Therefore, the risk of bias for missing outcome data was rated as low in all trials.

Conclusions

The reports related to the IANB technique combined with different local anesthetics in pediatric and adult patients have demonstrated that no relevant side effects were observed in any group, irrespective of the anesthetic solution employed. Nonetheless, it is important to acknowledge that the prevalence of temporary or even permanent injury due to IANB is considered to be very low, though not non-existent. Furthermore, it is also relevant to note that a follow-up period of 1 day may not be sufficient to observe the development of subsequent complications. Additionally, the fact that most of the studies excluded patients with systemic diseases could be a limitation of this study, both for adults and children.

The recommendation of local anesthetics is contingent upon the existence of high-quality trials with a low risk of bias. Future randomized controlled clinical trials with large samples are necessary to confirm these findings. Additionally, further studies are needed to enhance our comprehension of the distribution of complications associated with IANB using different local anesthetics at varying concentrations, with or without vasoconstrictor association, depending on patient age, the duration of action, the chronology of their onset and remission, and extended monitoring periods.

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.

Use of AI and AI-assisted technologies

Not applicable.

ORCID iDs

Maral Berenova https://orcid.org/0000-0002-0163-0085
Ifrah Sarfaraz https://orcid.org/0000-0002-1803-671X
Selma Pascoal https://orcid.org/0000-0002-9665-7183
João Gião Carvalho https://orcid.org/0000-0002-0716-1881
José Paulo Macedo https://orcid.org/0000-0003-3653-7267
Jorge Pereira https://orcid.org/0000-0003-1420-8331

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Review

Extraintestinal malignancies in inflammatory bowel disease – deciphering hazardous relationships: A literature review

Iga Gromny[1,A–F], Arkadiusz Aab[2,B–F], Maria Litwiniuk[2,B–F], Katarzyna Neubauer[1,A–F]

[1] Department of Gastroenterology, Hepatology and Internal Medicine, Faculty of Medicine, Wroclaw Medical University, Poland
[2] Department of Gastroenterology, Hepatology and Internal Medicine, University Teaching Hospital, Wroclaw, 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

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Address for correspondence
Iga Gromny
E-mail: iga.gromny@umw.edu.pl

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Abstract

Inflammatory bowel disease (IBD) is a chronic, systemic disease with complex and unclear pathogenesis, primarily affecting the gastrointestinal tract. Inflammatory bowel disease is associated with a wide spectrum of extraintestinal complications, among which cancer is of particular importance. It is well known that IBD is associated with a higher risk of colorectal cancer (CRC). Yet, the incidence of CRC in this group of patients has decreased due to the development of surveillance techniques and therapy. In contrast, the relationship between IBD and extraintestinal malignancies (EMs) remains unclear, and it is taking on new significance in light of the rise in the incidence of malignant tumors, both in IBD patients and in the general population. Based on the literature review, it can be stated that the available studies suggest a possible association between IBD and oral, pancreatic and hepatobiliary malignancies. However, the dynamic epidemiological situation, combined with the methodological limitations of many existing studies, underscores the need for further research to better understand the relationship between IBD and cancer. In this group of patients, special oncological vigilance, the employment of the available prevention methods (e.g., vaccination), patient education, and, when recommended, screening tests are required. A clinical challenge involving a multidisciplinary approach is the treatment of IBD in cancer patients, especially during disease exacerbation, as well as cancer therapy in IBD patients.

Keywords: inflammation, neoplasms, inflammatory bowel disease, immunosuppression therapy

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Highlights

- Inflammatory bowel disease (IBD) significantly increases the risk of colorectal cancer (CRC), although modern surveillance strategies and advanced therapies have reduced the overall CRC incidence in IBD patients.
- The relationship between IBD and extraintestinal malignancies (EMs) remains uncertain; however, it is gaining importance as the global cancer rates continue to rise.
- Current evidence from our literature review suggests a potential association between IBD and several extraintestinal cancers, including oral, pancreatic and hepatobiliary malignancies.

Introduction

Inflammatory bowel disease (IBD), encompassing Crohn's disease (CD) and ulcerative colitis (UC), is a chronic disease of complex and unclear etiology. It is assumed that in genetically predisposed individuals, environmental factors, such as impaired intestinal permeability and dysbiosis, lead to the dysregulation of the immune system and chronic intestinal inflammation. Inflammation can affect other organs, leading to extraintestinal manifestations in IBD patients.[1]

Currently, nearly 3.9 million females and 3.0 million males are living with IBD worldwide, and a considerable rise in both incidence and prevalence of IBD has been observed. Inflammatory bowel disease is an incurable disease with a difficult-to-predict course, and cancer is the second most common cause of death for IBD patients after cardiovascular disease.[2,3]

Many researchers are striving to elucidate the molecular mechanisms underlying cancer development in patients with IBD, which are gradually becoming better understood. Most scientific studies focus on colorectal cancer (CRC). In their recent study, Hisamatsu et al. found that chronic inflammation in IBD promotes tumor development by activating several key pathways, including NF-κB, JAK/STAT and Wnt/β-catenin, which drive cell proliferation and survival.[4] In addition to neoplastic transformation via DNA damage, the oxidative stress caused by chronic inflammation is also an important factor inducing the mutations and chromosomal instability required for cancer progression. Advances in epigenetics, in turn, have shown that CpG island hypermethylation and mismatch repair defects contribute to early carcinogenesis in colitis-induced cancer.[4] Chronic inflammation due to IBD results in systemic immune activation and epithelial damage, which may promote carcinogenesis not only in the colon, but also across many organ systems. Immunosuppressive therapies predispose to immune deregulation, leading to an enhanced risk for virus-related malignancies (e.g., lymphoma) and skin cancers. Additionally, continuous inflammatory signals and cytokine imbalance may lead to the formation of an in vivo microenvironment for malignancies beyond the gut mucosa, contributing to an increased incidence of cholangiocarcinoma (CCa)/urothelial carcinoma. This seems to imply that a set of both iatrogenic and intrinsic immune pathologies can be postulated as drivers of a systemic cancer risk in IBD patients.[5]

Another relevant factor is the influence of gut microbiota disorders on the process of carcinogenesis. Minervini et al. in their review showed that gut dysbiosis, or the imbalance of gut microbiota, promotes the process of carcinogenesis due to the production of toxins that have a harmful effect on the genome, the induction of inflammation and the disruption of the host immune response.[6] Bacteria that exert a harmful effect include *Fusobacterium nucleatum*, *Escherichia coli* and *Bacteroides fragilis*. These microorganisms, by weakening the host immune response, may allow cancer cells to evade elimination by the immune system.[6]

Banthia et al. in their review indicate that there is an association between periodontal disease and an increased risk of cancer, especially in the case of head and neck, esophageal, lung, and gastrointestinal cancers.[7] However, the researchers present no evidence of an association between periodontal disease and the risk of metastasis in already diagnosed cancers. It is important to remember that chronic inflammation and the changes in the oral microbiome involving specific pathogens may promote cancer development.[7]

Patients with IBD are at significantly increased risk of CRC, mainly due to the pro-neoplastic effects of chronic intestinal inflammation. The most important risk factors for CRC in IBD patients are the duration of the disease, its extent and severity, the presence of post-inflammatory pseudopolyps, the coexistence of primary sclerosing cholangitis (PSC), and a family history of CRC.[8–10] The worldwide incidence rate of CRC in CD is estimated to be between 19.5 and 344.9/100,000 per year, and between 54.5 and 543.5/100,000 per year in patients with UC.[11] It is estimated that in the cases of long-standing UC and Crohn's colitis (except for proctitis), there is a 2–3-fold increased risk of CRC as compared to the general population. Nevertheless, the available studies indicate that the rates of CRC in IBD are decreasing over time, likely due to improved medical therapies and endoscopic surveillance.

In contrast, the link between IBD and extraintestinal malignancies (EMs) remains unclear. In general, the incidence of malignant tumors is on the rise, both in the general population and in patients with IBD. The treatment of patients with IBD has advanced with the introduction of novel therapies. However, it is important to note the increased risk of cancer associated with immunosuppressive treatment.

Our review is innovative, since it is one of the few that focus on the association between IBD and the risk of less frequently discussed EMs. The review aims to summarize the available data and identify potential pathophysiological mechanisms that may link chronic inflammation to the development of malignancies at sites remote from the gut. Analyses such as ours may contribute to better identification of risk groups and the development of more effective cancer surveillance strategies in patients with IBD. The present review explores the relationship between IBD and EMs, including their characteristics and risk factors. Understanding these associations may help inform clinical decision-making and improve cancer surveillance in patients with IBD.

Material and methods

The literature search was performed in the Embase and PubMed databases, using the combinations of the following keywords: ("cancer*" OR "malignancy*" OR "tumor*" AND "Crohn's disease" OR "ulcerative colitis" OR "inflammatory bowel disease*" OR "IBD"). The search was limited to the articles published between January 2015 and August 2024. The asterisks allowed us to retrieve records where the query words appeared with suffixes. The exclusion criteria were as follows: experimental studies (including animal studies and in vitro research); non-IBD studies; studies solely on CRC; non-original articles; non-English language; abstracts; and posters. The evidence-based medicine (EBM) pyramid was used to assess the quality of the studies.[12] This narrative review followed the Scale for the quality Assessment of Narrative Review Articles (SANRA) criteria and the SANRA score for the review is presented in Fig. 1.[13]

Results

We found 39 studies that examined the link between EMs and IBD, along with 20 studies on the connection between EMs and IBD treatment, that met the inclusion criteria.

We systematized the search results according to the location of the tumor: oral, head and neck cancers; thyroid cancer (TC); lung cancer (LC); breast cancer (BC); pancreatic cancer (PC); hepatobiliary cancer; urinary tract cancer; prostate cancer (PCa); reproductive system cancers; central nervous system malignancies; hematological malignancies; and skin cancers.

Scale for the quality Assessment of Narrative Review Articles – SANRA

1. Justification of the article's importance for the readership
   - The importance is not justified. 0
   - The importance is alluded to, but not explicitly justified. 1
   - The importance is explicitly justified. 2

2. Statement of concrete aims or formulation of questions
   - No aims or questions are formulated. 0
   - Aims are formulated generally, but not concretely or in terms of clear questions. 1
   - One or more concrete aims or questions are formulated. 2

3. Description of the literature search
   - The search strategy is not presented. 0
   - The literature search is described briefly. 1
   - The literature search is described in detail, including the search terms and the inclusion criteria. 2

4. Referencing
   - Key statements are not supported by references. 0
   - The referencing of key statements is inconsistent. 1
   - Key statements are supported by references. 2

5. Scientific reasoning (e.g., the incorporation of appropriate evidence, such as RCTs in clinical medicine)
   - The article's point is not based on appropriate arguments. 0
   - Appropriate evidence is introduced selectively. 1
   - Appropriate evidence is generally present. 2

6. Appropriate presentation of data (e.g., absolute vs. relative risk, effect sizes without confidence intervals)
   - Data is presented inadequately. 0
   - Data is often not presented in the most appropriate way. 1
   - Relevant outcome data is generally presented appropriately. 2

SUMSCORE: 11

Fig. 1. Scale for the quality Assessment of Narrative Review Articles (SANRA)

Inflammatory bowel diseases and oral, head and neck cancers

Most head and neck cancers (HNC) originate from the mucosal epithelium of the oral cavity, pharynx and larynx, and are known as head and neck squamous cell carcinoma (HNSCC). Cancers of the oral cavity and larynx are generally linked to tobacco consumption, alcohol abuse, or both, while pharynx cancers are increasingly associated with human papillomavirus (HPV) infection, primarily HPV-16.[14]

The relationship between IBD and an increased risk of oral cancer has been the subject of many studies. In IBD, disturbances occur not only in the gut microbiome, but also in the oral microbiome.[15,16] The resulting dysbiosis contributes to an increase in pro-inflammatory cytokines, leading to the development of periodontitis.[17,18] The presence of chronic inflammation promotes oxidative stress and DNA damage, which increases the risk of the neoplastic transformation of oral epithelial cells. It should also be noted that patients with IBD undergoing immunosuppressive therapy are at increased risk of de-

veloping dysplastic lesions in the oral cavity. Immunosuppression weakens the body's natural immune defense mechanisms, reducing the ability to eliminate cells with tumorigenic potential, while simultaneously increasing susceptibility to HPV infection, a key risk factor for the development of oral cancer.[19–21]

We identified 6 studies examining the association between IBD and cancers of the oral cavity, head and neck (Table 1).[20,22–26] The results of all the studies, including a recently published Mendelian randomization study, were consistent and suggested an increased risk of oral cancer in IBD patients. In the study by Katsanos et al., it was demonstrated that patients with IBD had an elevated risk of malignant tumors in the oral cavity, particularly tongue cancer.[20] Immunosuppression, dysbiosis, a weakened immune system, and HPV infection are key risk factors. In light of these findings, the importance of regular dental examinations, patient education and preventive HPV vaccination in patients with IBD should be emphasized. Omitting HPV vaccination in patients with IBD is acknowledged as a significant vaccination oversight.[27] Studies showing no association between HPV vaccination and the IBD risk are also relevant.[28]

Thyroid cancer

Thyroid cancer includes papillary TC, follicular cancer, medullary cancer, and poorly or undifferentiated cancer. Thyroid cancer stands as the most prevalent form of endocrine cancer, with papillary cancer comprising over 80% of all cases.[29] It predominantly affects women and is more prevalent in white individuals than in African Americans.[30] In our review, we identified 5 studies that evaluated the risk of TC in patients with IBD (Table 2).[22,31–34] The results of the abovementioned studies are inconclusive. So et al. demonstrated that the risk of TC was not significantly higher in IBD patients as compared to the healthy population.[22] In 2 studies, the risk of TC was higher only in patients with CD.[31,32] On the other hand, other studies indicate an elevated risk of this cancer only in patients with UC.[33,34]

Lung cancer

Lung cancer (LC) is the most commonly diagnosed cancer and the leading cause of cancer-related deaths, with an estimated 1.8 million deaths worldwide.[35] The available studies are inconclusive in assessing the relationship between IBD and LC (Table 3).[33,36–40] Jung et al. showed that the risk of LC in IBD patients was similar to that in the general population.[33] In turn, other studies suggest a link between CD and an increased risk of LC, which is not confirmed in UC, even indicating a reduced risk of lung cancer in this group of patients.[36] Yet, the meta-analysis published in 2021 by Lo et al. suggests an increased risk of LC in IBD patients.[40]

Breast cancer

Breast cancer is the most prevalent cancer in women, with an estimated 2.3 million new cases diagnosed globally each year.[41] The known risk factors for BC are increasing age, prolonged estrogen exposure, obesity in postmenopausal women, reproductive and genetic factors, western lifestyle, as well as smoking and alcohol consumption.[41] It seems that IBD patients have a shorter period of estrogen exposure as compared to the general population. This is due to a later onset of menarche and earlier menopause, which could be linked to a reduced risk of BC.[42] In our review, only Van den Heuvel et al. noted a decreased risk of BC in female patients with IBD.[43] Three out of the 5 studies showed an increased risk of BC in IBD patients, while the rest reported a risk similar to that in the general population (Table 4).[37,39,43–45]

Pancreatic cancer

The findings are consistent and indicate a higher risk of PC in patients with IBD (Table 5).[33,46–51] A recently published meta-analysis of 11 cohort studies unequivocally showed a moderate increase in the PC risk in patients with IBD.[51] Moreover, the study demonstrated a significantly higher PC risk in men with IBD as compared to women.[51] The incidence of PC has been increasing worldwide yearly. The link between PC and IBD is primarily attributed to the chronic systemic inflammation caused by the disease. Kimchy et al. suggests that this increasing trend observed in the IBD population parallels the increase in the incidence of PC reported among the general population, but at a much greater rate.[49] In their study, Jung et al. observed an increased risk of PC, specifically in women with CD.[33] On the other hand, Burish et al. noted an increased risk of this cancer in patients with UC.[48] A particular factor that increases the risk of PC in patients with IBD is the presence of PSC. In a recently published systematic review and meta-analysis of cohort studies, the summary relative risk (*RR*) (95% confidence interval (*CI*)) comparing persons with PSC to persons without PSC was 7.56 (2.42–23.62; I^2 = 0%, n = 3) for PC.[52]

Hepatobiliary cancer

The available studies suggest that in patients with IBD there is a higher risk of hepatobiliary tumors (Table 6).[33,37,53] The risk is especially high in patients with PSC. Concomitant IBD is reported in up to 60–80% of PSC patients.[54] There are strong positive associations between PSC and several tumors. For instance, in a recently published systematic review and meta-analysis of cohort studies, the summary *RR* (95% *CI*) comparing persons with PSC to persons without PSC was 584.37 (269.42–1,267.51; I^2 = 89%; n = 4) for cholangiocarcinoma, 155.54 (125.34–193.02; I^2 = 0%; n = 3) for hepatobiliary cancer and 30.22 (11.99–76.17; I^2 = 0%; n = 2) for liver cancer.[52]

Table 1. Inflammatory bowel disease (IBD) and oral, head and neck cancers

Study, year, country	Study design/ quality level*	Number of participants (*n*)/ type of cancer	Age [years] and gender of the participants	Inclusion criteria	Exclusion criteria	Findings
Katsanos et al.[20] 2016 USA	retrospective study *C	7,294 IBD patients 11 OCC (7 CD, 4 UC): squamous cell cancer of the tongue and palate ($n = 6$); tonsillar, buccal and mandibular sarcomas ($n = 3$)	41–81 3,509 M 3,785 F	the IBD diagnosis	oral neoplastic lesions diagnosed before the IBD diagnosis	and increased risk of oral cancer in IBD patients (*SIR*: 9.77; 95% *CI*: 5.14–16.98), particularly in women
So et al.[22] 2017 China	population-based cohort study *C	2,621 IBD patients 1,603 UC 1,018 CD	all age categories 1,559 M 1,062 F	the IBD diagnosis	patients with IBD diagnosed before 1990 and whose dates of the IBD diagnosis were unknown	an increased risk of HNC in CD (*SIR*: 5.08; 95% *CI*: 1.64–15.76)
Nissen et al.[23] 2018 the Netherlands	retrospective case–control study *D	66 IBD patients and 2,141 controls with OCC 31 IBD patients and 1,552 controls with PxC 1,800 IBD controls	all age categories	IBD patients with primary OCC and PxC	OCC and PxC in situ; lymphoma; the IBD diagnosis >3 months after the OCC or PxC diagnosis; the OCC or PxC diagnosis before 1993 or after 2012; no confirmed IBD diagnosis	an older age at the IBD diagnosis was a risk factor for both OCC and PxC development; CD (*OR*: 1.04; 95% *CI*: 1.02–1.07) and UC (*OR*: 1.03; 95% *CI*: 1.01–1.06) were risk factors for OCC development; the proximal disease localization in CD was a risk factor for OCC development (*OR*: 1.103; 95% *CI*: 1.040–1.170; $p = 0.028$)
Van de Ven et al.[24] 2020 Denmark	retrospective case–control study *D	1,855 IBD patients 1,004 UC 796 CD 55 LxC	NA 863 M 992 F	the IBD diagnosis	LxC in situ; the IBD diagnosis >3 months after the LxC diagnosis; no confirmed diagnosis of IBD or LxC; the diagnosis before 1993 or after 2012; laryngeal lymphoma	the male sex was a risk factor for LxC in IBD patients; an older age at the IBD diagnosis was a risk factor for LxC development in UC; tobacco use, stricturing and penetrating disease were risk factors for LxC development in CD; IBD was not associated with impaired LxC survival
Gao et al.[25] 2023 European countries	Mendelian randomization study *B	12,882 IBD patients 6,968 UC 5,956 CD	NA	the IBD diagnosis	NA	IBD, UC and CD have potential causal associations with OCC (IBD – *OR*: 1.180; 95% *CI*: 1.059–1.316; $p = 0.003$; UC – *OR*: 1.158; 95% *CI*: 1.041–1.288; $p = 0.007$; CD – *OR*: 1.112; 95% *CI*: 1.008–1.227; $p = 0.034$)
Harjunen et al.[26] 2023 Finland	retrospective study *C	70,567 IBD patients 50,873 UC 19,694 CD 89 HNSCC	18–91 35,885 M 34,682 F	the IBD diagnosis; age ≥18 years	HNC diagnosed before the IBD diagnosis	the incidence of HNSCC was increased in IBD patients as compared to the estimates for the Finnish population in general (*SIR*: 1.300: 95% *CI*: 1.065–1.614; $p = 0.062$); only individuals with CD had a statistically significantly increased incidence of HNSCC (*SIR*: 1.715; 95% *CI*: 1.156–2.431; $p = 0.034$); the incidence was increased for men with CD (*SIR*: 1.951; 95% *CI*: 1.216–2.935; $p = 0.025$), but not for women (*SIR*: 1.317; 95% *CI*: 0.602–2.451; $p = 0.873$)

* B – randomized-controlled study; C – cohort study; D – case-controlled study; OCC – oral cavity cancer; CD – Crohn's disease; UC – ulcerative colitis; PxC – pharyngeal cancer; LxC – laryngeal cancer; HNSCC – head and neck squamous cell carcinoma; HNC – head and neck cancer; M – male; F– female; *SIR* – standardized incidence ratio; *CI* – confidence interval; *OR* – odds ratio; NA – not applicable.

Table 2. Inflammatory bowel disease (IBD) and thyroid cancer (TC)

Study, year, country	Study design/ quality level*	Number of participants (*n*)/ type of cancer	Age [years] and gender of the participants	Inclusion criteria	Exclusion criteria	Findings
So et al.[22] 2017 China	population-based cohort study *C	2,621 IBD patients 1,603 UC 1,018 CD	all age categories 1,559 M 1,062 F	the IBD diagnosis	patients with IBD diagnosed before 1990 and whose dates of the IBD diagnosis were unknown	the risk of TC was not significantly higher in IBD patients as compared to the healthy population
Wadhwa et al.[31] 2016 USA	case–control study *D	289,935 IBD patients 315,145 with diverticulitis	≥18	the IBD diagnosis	NA	CD, not UC, is associated with a higher risk of TC
Taborelli et al.[32] 2020 Italy	population-based cohort study *C	3,664 IBD patients 2,358 UC 1,306 CD	18–84 1,911 M 1,753 F	the IBD diagnosis	<5 years of residence in the Friuli-Venezia Giulia region preceding the IBD diagnosis; follow-up <90 days; codes for both UC and CD	a higher risk of TC only in CD (*SIR*: 5.58; 95% *CI*: 2.41–11.00)
Jung et al.[33] 2017 South Korea	retrospective study *C	10,049 UC 5,595 CD	all age categories 9,743 M 5,548 F	the IBD diagnosis	previous cancer recognition	an increased risk of TC (*SIR*: 2.2; 95% *CI*: 1.1–3.9) in men with UC
Cao[34] 2018 China	case–control study and meta-analysis *D/A	case–control study: 1,392 IBD patients 1,022 UC 370 CD control group: 1,392 patients with diverculitis meta-analysis: 334,015 IBD patients	≥18	the IBD diagnosis	NA	in the case–control study, TC was more common in IBD patients than in controls ($p = 0.032$); in the meta-analysis, IBD patients showed an increased risk of TC; assessing UC and CD separately, the risk of TC was increased only in UC

* A – meta-analysis; B – randomized-controlled study; C – cohort study; D – case-controlled study; NA – not applicable.

Table 3. Inflammatory bowel disease (IBD) and lung cancer (LC)

Study, year, country	Study design/ quality level*	Number of participants (*n*)/ type of cancer	Age [years] and gender of the participants	Inclusion criteria	Exclusion criteria	Findings
Jung et al.[33] 2017 South Korea	retrospective study *C	10,049 UC 5,595 CD	all age categories 9,743 M 5,548 F	the IBD diagnosis	previous cancer recognition	the risk of LC in UC (*SIR*: 1.25; 95% *CI*: 0.62–2.23) and CD (*SIR*: 1.66; 95% *CI*: 0.34–4.85) was comparable to that in the general population
Wilson et al.[36] 2016 Switzerland	nested case–control study *D	39,294 IBD patients 1,603 UC 1,108 CD	NA	the IBD diagnosis	NA	an increased risk of trachea/ lungs cancer in CD (*SIR*: 2.91) and a reduced risk of LC in UC (*SIR*: 0.79)
Hovde et al.[37] 2017 Norway	prospective population-based study *B	756 IBD patients 519 UC 237 CD	4–89 370 F	the IBD diagnosis	NA	in CD, an increased risk for trachea/lungs cancer as compared to controls
Biancone et al.[38] 2020 Italy	prospective case–control study *B	204 CD 199 UC 29 LC 806 IBD controls	≥17 214 M 189 F	the CD or UC diagnosis; IBD diagnosis >3 months after the LC diagnosis; regular clinical assessments (≥2 visits/year)	previous malignancies; age <17 years	an increased risk of LC in both CD and UC
Loo et al.[39] 2019 Canada	population-based study *C	35,985 IBD patients 20,644 CD 14,000 UC 1,341 IBD-U 225 ECs	≥18 16,563 M 19,422 F	the IBD diagnosis; age ≥18 years	prevalent IBD; less than 1 day of follow-up	an increased risk of respiratory cancers (*SIR*: 1.16)
Lo et al.[40] 2021 multi-national study	meta-analysis of population-based cohort studies *A	NA	NA	the IBD diagnosis	NA	CD patients had an increased risk of LC (*IRR*: 1.53; 95% *CI*: 1.23-1.91), while UC patients had a borderline significantly lower risk

* A – meta-analysis; B – randomized-controlled study; C – cohort study; D – case-controlled study; IBD-U – inflammatory bowel disease unclassified; EC – extracolonic cancers; *IRR* – incidence rate ratio; NA – not applicable.

Table 4. Inflammatory bowel disease (IBD) and breast cancer (BC)

Study, year, country	Study design/ quality level*	Number of participants (*n*)/ type of cancer	Age [years] and gender of the participants	Inclusion criteria	Exclusion criteria	Findings
Hovde et al.[37] 2017 Norway	prospective population-based study *B	756 IBD patients 519 UC 237 CD	4–89 370 F	the IBD diagnosis	NA	BC was observed more often in both, UC and DC
Loo et al.[39] 2019 Canada	population-based study *C	35,985 IBD patients 20,644 CD 14,000 UC 1,341 IBD-U 225 ECs	≥18 16,563 M 19,422 F	the IBD diagnosis; age ≥18 years	prevalent IBD; less than 1 day of follow-up	an increased risk of BC (*SIR*: 1.13) was noted
Van den Heuvel et al.[43] 2016 Denmark	population-based cohort study *C	2,325 IBD patients 1,515 UC 810 CD	≥18	the IBD diagnosis	NA	a decreased risk of BC in IBD patients (*SIR*: 0.11)
Tsai et al.[44] 2015 Taiwan	population-based cohort study *C	4,856 women with IBD 19,424 control female patients	≥18 only F	the IBD diagnosis	NA	the incidence of BC was similar in the IBD and control cohorts (1.31 vs. 1.25 per 1,000 person-years); the *aHR* of BC was 0.95 (95% *CI*: 0.66–1.36) for IBD patients
Mansoor et al.[45] 2020 USA	retrospective population-based study *C	306,390 IBD patients 165,750 CD 140,640 UC 6,500 BC	≥18	IBD female patients	NA	the prevalence of BC in individuals without IBD was 1.1%; the prevalence of BC was increased at 1.9% in CD (*OR*: 1.79; 95% *CI*: 1.73–1.85; $p < 0.0001$) and at 2.3% in UC (*OR*: 2.24; 95% *CI*: 2.17–2.32; $p < 0.0001$)

* B – randomized-controlled study; C – cohort study; *aHR* – adjusted hazard ratio; NA – not applicable.

Table 5. Inflammatory bowel disease (IBD) and pancreatic cancer (PC)

Study, year, country	Study design/ quality level*	Number of participants (*n*)/ type of cancer	Age [years] and gender of the participants	Inclusion criteria	Exclusion criteria	Findings
Jung et al.[33] 2017 South Korea	retrospective study *C	10,049 UC 5,595 CD	all age categories 9,743 M 5,548 F	the IBD diagnosis	previous cancer recognition	there was a significantly increased risk of PC (8.6; 1.0–31.0) in female CD patients
Mosher et al.[46] 2018 USA	case–control study *D	2,080 IBD patients	NA	the IBD diagnosis	a history of heritable cancer syndromes	IBD patients were at significantly elevated risk of PC (*RR*: 4.23; 95% *CI*: 1.35–13.29)
Everhov et al.[47] 2020 Denmark, Sweden	retrospective register-based cohort study *C	161,926 IBD patients/442 PC 97,514 UC 47,402 CD 17,010 IBD-U 1,599,024 control group/3,386 PC	all age categories 79,898 M 82,028 F	the IBD diagnosis	NA	the HR for PC was increased overall: 1.43 (1.30–1.58) in subtypes (UC: 1.35 (1.19–1.53); CD: 1.44 (1.18–1.74); IBD-U: 1.99 (1.50–2.64)), and especially in IBD patients with PSC: 7.55 (4.94–11.5)
Burisch et al.[48] 2022 Denmark	retrospective study *C	1,161 UC	2–88	the UC diagnosis	NA	an increased risk of PC in patients with UC
Kimchy et al.[49] 2023 USA	retrospective study analysis of hospitalizations of patients with IBD associated with PC *C	2,235,413 CD; 3,590 (0.16%) were found to be related to PC among the hospitalizations 1,324,746 UC; 2,878 (0.22%) were found to be related to PC among the hospitalizations	≥18	the IBD diagnosis; the PC diagnosis	NA	increased prevalence of PC in hospitalized IBD patients
Coward et al.[50] 2023 Canada	retrospective population-based study *C	35,763 IBD patients 289,212 controls	all age categories	the IBD diagnosis	NA	IBD patients had a higher risk of PC (7.79; 5.53–10.97)
Zamani et al.[51] 2024	meta-analysis *A	11 cohort studies	NA	studies: (1) involving ≥100 adult subjects (aged ≥18 years) without a preexisting diagnosis of PC at the enrollment; (2) diagnosing IBD through endoscopy/ colonoscopy or histological examinations; (3) reporting new cases of incident PC after the IBD diagnosis; (4) providing any statistical measures of association (compared with healthy populations) adjusted for potential confounding factors with their corresponding 95% *CI*s	(1) reviews, case reports, editorials, or letters to the editor; studies: (2) being published as duplicates or appraising the same population; (3) conducted on children; (4) lacking a clear presentation of the information concerning their methodology or the findings related to the study outcomes	the risk of PC increased by 79% in IBD patients (*RR*: 1.79; 95% *CI*: 1.16– 2.75; $I^2 = 95.7\%$); patients either with CD (RR: 1.42; 95% *CI*: 1.24–1.63) or UC (*RR*: 1.50; 95% *CI*: 1.17–1.92) had an increased risk (*p* for interaction = 0.72); the annual incidence of PC potentially attributable to IBD increased by 55 cases (95% *CI*: 17–103) per million

* A – meta-analysis; C – cohort study; D – case-controlled study; *RR* – relative risk; *HR* – hazard ratio; PSC – primary sclerosing cholangitis; NA – not applicable.

Table 6. Inflammatory bowel disease (IBD) and hepatobiliary cancer

Study, year, country	Study design/ quality level*	Number of participants (*n*)/ type of cancer	Age [years] and gender of the participants	Inclusion criteria	Exclusion criteria	Findings
Jung et al.[33] 2017 South Korea	retrospective study *C	10,049 UC 5,595 CD	all age categories 9,743 M 5,548 F	the IBD diagnosis	previous cancer recognition	an increased risk of liver cancers for women in UC (*SIR*: 4.4) and CD (*SIR*: 15.3)
Hovde et al.[37] 2017 Norway	prospective population-based study *B	756 IBD patients 519 UC 237 CD	4–89 370 F	the IBD diagnosis	NA	an increased risk of liver/ biliary cancer in UC (*SIR*: 2.85)
Madanchi et al.[53] 2016 Switzerland	retrospective study *C	1,026 IBD patients	19–102	the IBD diagnosis	NA	the observed incidence of CC (10.8/100,000) was higher than expected and known from the general population

* B – randomized-controlled study; C – cohort study; CCa – cholangiocarcinoma; NA – not applicable.

Urinary tract cancer

Urinary tract cancers comprise urinary bladder cancer (UBC), which tends to develop in older males, particularly those who smoke or have chronic inflammation, and renal cell carcinoma (RCC), which is associated with smoking, obesity and hypertension.[55,56] In the early stages of RCC, elevated levels of tumor necrosis factor (TNF), a crucial mediator of cancer-related inflammation, have been observed.[57] Also in the case of urinary tract cancers, the results of the studies included in our review are ambiguous (Table 7).[22,32,33,46,53,58–60] Importantly, an association between urinary tract cancer and thiopurines was suggested. In a French study, the incidence rates of urinary tract cancer were 0.48/1,000 patient-years in patients receiving thiopurines (95% *CI*: 0.21–0.95), 0.10/1,000 patient-years in patients who discontinued thiopurines (95% *CI*: 0.00–0.56) and 0.30/1,000 patient-years in patients never treated with thiopurines (95% *CI*: 0.12–0.62) at entry.[60] In turn, in another study, IBD patients had a significantly lower age at the RCC diagnosis, lower N-stage and lower M-stage, underwent more frequent surgical treatment for RCC as compared to the general population, and had a better survival independent of immunosuppression.[59]

Prostate cancer

In men, prostate cancer (PCa) ranks as the most frequently diagnosed cancer in 118 countries.[61] Men with IBD demonstrated a shorter time to develop PCa. An increasing adjusted hazard ratio (*aHR*) across years since the IBD diagnosis was observed (≤20 years, *aHR*: 1.22; >20 years, *aHR*: 1.49; p-trend = 0.018).[62] Men with IBD had higher rates of clinically significant PCa when compared with age- and race-matched controls.[63] In the majority of the analyzed studies, an increased risk of PCa was observed in patients with UC (Table 8).[22,33,36,43,46,62–67]

Reproductive system cancers

The available studies regarding the association between IBD and reproductive cancers are inconclusive (Table 9).[32,33,68–71] Rungoe et al. investigated the association between the occurrence of precancerous cervical lesions, finding that both CD and UC patients had an increased risk of low-grade and high-grade squamous intraepithelial lesion (SIL) as compared to the control group.[68] However, the risk of cervical cancer (CC) was elevated only in patients with CD.[68] Goetgebuer et al. also observed an increased risk of precancerous cervical lesions of cervical intraepithelial neoplasia (CIN) 2+ grade in patients with IBD.[70] The 2023 study by Hamid et al. reported a higher annual incidence of cervical, ovarian, endometrial, and vulvar cancers in female patients with IBD as compared to the general population.[71] On the other hand, Kim et al. did not confirm an association between CC and IBD.[69]

Table 7. Inflammatory bowel disease (IBD) and urinary tract cancers

Study, year, country	Study design/ quality level*	Number of participants (*n*)/ type of cancer	Age [years] and gender of the participants	Inclusion criteria	Exclusion criteria	Findings
So et al.[22] 2017 China	population-based cohort study *C	2,621 IBD patients 1,603 UC 1,018 CD	all age categories 1,559 M 1,062 F	the IBD diagnosis	patients with IBD diagnosed before 1990 and whose dates of the IBD diagnosis were unknown	an increased risk of RCC in CD (*SIR*: 6.89)
Taborelli et al.[32] 2020 Italy	population-based cohort study *C	3,664 IBD patients 2,358 UC 1,306 CD	18–84 1,911 M 1,753 F	the IBD diagnosis	<5 years of residence in the Friuli-Venezia Giulia region preceding the IBD diagnosis; follow-up <90 days; codes for both UC and CD	an increased risk of KC in UC (*SIR*: 2.06)
Jung et al.[33] 2017 South Korea	retrospective study *C	10,049 UC 5,595 CD	all age categories 9,743 M 5,548 F	the IBD diagnosis	previous cancer recognition	in male and female patients with UC and CD, the risk of UBC and KC were comparable with that in the general population
Mosher et al.[46] 2018 USA	case–control study *D	2,080 IBD patients	NA	the IBD diagnosis	a history of heritable cancer syndromes	IBD patients were at significantly elevated risk for KC (*RR*: 2.90; 95% *CI*: 1.46–5.84)
Madanchi et al.[53] 2016 Switzerland	retrospective study *C	1,026 IBD patients	19–102	the IBD diagnosis	NA	the observed incidence of UBC (21.7/100,000), was higher than expected and known from the general population
Algaba et al.[58] 2015 Spain	retrospective cohort study *C	9,100 IBD patients 4,550 CD 4,326 UC 224 IBD-U 5 UBC in CD 3 UBC in UC 6 KC in CD 4 KC in UC	all age categories	the IBD diagnosis	NA	there was no statistically significantly increased risk of UBC and KC in IBD patients (UBC IBD – 1.83 (0.28–1.11); UBC CD – 2.00 (0.29–1.63); UBC UC – 1.41 (0.14–1.30); KC IBD – 1.83 (0.90–3.19); KC CD – 2.00 (0.90–4.48); KC UC – 1.41 (0.53–3.74))
Derikx et al.[59] 2015 Denmark	case–control study *D	NA	all age categories	NA	NA	pancolitis (*OR*: 1.8–2.5), penetrating CD (*OR*: 2.8), IBD-related surgery (*OR*: 3.7–4.5); male gender (*OR*: 3.2–5.0), and an older age at the IBD onset (*OR*: 1.0–1.1) were identified as independent risk factors for RCC
Bourrier et al.[60] 2016 France	prospective observational cohort study *C	19,486 IBD patients 11,759 CD 7,727 UC or IBD-U	all age categories	the IBD diagnosis	NA	an increased risk of urinary tract cancers (*HR*: 2.82; 95% *CI*: 1.04–7.68; $p = 0.04$)

* C – cohort study; D – case-controlled study; UBC – urinary bladder cancer; KC – kidney cancer; RCC – renal cell carcinoma; NA – not applicable.

Table 8. Inflammatory bowel disease (IBD) and prostate cancer (PCa)

Study, year, country	Study design/ quality level*	Number of participants (*n*)/ type of cancer	Age [years] and gender of the participants	Inclusion criteria	Exclusion criteria	Findings
So et al.[22] 2017 China	population-based cohort study *C	2,621 IBD patients 1,603 UC 1,018 CD	all age categories 1,559 M 1,062 F	the IBD diagnosis	patients with IBD diagnosed before 1990 and whose dates of the IBD diagnosis were unknown	an increased risk of PCa in UC (*SIR*: 2.47; 95% *CI*: 1.24–4.95)
Jung et al.[33] 2017 South Korea	retrospective study *C	10,049 UC 5,595 CD	all age categories 9,743 M 5,548 F	the IBD diagnosis	previous cancer recognition	an increased risk of PCa in UC (*SIR*: 3.47; 95% *CI*: 2.06–5.48)
Wilson et al.[36] 2016 Switzerland	nested case–control study *D	39,294 IBD patients 1,603 UC 1,108 CD	NA	the IBD diagnosis	NA	a reduced risk of PCa in IBD patients taking aminosalicylates
Van den Heuvel et al.[43] 2016 Denmark	population-based cohort study *C	2,325 IBD patients 1,515 UC 810 CD	≥18	the IBD diagnosis	NA	UC patients were not at increased risk of cancer overall (*SIR*: 1.12; 95% *CI*: 0.97–1.28) despite an increased risk of PCa (*SIR*: 1.82; 95% *CI*: 1.17–2.71)
Mosher et al.[46] 2018 USA	case–control study *D	2,080 IBD patients	NA	the IBD diagnosis	a history of heritable cancer syndromes	IBD patients were at significantly elevated risk of PCa (*RR*: 1.70; 95% *CI*: 1.28–2.27)
Meyers et al.[62] 2020 UK	prospective population-based study *B	2,311 men with IBD 1,488 UC 643 CD 215,773 men without IBD (control group) 4,681 new cases of PCa in men without IBD and 66 in men with IBD (after a median follow-up of 78 months)	40–69 only M 218,084	IBD male patients	a prior history of a malignant cancer (any site) or the timing of the malignant cancer diagnosis relative to baseline could not be determined; the surgical removal of the prostate; earlier recorded death date; individuals whose genetically inferred sex was female	the association with PCa was only among men with UC (*aHR*: 1.47; 95% *CI*: 1.11–1.95; $p = 0.007$), and not CD (*aHR*: 1.06; 95% *CI*: 0.63–1.80; $p = 0.820$)
Burns et al.[63] 2019 USA	retrospective matched-cohort study *C	1,033 male patients with IBD 9,306 men without IBD (control group) 715 PCa	only M 10,339	IBD male patients	female patients	the incidence of PCa at 10 years was 4.40% among men with IBD and 0.65% among controls
Khan et al.[64] 2017 USA	retrospective cohort study *C	59,916 IBD 35,437 UC 28,332 CD 204 PCa	≥18	the IBD diagnosis	a diagnosis of rheumatoid arthritis or psoriasis; biological treatment, immuno-modulator or corticosteroids; age <18 years	the elderly with IBD have a higher risk of malignancy, specifically PCa, when compared with younger IBD patients and the general age-matched population
Ge et al.[65] 2020	meta-analysis *A	NA	NA	the IBD diagnosis	NA	the risk of PCa was higher in UC (pooled *SIR*: 1.58, 95% *CI*: 1.08–2.30), but not in patients with CD (pooled *SIR*: 1.12; 95% *CI*: 0.97–1.31)
Na et al.[66] 2022 South Korea	retrospective population-based cohort study *C	14,761 IBD patients 59,044 non-IBD patients	≥40 only M	IBD male patients	follow-up loss; any cancer development before the index date; follow-up <1 year from the index date; age <40 years; the female sex	the IBD status was not associated with the risk of PCa as compared to non-IBD (*aHR*: 0.93; 95% *CI*: 0.80–1.08; $p = 0.320$)
Zhou et al.[67] 2023	meta-analysis of cohort studies *A	592,853 participants	NA	the IBD diagnosis	NA	IBD was linked to an elevated risk of incident PCa (*HR*: 1.20; 95% *CI*: 1.06–1.37; $p = 0.004$); UC was associated with an increased risk of incident PCa (*HR*: 1.20; 95% *CI*: 1.06–1.38; $p = 0.006$), while CD was not significantly linked to a higher risk of PCa (*HR*: 1.03, 95% *CI*: 0.91–1.17; $p = 0.650$)

* A – meta-analysis; B – randomized-controlled study; C – cohort study; D – case-controlled study; NA – not applicable.

Table 9. Inflammatory bowel disease (IBD) and reproductive system cancers

Study, year, country	Study design/ quality level*	Number of participants (*n*)/ type of cancer	Age [years] and gender of the participants	Inclusion criteria	Exclusion criteria	Findings
Taborelli et al.[32] 2020 Italy	population-based cohort study *C	3,664 IBD patients 2,358 UC 1,306 CD	18–84 1,911 M 1,753 F	the IBD diagnosis	<5 years of residence in the Friuli-Venezia Giulia region preceding the IBD diagnosis; follow-up <90 days; codes for both UC and CD	there was an increased risk of corpus uteri cancer in UC (*SIR*: 2.67)
Jung et al.[33] 2017 South Korea	retrospective study *C	10,049 UC 5,595 CD	all age categories 9,743 M 5,548 F	the IBD diagnosis	previous cancer recognition	there was an increased risk of CC in UC (SIR: 5.7)
Rungoe et al.[68] 2015 Denmark	population-based cohort study *C	27,398 female patients with IBD 18,681 UC 8,717 CD 1,508,334 women (control group)	NA	the IBD diagnosis	NA	patients with UC had an increased risk of low-grade (*IRR*: 1.15; 95% *CI*: 1.00–1.32) and high-grade (*IRR*: 1.12; 95% *CI*: 1.01–1.25) SIL, whereas patients with CD had an increased risk of low-grade (*IRR*: 1.26; 95% *CI*: 1.07–1.48), and high-grade (*IRR*: 1.28; 95% *CI*: 1.13–1.45) SIL, and CC as compared to controls (*IRR*: 1.53; 95% *CI*: 1.04–2.27)
Kim et al.[69] 2015 USA	population-based study *C	133,333 female patients with systemic inflammatory diseases 25,176 IBD	only F	a diagnosis of systemic inflammatory disease	NA	no association between IBD and CC
Goetgebuer et al.[70] 2021 Denmark	case–control cohort study *D	2,098 female patients with IBD	only F	the IBD diagnosis	no cytological or histological results during the study period	the CIN2+ detection rate was higher in the IBD cohort than in the matched cohort (*SDR*: 1.27, 95% *CI*: 1.05–1.52); women with IBD had an increased risk of CIN2+ (*IRR*: 1.66; 95% *CI*: 1.21–2.25), and persistent or recurrent CIN during follow-up (*OR*: 1.89; 95% *CI*: 1.06–3.38)
Hamid et al.[71] 2023 USA	population-based control study *C	264,984 female patients with IBD 4,563 (1.7%) developed gynecological cancer 1,225 IBD and CC 1,395 IBD and ovarian cancer 1,567 IBD and endometrial cancer 376 IBD and vulvar cancer	all age categories only F	IBD female patients	a history of gynecological cancers prior to the IBD diagnosis	comparing the IBD population to the non-IBD general population, higher incidence proportion per year and prevalence of all 4 types of gynecological cancers were observed: for CC, the annual incidence proportion was 0.033% among women with IBD, while it was 0.014% in non-IBD patients; in ovarian cancer, the annual incidence proportion was 0.042% in the IBD group as compared to 0.021% in non-IBD patients; endometrial cancer showed an annual incidence proportion of 0.047% in women with IBD as compared to 0.028% in non-IBD patients, and vulvar cancer had an annual incidence proportion of 0.012% in IBD patients as compared to 0.004% in non-IBD patients

* C – cohort study; D – case-controlled study; CC – cervical cancer; SIL – squamous intraepithelial lesion; CIN – cervical intraepithelial neoplasia; CIN2+ – high-grade dysplasia and cervical cancer; *SDR* – standardized detection ratio; NA – not applicable.

Central nervous system malignancies

The number of studies assessing the risk of central nervous system tumors in patients with IBD is limited (Table 10).[25] In 2023, a large, multicenter, prospective study was published, which included 12,882 patients with IBD; it was found that there was a relationship between IBD, CD and brain malignancies.[25] More population studies are required to validate this correlation.

Hematological malignancies

The relationship between hematological malignancies and IBD is of great interest. Two large meta-analyses focusing on this relationship have been published recently.[51,67] They have shed new light on the not always consistent results of single studies.[32,53] Although further research work is needed, the abovementioned studies organize current knowledge, and provide a practical signpost for gastroenterologists and oncologists.

Zhou et al. demonstrated that the incidence of hematologic malignancies in the IBD cohort, both CD and UC patients, was higher than in non-IBD individuals.[67] Furthermore, the incidence of specific malignancies – non-Hodgkin's lymphoma, Hodgkin's lymphoma and leukemia – was also higher in IBD patients.[67]

Zamani et al. published a systematic review and meta-analysis of population-based cohort studies that evaluated the risk of lymphoma in patients with IBD in comparison with those without IBD.[51] The research demonstrated that the risk was moderately increased in patients with IBD, with CD having a slightly higher risk than UC. Considering the strengths of the study, especially the type of studies included and a very high number of participants, the significance of the findings showing a 30% higher risk of lymphoma in patients with IBD should be highlighted. Furthermore, based on the meta-regression and sensitivity analysis, Zamani et al. concluded that the overall increased risk of lymphoma in IBD was probably independent of the effects of medications. This is another important finding of the study considering the association between drugs, especially biologics and immunomodulators, and the risk of lymphoma.[51] All the mentioned studies are presented in Table 11.[32,51,53,67]

Skin cancers

There are well-recognized associations between IBD and skin cancers, which are most frequently linked to specific therapies (Table 12).[22,39,43,46,72] The risk of basal cell carcinoma (BCC) and melanoma was increased in thiopurine and anti-TNF users, and the risk of squamous cell carcinoma (SCC) was increased only in thiopurine users.[72]

Table 10. Inflammatory bowel disease (IBD) and central nervous system malignancies

Study, year, country	Study design/ quality level*	Number of participants (*n*)/ type of cancer	Age [years] and gender of the participants	Inclusion criteria	Exclusion criteria	Findings
Gao et al.[25] 2023 European countries	Mendelian randomization study *B	12,882 IBD patients 6,968 UC 5,956 CD	NA	the IBD diagnosis	NA	there is a link between IBD and brain cancer (*OR*: 1.104; 95% *CI*: 1.003–1.216; $p = 0.043$); a relationship between CD and brain cancer was found (*OR*: 1.105; 95% *CI*: 1.013–1.205; $p = 0.024$)

* B – randomized-controlled study; NA – not applicable.

Table 11. Inflammatory bowel disease (IBD) and hematological malignancies

Study, year, country	Study design/ quality level*	Number of participants (*n*)/ type of cancer	Age [years] and gender of the participants	Inclusion criteria	Exclusion criteria	Findings
Taborelli et al.[32] 2020 Italy	population-based cohort study *C	3,664 IBD patients 2,358 UC 1,306 CD	18–84 1,911 M 1,753 F	the IBD diagnosis	<5 years of residence in the Friuli-Venezia Giulia region preceding the IBD diagnosis; follow-up <90 days; codes for both UC and CD	there was no increased risk of hematological malignancies in IBD patients
Zamani et al.[51] 2024	meta-analysis *A	23 studies 2,078 lymphoma events in 656,731 IBD patients	NA	studies: (1) involving adult subjects without a preexisting diagnosis of lymphoma at the enrollment; (2) diagnosing IBD through histological or radiological examinations; (3) reporting new cases of lymphoma after the IBD diagnosis; (4) providing any statistical measures of association (compared with healthy populations)	(1) reviews, case reports, editorials, or letters to the editor; studies: (2) being published as duplicates or appraising the same population; (3) conducted on children; (4) specifically dealing with cutaneous lymphoma (5) lacking a clear presentation of the information concerning their methodology or the findings related to the study outcomes	patients with IBD had 30% higher odds of lymphoma (*RR*: 1.30; 95% *CI*: 1.21–1.40); the risk of developing both NHL (*RR*: 1.31; 95% *CI*: 1.20–1.42) and HL (*RR*: 1.29; 95% *CI*: 1.06–1.53) was increased in patients with IBD (p for interaction = 0.881); an increased risk of lymphoma was observed in both CD (*RR*: 1.54; 95% *CI*: 1.27–1.80) and UC (*RR*: 1.22; 95% *CI*: 1.09–1.35; p for interaction = 0.026)
Madanchi et al.[53] 2016 Switzerland	retrospective study *C	1,026 IBD patients	19–102	the IBD diagnosis	NA	the observed incidence of lymphoma (32.5/100,000) was higher than expected and known from the general population
Zhou et al.[67] 2023	meta-analysis *A	20 cohort studies 756,377 participants	NA	(1) population: people without hematologic malignancies or related high-risk factors; (2) exposure: patients diagnosed with IBD; (3) control: people who did not suffer from IBD; (4) outcome: the incidence of hematological malignancies (5) cohort studies	(1) full text not available; (2) articles published before 2000; (3) articles not in English; (4) the patients had developed hematological malignancies before the IBD diagnosis; (5) different articles with the same source of the cohort data; more comprehensive or newer, updated studies were included	in comparison with the non-IBD cohort, the incidence of hematological malignancies in IBD patients was higher (*SIR*: 3.05; $p < 0.001$); in comparison with the non-IBD cohort, the incidence of hematological malignancies in CD (*SIR*: 3.56; $p = 0.005$) and UC (*SIR*: 2.29; $p = 0.005$) was higher; the incidence of NHL (*SIR*: 1.70; $p = 0.010$), HL (*SIR*: = 3.47; $p = 0.002$) and leukemia (*SIR*: 3.69; $p < 0.001$) was higher in the IBD cohort

* A – meta-analysis; C – cohort study; NHL – non-Hodgkin's lymphoma; HL – Hodgkin's lymphoma; NA – not applicable.

Table 12. Inflammatory bowel disease (IBD) and skin cancers

Study, year, country	Study design/ quality level*	Number of participants (*n*)/ type of cancer	Age [years] and gender of the participants	Inclusion criteria	Exclusion criteria	Findings
So et al.[22] 2017 China	population-based cohort study *C	2,621 IBD patients 1,603 UC 1,018 CD	all age categories 1,559 M 1,062 F	the IBD diagnosis	a previous history of cancer before IBD; cancer diagnosed within 6 months of the IBD diagnosis	an increased risk of NMSC in UC (SIR: 9.05) and CD (*SIR*: 13.88)
Loo et al.[39] 2019 Canada	population-based study *C	35,985 IBD patients 20,644 CD 14,000 UC 1,341 IBD-U 225 ECs	≥18 16,563 M 19,422 F	the IBD diagnosis; age ≥18 years	prevalent IBD; less than 1 day of follow-up	an increased risk of NMSC (*SIR*: 22.62)
Van den Heuvel et al.[43] 2016 Denmark	population-based cohort study *C	2,325 IBD patients 1,515 UC 810 CD	≥18	the IBD diagnosis	NA	an increased risk of skin cancer in CD (*SIR*: 1.55)
Mosher et al.[46] 2018 USA	case–control study *D	2,080 IBD patients	NA	the IBD diagnosis	a history of heritable cancer syndromes	an increased risk of NMSC (*RR*: 2.38) and MSC (*RR*: 2.85) in IBD patients
Narous et al.[72] 2023 Canada	retrospective study *C	11,228 IBD patients 5,839 UC 5,389 CD BCC 647 SCC 169 other NMSCs 22 MSC 56	5,227 M 6,001 F	the IBD diagnosis	NA	patients with UC were more likely to have BCC predating their UC diagnosis (*OR*: 1.32; 95% *CI*: 1.08–1.60); for the post-IBD diagnosis, the risk of BCC (*HR*: 1.53; 95% *CI*: 1.37–1.70) and SCC (*HR*: 1.61; 95% *CI*: 1.29–2.01) were significantly increased across all IBD groups, except for SCC in UC; there was no significant association between melanoma and IBD post-IBD diagnosis

* C – cohort study; D – case-controlled study; BCC – basal cell carcinoma; SCC – squamous cell carcinoma; NMSC – non-melanoma skin cancer; MSC – melanoma skin cancer; NA – not applicable.

Effect of the use of thiopurines on the risk of developing extraintestinal malignancies

Despite the development of molecularly oriented therapeutic strategies, thiopurines, such as azathioprine and 6-mercaptopurine, still play an important role in treating IBD. Yet, the treatment with thiopurines in IBD patients is associated with an increased risk of developing hematological malignancies, non-melanoma skin cancer (NMSC), urinary tract cancers, and CC. However, the latter association is uncertain.

Hematological malignancies

Several studies have found that the use of immunosuppressive treatment with thiopurines may increase the risk of hematological malignancies in patients with IBD. A prospective observational study involving 19,486 IBD patients included in the Cancer et Sur-risque Associé aux Maladies Inflammatoires Intestinales en France (CESAME) showed that IBD patients who were currently treated with thiopurines and those who had never received the drugs showed no increased overall risk of lymphoproliferative neoplasms, including acute myeloid leukemia and myelodysplastic syndrome.[73] In contrast, patients who had been exposed to thiopurines in the past had a 7-fold increased risk of developing lymphoproliferative neoplasms.[73] Kotlyar et al. in a meta-analysis proved that the incidence of lymphoma during thiopurine use was almost 6 times higher, but this risk did not persist after thiopurines were discontinued.[74] Selected studies on the association between thiopurine therapy in IBD and hematological malignancies are presented in Table 13.[67,74–76]

Urinary tract cancers

Studies on thiopurines and the urinary tract cancer risk present conflicting results. A cohort study involving 1,986 IBD patients, 30.1% of whom were receiving thiopurines, found that patients treated with thiopurines were at increased risk of developing urinary tract cancer.[60] In contrast, in a retrospective study, Caviglia et al. found no clinically significant association between thiopurines use and urothelial carcinoma.[77] The analyzed studies are presented in Table 14.[60,75,77]

Cervical cancer

The effect of immunosuppressive drugs on the development of dysplasia and CC in IBD has been addressed many times and is still controversial.

A meta-analysis published in 2015 found an increased overall risk of cervical dysplasia and CC in IBD patients with current or prior treatment with immunosuppressive drugs as compared to the general population.[78] However,

Table 13. Thiopurines and hematological malignancies

Study, year, country	Study design/ quality level*	Number of participants (*n*)/ type of cancer	Age [years] and gender of the participants	Inclusion criteria	Exclusion criteria	Findings
Zhou et al.[67] 2023	meta-analysis *A	20 cohort studies 756,377 participants 4 studies reported the percentage of TP patients with IBD	NA	the IBD diagnosis	patients developed hematological malignancies before being diagnosed with IBD	for patients with IBD who used TP – *SIR*: 3.80; 95% *CI*: 2.46–5.87; $p = 0.001$); the type of hematological malignancy was not specified
Kotlyar et al.[74] 2015 USA	meta-analysis *A	24,029 IBD patients	NA	the IBD diagnosis; AZA or 6-MP treatment	anti-TNF treatment	current TP treatment – *SIR*: 5.71; 95% *CI*: 3.72–10.1; past TP treatment – *SIR*: 1.42; 95% *CI*: 0.86–2.34
Ardabili et al.[75] 2022 the Netherlands	retrospective cohort study *C	1,016 IBD patients 643 CD 373 UC 653 patients used TP in monotherapy for at least 12 months	≥18	the IBD diagnosis; monotherapy with TP (AZA, 6-MP, 6-TG)	UC patients with a history of colectomy; patients starting combination therapy or using other immuno-suppressive drugs	the incidence rate of lymphoma was 1.04 (95% *CI*: 0.38–2.31) per 1,000 person-years
Lemaitre et al.[76] 2017 France	cohort study *C	189,289 IBD patients 50,405 were exposed to TP monotherapy lymphoma (70 patients exposed to thiopurine monotherapy)	≥18	the IBD diagnosis	HIV infection; organ transplant recipients; cancer before inclusion or cancer <3 months after the IBD diagnosis; an uncertain IBD diagnosis	the risk of lymphoma was found to be higher in patients exposed to TP monotherapy (*aHR*: 2.60; 95% *CI*: 1.96–3.44; $p < 0.001$), anti-TNF monotherapy (*aHR*: 2.41; 95% *CI*: 1.60–3.64; $p < 0.001$), or combination therapy (*aHR*: 6.11; 95% *CI*: 3.46–10.8; $p < 0.001$)

* A – meta-analysis; C – cohort study; TP – thiopurine; AZA – azathioprine; 6-MP – 6-mercaptopurine; 6-TG – 6-thioguanine; TNF – tumor necrosis factor; HIV – human immunodeficiency virus; NA – not applicable.

Table 14. Thiopurines and urinary tract cancers

Study, year, country	Study design/ quality level*	Number of participants (*n*)/ type of cancer	Age [years] and gender of the participants	Inclusion criteria	Exclusion criteria	Findings
Bourrier et al.[60] 2016 France	prospective observational cohort study *C	1,986 IBD patients 456 patients used TP in monotherapy UBC 6 KC 10	≥18	the IBD diagnosis	NA	an increased risk of urinary tract cancers (*HR*: 2.82; 95% *CI*: 1.04–7.68; $p = 0.04$)
Ardabili et al.[75] 2022 the Netherlands	retrospective cohort study *C	1,016 IBD patients 643 CD 373 UC 653 patients used TP in monotherapy for at least 12 months	≥18	the IBD diagnosis; monotherapy with TP	UC patients with a history of colectomy; patients starting combination therapy or using other immuno-suppressive drugs	no increased risk of urinary tract cancers during TP treatment
Caviglia et al.[77] 2021 Italy	retrospective cohort study *C	5,739 IBD patients	≥16	the IBD diagnosis	a lack of data on the presence of tumor comorbidities	treatment with TP was not a risk factor for urinary tract cancers (*OR*: 0.57; 95% *CI*: 0.19–1.69)

* C – cohort study; NA – not applicable.

this study has a limitation, as it did not take into account the effect of specific immunosuppressive drugs, the actual or cumulative dose, or the duration of therapy.[78] Of a different opinion are Mann et al., who in their meta-analysis did not confirm an increased risk of CC for patients using thiopurines.[79] In turn, Goetgebuer et al. found an increase in the risk of CIN2+ due to exposure to immunosuppressants.[70] A detailed overview of the studies under analysis is provided in Table 15.[70,78,79]

Biological treatment and extraintestinal malignancies

The invention of biological drugs, and consequently their incorporation into the treatment standards for individuals with IBD, has significantly changed the approach to patient management. In addition to the obvious benefits of better control of the underlying disease, several challenges have also emerged that need to be addressed. Biological drugs have various mechanisms of action, but their common feature is the suppression of the immune system. The biologics used in IBD include: TNF-α inhibitors (infliximab, adalimumab, certolizumab, and golimumab); anti-integrin drugs (vedolizumab – a humanized immunoglobulin G1 (IgG1) monoclonal antibody that blocks the binding of integrin α4β7 to MAdCAM-1, preventing lymphocyte migration to the intestines); drugs targeting the interleukin (IL) IL-12/IL-23 pathway (ustekinumab – a human monoclonal antibody targeting the p40 subunit of IL-12 and IL-23, inhibiting the binding of Il-12 and Il-23 to their respective receptors on the surface of T and NK cells, others – mirikizumab, rizankizumab).[80,81]

TNF-α inhibitors and the risk of extraintestinal malignancies

Several studies have addressed the issue of the impact of using anti-TNF inhibitors in the treatment of IBD on the development of extraintestinal cancers (Table 16).[76,82–84] Lemaitre et al., based on data from a cohort study involving 189,289 patients with IBD, concluded that there was a small, but statistically significant increase in the risk of lymphoma associated with the use of TNF-alpha inhibitors.[76] The risk of cancer was even higher when TNF-alpha inhibitors and thiopurines were used concurrently.[76] Chaparro et al., in their observational cohort study involving 11,011 patients from the Spanish ENEIDA registry, did not observe an association between the use of immunosuppressants (mainly thiopurines) nor anti-TNF drugs and an increased risk of EMs in individuals with IBD.[83] The study considered lymphomas, leukemia, NMSC, and melanoma skin cancer (MSC).[83] Similarly, D'Haens et al.[82] and Kopylov et al.[84] did not describe an association between the use of anti-TNF inhibitors and an increased risk of EMs.

Anti-integrin drugs (vedolizumab) and the risk of extraintestinal malignancies

Colombel et al. in their meta-analysis found no significant association between the use of vedolizumab and an increased risk of EMs.[85] Furthermore, all patients exposed to vedolizumab who were diagnosed with skin cancer had a history of azathioprine therapy, and 2 continued to use azathioprine in the study.[85] Card et al., based on data from the GEMINI long-term safety (LTS) study, found that the number of malignancies occurring in patients using vedolizumab was similar to the expected number of malignancies in a population of IBD patients not using vedolizumab.[86] Singh et al. demonstrated that the use of vedolizumab did not increase the risk of malignancy as compared to infliximab.[87] In a meta-analysis conducted by Cohen et al. on a very large number of patients, we can find information that among EMs in patients with CD, there was UBC and KC, and then skin cancer, and among patients with UC – lymphomas and respiratory malignancies.[88] The study did not note any new safety concerns. The incidence of adverse events, such as malignancies, was low enough considering the patient-years of exposure to confirm the favorable safety profile of vedolizumab.[88] Additionally, a recent multicenter cohort study demonstrated no difference in cancer incidence in the IBD patients with prior non-digestive malignancy, treated with vedolizumab or anti-TNF.[89] The studies included in the analysis are listed in Table 17.[85–88]

Drugs targeting the IL-12/IL-23 pathway (ustekinumab) and the risk of extraintestinal malignancies

In their study, Hong et al. showed that there was no association between the use of ustekinumab and the occurrence of malignancy in patients with prior malignancy.[90] Sandborn et al. showed in their meta-analysis that taking ustekinumab did not increase the risk of malignancy as compared to placebo.[91] Table 18 provides a summary of the study results.[90,91]

Small-molecule drugs and extraintestinal malignancies

There are 2 basic groups of small-molecule drugs: Janus-activated kinase (JAK) inhibitors (including tofacitinib, filgotinib, upadacitinib); and sphingosine-1-phosphate receptor modulators (including ozanimod), increasingly used to treat IBD.[92] Chen et al. in their meta-analysis did not show any association between the use of small-molecule drugs and the occurrence of malignancy.[93] Goessens et al., in their observational study of IBD patients receiving combination therapy consisting of biologics and small-molecule

Table 15. Thiopurines and cervical cancer (CC)

Study, year, country	Study design/ quality level*	Number of participants (*n*)/ type of cancer	Age [years] and gender of the participants	Inclusion criteria	Exclusion criteria	Findings
Goetgebuer et al.[70] 2021 Denmark	case–control cohort study *D	2,098 female patients with IBD 1,382 CD 716 UC 1,030 using immuno-suppresants	≥18 only F	the IBD diagnosis	no cytological or histological results during the study period	the CIN2+ detection rate was higher in the IBD cohort than in the matched cohort (*SDR*: 1.27, 95% *CI*: 1.05–1.52); women with IBD had an increased risk of CIN2+ (*IRR*: 1.66; 95% *CI*: 1.21–2.25), and persistent or recurrent CIN during follow-up (*OR*: 1.89; 95% *CI*: 1.06–3.38)
Allegretti et al.[78] 2015 multi-national study	meta-analysis *A	77,116 IBD patients high-grade cervical dysplasia/ cancer: 995 cases	≥16 only F	the IBD diagnosis; immune-suppressive medications (including, but not restricted to, AZA, 6-MP, MTX); at least one Pap test within the defined study period	no immune-suppressive medications	IBD patients had an increased risk of cervical high-grade dysplasia/ cancer as compared to healthy controls (*OR*: 1.34; 95% *CI*: 1.23–1.46)
Mann et al.[79] 2022 multi-national study	meta-analysis *A	74,310 IBD patients	only F	the IBD diagnosis	NA	no statistically significantly increased risk of cervical cancer in IBD patients (*HR*: 1.24; 95% *CI*: 0.94–1.63); an increased risk of low-grade cervical lesions in IBD patients (*HR*: 1.15; 95% *CI*: 1.04–1.28); no statistically significantly increased risk in CD (*HR*: 1.36; 95% *CI*: 0.83–2.23) or UC (*HR*: 0.95; 95% *CI*: 0.72–1.25)

* A – meta-analysis; D – case-controlled study; MTX – methotrexate; NA – not applicable.

Table 16. Tumor necrosis factor-alpha (TNF-α) inhibitors and the risk of extraintestinal malignancies (EMs)

Study, year, country	Study design/ quality level*	Number of participants (*n*)/ type of cancer	Age [years] and gender of the participants	Inclusion criteria	Exclusion criteria	Findings
Lemaitre et al.[76] 2017 France	nationwide cohort study *C	189,289 IBD patients lymphoma (32 patients exposed to anti-TNF monotherapy)	≥18	the IBD diagnosis	HIV infection; organ transplant recipients; cancer before inclusion or cancer <3 months after the IBD diagnosis; an uncertain IBD diagnosis	anti-TNF monotherapy (infliximab or adalimumab) was associated with a small, but statistically significant increase in the risk of lymphoma as compared to exposure to neither medication (*aHR*: 2.41; 95% *CI*: 1.60–3.64; $p < 0.001$)
D'Haens et al.[82] 2017 8 EU countries and UK	prospective observational study *B	2,662 IBD patients lymphoma (9 patients) BCC 5 BC 5	≥18	the CD diagnosis	active and untreated latent tuberculosis; lympho-proliferative disorders; malignancies; moderate or severe heart failure NYHA class III/IV diagnosed before inclusion	anti-TNF monotherapy (infliximab) vs. conventional therapy (without infliximab) was not associated with an increased risk of lympho-proliferative disorder/ malignancy (*HR*: 1.44; 95% *CI*: 0.86–2.42; $p = 0.163$)
Chaparro et al.[83] 2017 Spain	observational cohort study *C	11,011 IBD patients lymphoma (5 patients*) leukemia (4 patients*) NMSC 4* MSC 3* * exposed to both anti-TNF drugd and immune-suppressants (mainly thiopurines)	NA	the IBD diagnosis	cancer development before the IBD diagnosis; receiving an immune-suppressant other than thiopurines, MTX or an anti-TNF agent (such as cyclosporine or tacrolimus)	TNF-α inhibitors (vs. no treatment) did not increase the risk of EMs (*HR*:= 0.72; 95% *CI*: 0.52–1.00)
Kopylov et al.[84] 2015 Canada	nested case–control cohort study *D	19,582 IBD	NA	the IBD diagnosis	<1 year of follow-up; never exposed to IBD-related medications; the cancer diagnosis predating the inclusion in the database or within the first year	no association was found between the risk of the evaluated malignancies and anti-TNF-α medications

* B – randomized-controlled study; C – cohort study; D – case-controlled study; NYHA – New York Heart Association classification; NA – not applicable.

Table 17. Anti-integrin drugs (vedolizumab) and the risk of extraintestinal malignancies (EMs)

Study, year, country	Study design/ quality level*	Number of participants (*n*)/ type of cancer	Age [years] and gender of the participants	Inclusion criteria	Exclusion criteria	Findings
Colombel et al.[85] 2017	meta-analysis based on 6 studies *A	2,830 patients HCC KC NMSC B cell lymphoma	NA	the IBD diagnosis; phase 2 and phase 3 clinical studies; -patients who received at least one dose of vedolizumab	data from a phase 1 clinical study	18 vedolizumab-exposed patients (<1%) were diagnosed with a malignancy; the incidence of NMSC in vedolizumab-exposed patients was not greater than in patients who received placebo
Card et al.[86] 2020	meta-analysis based on the GEMINI long-term safety (LTS) study and post-marketing (PM) setting *A	1,785 patients TC respiratory tract cancer UBC KC	NA	the IBD diagnosis	patients with a history of malignancy prior to starting treatment with vedolizumab or with a diagnosis of malignancy within 1 year following the initiation of vedolizumab	the total number of the observed malignancies was similar to that expected after standardizing against patients with IBD in the databases
Singh et al.[87] 2022 USA	retrospective cohort study *C	4,807 patients solid organs cancer hematological cancer skin cancer	18–89	the IBD diagnosis	patients with HIV infection, congenital immune-deficiency or organ transplantation; a diagnosis of any malignancy within the baseline12 months prior to index biologic initiation date; a concomitant diagnosis of rheumatoid arthritis, ankylosing spondylitis, psoriasis, or psoriatic arthritis within the baseline 12 months prior to index biologic initiation date	no difference was observed in time to incident malignancy between the vedolizumab and TNF-α antagonists groups (*HR*: 1.15; 95% *CI*: 0.61–2.19)
Cohen et al.[88] 2020	meta-analysis of cohort studies *A	32,752 patients respiratory tract cancer UBC KC lymphoma skin cancer	NA	the IBD diagnosis	NA	in patients with CD, UBC and KC (18 events), skin cancer – unspecified and other (12 events); in patients with UC, respiratory tract cancer (11 events) and lymphoma (14 events)

* A – meta-analysis; C – cohort study; HCC – hepatocellular carcinoma; NA – not applicable.

Table 18. Anti-interleukin drugs and the risk of extraintestinal malignancies (EMs)

Study, year, country	Study design/ quality level*	Number of participants (*n*)/ type of cancer	Age [years] and gender of the participants	Inclusion criteria	Exclusion criteria	Findings
Hong et al.[90] 2022 USA	retrospective cohort study *C	390 patients hematological cancer dermatological cancer	adults M and F	the IBD diagnosis	patients with exposure to multiple agents were excluded from the primary analysis	no increase in the risk of subsequent cancer with ustekinumab (*aHR*: 0.96; 95% *CI*: 0.17–5.41)
Sandborn et al.[91] 2021 USA	meta-analysis *A	2,574 patients papillary RCC PCa testis cancer plasma cell myeloma (multiple myeloma)	NA	the IBD diagnosis; data from phase 2 and phase 3 clinical trials	NMSC	the risk of malignancy was similar in placebo and ustekinumab; placebo – 0.17 (95% *CI*: 0.00–0.93) vs. ustekinumab – 0.40 (95% *CI*: 0.16–0.83)

* A – meta-analysis; C – cohort study; NA – not applicable.

drugs, observed that in patients with a previous history of malignancy there was no recurrence of cancer.[94] The results of the discussed studies are presented in Table 19.[93,94]

Molecular and immunological mechanisms of extraintestinal malignancies in inflammatory bowel diseases

One of the leading factors contributing to an increased risk of cancer in IBD is the chronic inflammatory process.[95] The available studies indicate that this factor plays a key role not only in the development of CRC or small bowel adenocarcinoma, but also EMs. Other factors, such as an older age, smoking, HPV infection, and the coexisting PSC, may independently increase the risk of developing EMs in IBD.[96] Chronic inflammation in IBD leads to the release of pro-inflammatory mediators, like TNF-α, IL-6 and IL-17, which drive cancer development by activating key signaling pathways, such as NF-κB, JAK/STAT, Wnt/β-catenin, and PI3K/AKT. Inflammation leads to oxidative stress by overproducing reactive oxygen species, which can damage DNA and contribute to cancer-related genetic changes.[97] Gut microbiota is the key to maintaining intestinal balance and immune regulation. Dysbiosis is strongly associated with IBD, colitis-associated cancer, and could also contribute to the formation of EMs. Harmful bacteria, like *F. nucleatum* and certain *E. coli* strains, can disrupt the mucosal barrier, trigger inflammation and promote carcinogenesis through mechanisms such as toxin production and DNA damage.[98]

Limitations

Our review has several limitations. Our literature search was limited to the papers published after January 1, 2015, to review the most recent studies. Due to the changing incidence of cancer and dynamic changes in IBD therapy, we decided to limit our search to the last 10 years. Studies of EMs in IBD were characterized by high heterogeneity. Several studies were of retrospective nature and referred to a small sample of IBD patients. The results of these studies do not provide strong evidence and must be interpreted with extreme caution.

Conclusions

An increased risk of oral, pancreatic and hepatobiliary malignancies is observed among EMs in IBD patients (Fig. 2). As for other types of EMs, the results of the presented research are inconsistent and require verification in large population studies.

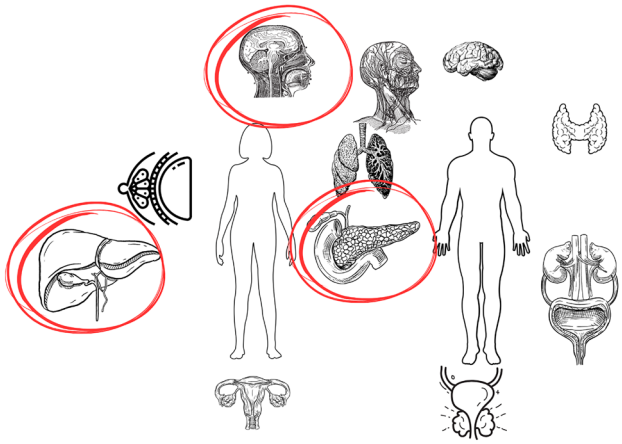

Fig. 2. Extraintestinal cancers in inflammatory bowel disease (IBD)

The dynamic epidemiological situation, combined with the methodological limitations of many existing studies, underscores the need for further research to better understand the relationship between IBD and cancer. In this group of patients, special oncological vigilance, the employment of the available prevention methods (e.g., vaccination), patient education, and, when recommended, screening tests are required. A clinical challenge involving a multidisciplinary approach is the treatment of IBD in cancer patients, especially during disease exacerbation, as well as cancer therapy in IBD patients.

It should be emphasized that the overall risk of EMs in IBD patients remains low, and even if the therapies used are associated with an increase in this risk, it should not influence the decision on the optimal available therapy.

Ethics approval and consent to participate

Not applicable.

Data availability

Not applicable.

Consent for publication

The datasets supporting the findings of the current study are available from the corresponding author on reasonable request.

Use of AI and AI-assisted technologies

The graphical abstract and the figures were created using Canva.

ORCID iDs

Iga Gromny https://orcid.org/0000-0001-6858-176X
Arkadiusz Aab https://orcid.org/0009-0002-3329-2620
Maria Litwiniuk https://orcid.org/0009-0004-5396-7482
Katarzyna Neubauer https://orcid.org/0000-0003-3650-9311

Table 19. Small-molecule drugs and the risk of extraintestinal malignancies (EMs)

Study, year, country	Study design/ quality level*	Number of participants (*n*)/ type of cancer	Age [years] and gender of the participants	Inclusion criteria	Exclusion criteria	Findings
Chen et al.[93] 2024 multi-national study	meta-analysis of randomized controlled trials (RCT) *A	10,623 different types of cancer (including NMSC and other EMs)	adults M and F	patients diagnosed with IBD, receiving the administered SMDs	pediatric patients with IBD	in general, no malignancies were linked to SMDs; using SMDs was not significantly associated with the risk of developing malignant tumors, excluding NMSC (*RR*: 1.20; 95% *CI*: 0.55–2.59); using SMDs was not associated with an increased risk of developing NMSC (*RR*: 1.14; 95% CI: 0.41–3.23)
Goessens et al.[94] 2021 multi-national study	retrospective observational study *C	98	adults M and F	IBD patients with the concomitant use of 2 biologics or 1 biologic with SMDs	pediatric patients with IBD	4% of the study population had a previous history of cancer before undergoing combination therapy, with no recurrence noted after a median follow-up of 14.5 months

* A – meta-analysis; C – cohort study; SMDs – small-molecule drugs; NA – not applicable.

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Review

Smoldering inflammation: The silent flame driving heart failure

Michał Maksymilian Wilk*[1,2,A–F], Jakub Wilk*[1,2,A–F], Kamila Florek*[1,3,A–F], Wojciech Jan Zimoch[1,4,C,E,F]

* first authors
[1] Institute of Heart Diseases, Wroclaw Medical University, Poland
[2] Student Scientific Organization, Institute of Heart Diseases, Wroclaw Medical University, Poland
[3] Students' Scientific Group of Invasive Cardiology, Institute of Heart Diseases, Wroclaw Medical University, Poland
[4] Institute of Heart Diseases, Wroclaw University Hospital, 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

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Address for correspondence
Michał Maksymilian Wilk
E-mail: michalwilk200218@wp.pl

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Abstract

In recent years, significant advancements in the understanding of the processes underlying heart failure (HF) have been made, particularly regarding the role of chronic low-intensity inflammation or smoldering inflammation (SI). This review consolidates findings from the available literature and illustrates the relationships between inflammation, neurohormonal activation, metabolic derangements, and comorbidities in HF, with a focus on heart failure with preserved ejection fraction (HFpEF).

A comprehensive literature search was conducted using PubMed®, Wiley Online Library, Scopus, and Web of Science (limited to 2025). The search terms included "heart failure", "HFpEF", "inflammation", "smoldering inflammation", "biomarkers", "cytokines", "fibrosis", and "comorbidities". Peer-reviewed articles, reviews, as well as clinical and observational studies describing the mechanistic, prognostic and therapeutic aspects of SI in HF were included. Studies limited to acute coronary syndrome (ACS) were excluded.

Structural changes leading to hemodynamic perturbations in HFpEF are correlated with processes mediated by SI. Several biomarkers measure inflammation and provide diagnostic and prognostic value, including C-reactive protein (CRP), interleukin-6 (IL-6), soluble suppression of tumorigenicity 2 (sST2), galectin-3 (Gal-3), and iron homeostasis. Clinical trials demonstrate the efficacy of sodium–glucose cotransporter-2 (SGLT-2) inhibitors, glucagon-like peptide 1 (GLP-1) receptor agonists, and other targeted interventions in the modulation of SI.

Smoldering inflammation is a key mechanism in the pathogenesis of HFpEF and the progression of comorbidities. Understanding SI may improve risk stratification and management strategies. Both established and emerging anti-inflammatory therapies, when administered alone or in combination, may target SI in order to enhance HF management.

Keywords: inflammation, heart failure, cardiac remodeling, neurohormonal interactions

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Highlights

- Smoldering inflammation (SI) is a key factor in the pathophysiology of heart failure (HF).
- Understanding SI may enhance risk stratification and guide personalized management strategies in HF.
- Both established and emerging anti-inflammatory therapies, used alone or in combination, may effectively target SI to improve HF outcomes.

Introduction

Heart failure (HF) is a cardiac condition that has emerged as a global health concern, affecting approx. 64 million individuals worldwide.[1] Recent advancements in pharmacotherapy for one of the HF phenotypes, HF with reduced ejection fraction (HFrEF), have shifted the structure of patients presenting with a specific HF phenotype toward HFpEF. Nowadays, HFpEF accounts for more than half of the patients presenting with HF.[2] Due to its heterogeneous etiology and complexity of development, managing this condition is still a significant challenge. One of the potential factors contributing to the pathophysiology of HF, and especially to HFpEF, is smoldering inflammation (SI), which can be described as chronic low-grade inflammation, promoting maladaptive responses and triggering maladaptive mechanisms.[3]

Inflammation is associated with neurohormonal activation, fibroblast activation, and subsequent fibrosis, oxidative stress, vascular endothelial dysfunction, and ischemia.[3] These processes are the fundamental core of SI. The chronic nature of this condition complicates its observation and analysis. However, in recent decades, the complex relationship between metabolic pathways and the onset and development of HF has been revealed.[4] Consequently, we have identified specific inflammation-related biomarkers that might assist in predicting the future onset of HF that remains clinically silent.[5] Additionally, inflammatory indicators may prove useful in risk stratification and outcome assessment.[2,3,5–12]

Comorbidities that are prevalent in patients with HF should be perceived not only as additional conditions, but rather as a common result of a shared pathophysiological, inflammation-dependent process or even a direct cause of HF development.[13–22] It is important to distinguish the connection between HF and comorbidities because effective treatment of concomitant diseases is a promising way of preventing HF onset or progression. In the subset of HFpEF patients, SI appears to play a pivotal role in the pathogenesis process, simultaneously promoting the development of multimorbidity and exacerbating its severity.[5,23,24] Therefore, the role of SI in the etiology of the disease requires further evaluation.

Lastly, pioneering discoveries related to the role of inflammation in the exacerbation of HF provided a rationale for the evaluation of novel drugs for SI alleviation, as well as for the identification of effective responses to the growing number of patients presenting with HFpEF who require advanced treatment.[16,25–27] Numerous therapeutic avenues related to SI research offer optimism for the identification of efficacious, life-extending therapeutic modalities, but the results of these studies require careful analysis.

Even though numerous studies and reviews have explored the role of inflammation in cardiovascular disease (CVD) and HF, the majority of these studies have focused on either HFpEF or responses to acute inflammation. Smoldering inflammation has not been explored as a mechanism that connects comorbidities, metabolic dysfunction and structural cardiac remodeling in the context of HFpEF. Importantly, none of the reviews has systematically linked biomarkers, comorbidities or therapies into a single construct. Current studies describe biomarkers, comorbidities or therapies separately, and do not provide a comprehensive framework of how chronic, low-grade inflammation contributes to the presentation, progression and therapeutic response of HFpEF. By using available evidence regarding molecular pathways, biomarkers, comorbidities, and novel anti-inflammatory interventions, our objective is to identify and address this knowledge gap by offering a comprehensive view of SI in the context of HFpEF. We hope that our unique approach will compel others to emulate this model in clinical research and alternative therapeutic approaches.

Therefore, the aim of this review was to consolidate the current evidence regarding the role of SI in HF, with a particular focus on HFpEF. Particular attention has been placed on biomarkers, outcomes, comorbidities, and novel therapies to elucidate the associations between SI and the development, exacerbation and treatment of HFpEF.

Material and methods

In this review, a structured literature search was performed for studies assessing the role of SI in the field of HF, with a focus on HFpEF. The core databases used for the search were PubMed® and Scopus, with supplementary searches conducted in Web of Science and Wiley Online Library, if appropriate. The following Medical Subject Headings (MeSH) terms were used: "heart failure", "HFpEF", "inflammation", "smoldering inflammation", "biomarkers", "cytokines", "fibrosis", and "comorbidities".

These keywords were utilized to assess studies evaluating a range of pathophysiological mechanisms, as well as diagnostic and prognostic aspects of molecular pathways for SI and therapeutic measures implemented in HF.

The search encompassed articles published until 2025, with no other limitations regarding study design. To assess the multifactorial source of inflammation in HF, the study analyzed clinical trials, systematic reviews and observational cohort studies.

The inclusion criteria for the study encompassed peer-reviewed original articles, reviews, meta-analyses, and clinical trials that discussed inflammation, inflammatory biomarkers and inflammation-targeted therapies within the context of HF, with a particular focus on HFpEF. The exclusion criteria were studies that focused solely on acute coronary syndrome (ACS) or non-inflammatory origin of HF. The selection of articles was based on relevance, scientific quality and novelty.

Pathophysiology

The pathophysiology of HF is complex, with inflammation playing a pivotal role in this process. Nevertheless, the contribution of inflammation varies depending on the HF phenotype, with myocardial injury being the leading cause of HFrEF and inflammation in HFpEF.[3] Notably, these phenotypes are not distinct entities, with heart failure with mildly reduced ejection fraction (HFmrEF) serving as a buffer between them that can progress to either HFpEF or HFrEF.[4,28] The phenotypes of HF share some common pathways, some of which are more pronounced in HFpEF compared to HFrEF and vice versa.[3]

Heart failure with preserved ejection fraction is mainly preceded by metabolic diseases, including obesity and type 2 diabetes mellitus (T2DM). These conditions induce chronic low-grade inflammation, also referred to as metainflammation.[29] Metainflammation leads to coronary microvascular endothelial inflammation, which contributes to cardiac remodeling through fibrosis and hypertrophy.[30,31] The aforementioned mechanisms result in left ventricular (LV) stiffness and increased filling pressure at rest and during exercise.[32–35]

A prominent contributor to this comorbidity-driven inflammation is the nucleotide oligomerization domain-like receptor family pyrin domain containing 3 (NLRP3) inflammasome.[36,37] This cascade is triggered by interleukin (IL)-1 and tumor necrosis factor alpha (TNF-α), or by reactive oxygen species (ROS) with mitochondrial damage, both leading to the increased activation of NLRP3. Consequently, NLRP3, via adaptor protein, binds to the caspase-1, which cleaves pro-IL-1β and pro-IL-18 to their active forms.[38] An effect of IL-1β is seen as a further enhancement of nuclear factor kappa B (NF-κB) production and an increase in IL-6 level, directly stimulating the liver to synthesize highly sensitive C-reactive protein (hs-CRP), which is a widely available biomarker of inflammation.[39] On the other hand, higher oxidative stress observed in HFpEF contributes to the enhancement in ROS production with concomitant endothelial dysfunction, causing a drop in nitric oxide (NO) synthesis, which is a direct inhibitor of NLRP3. Nevertheless, NO is not only involved in the cross-talk between these pathways; its impaired production results in the suppression of the NO–soluble guanylate cyclase (cGMP)–protein kinase G (PKG) cascade, leading to vasoconstriction and fibrosis.[40] Moreover, NO–cGMP–PKG knockdown results in the hypophosphorylation of titin, which is a key sarcomeric protein involved in diastolic cardiomyocyte relaxation, consequently inducing cardiomyocyte stiffness.[41] In addition, PKG downregulation results in the hypophosphorylation of the protein RhoA, which reduces its protective role and leads to the hypertrophic and fibrotic activation of vascular smooth muscle cells (VSMCs).[41]

Another factor that exerts an antifibrotic and antihypertrophic effect on the heart is IL-33, which is released in response to cardiac mechanical stress and injury.[42,43] Importantly, the cardioprotective signaling pathway activated by IL-33 involves suppression of tumorigenicity 2 (ST2) with its 2 variants – transmembrane (suppression of tumorgenicity 2 ligand (ST2L)) located at myocytes, fibroblasts and inflammatory cells, which is activated by IL-33, inducing antihypertrophic and antifibrotic mechanisms that promote adaptive remodeling, and a soluble form (soluble suppression of tumorigenicity 2 (sST2)), which plays the role of the decoy receptor, sequestering IL-33.[43] On the other hand, in conditions of cardiac stress or damage, an increased level of sST2 is released, preventing the formation of the ST2–IL-33 complex and suppressing its cardioprotective effect.[43] Of note, IL-33 activates NF-κB, which has a dual role in acute hypoxia and cardiac injury and exerts a protective role by inhibiting NF-κB activation induced by angiotensin II, a hypertrophic stimulus.[44] However, its chronic activation promotes HF by enhancing the effects of IL-1, TNF-α and IL-6.[44]

Galectin-3 (Gal-3) is another molecule with a dual role. It is secreted by activated macrophages and plays apoptotic and antinecrotic roles. However, its long-term overexpression observed in HF enhances pro-inflammatory and pro-fibrotic processes.[45] Galectin-3, via fibroblast activation, stimulates extracellular matrix (ECM) components like collagen I and collagen III and the synthesis of cytoskeletal proteins, simultaneously inhibiting matrix metalloproteinase (MMP)-induced degradation.[45] It is worth mentioning that the disturbance between MMPs and their endogenous inhibitor may lead to excessive accumulation of collagen in the myocardium, which, in turn, further promotes fibrosis and secondary stiffness, ultimately impairing the diastolic function of the ventricles, especially in HFpEF.[46] Other factors contributing to collagen degradation are angiotensin II, aldosterone, TNF-α, and transforming growth factor beta (TGF-β).[47] Moreover,

Gal-3 induces aortic valve calcifications through NF-κB and, by the TGF-β1/Smad pathway, promotes atrial fibrillation (AF), subsequently leading to impaired diastolic filling.[48]

The cumulative effect of these molecular alterations — inflammation, fibrosis and endothelial dysfunction — leads to structural and functional impairment, characterized by myocardial stiffening and impaired ventricular relaxation, accompanied by increased filling pressures and atrial remodeling. As a result, the clinical manifestation of HF presents as dyspnea, exercise intolerance and systemic congestion. A complex metabolic interplay is demonstrated in Fig. 1, and key findings regarding biomarkers and pathways related to SI are summarized in Table 1.

Clinical role of inflammatory biomarkers in HFpEF

Heart failure is a progressive disease that develops through stages, as defined by the American College of Cardiology (ACC) and the American Heart Association (AHA).[49]

The presence of risk factors contributing to HF development, e.g., T2DM, corresponds to stage A, followed by asymptomatic structural heart diseases (stage B), the presence of clinical symptoms of HF (stage C), and end-stage or refractory HF described as stage D.[50] Even though the risk factors of HFpEF are well-defined, the determinants contributing to the progression of HFpEF from stage A to B, as well as more advanced stages, are under investigation.[51] Several biomarkers involved in the inflammatory processes underlying HFpEF development are considered to be predictors of disease progression and patients' outcomes, as well as therapeutic targets.[52]

A recent meta-analysis emphasized that high levels of CRP were associated with a 9% increase in the risk of HFpEF development.[6] In addition, a cohort study revealed the significant role of other NLRP3 inflammasome molecules, particularly IL-6 in HFpEF and TNF-α in the entire HF group.[53] Importantly, a prospective study within a PREVEND cohort demonstrated strong evidence of IL-6's predictive role in new-onset HFpEF, underscoring its significance in HFpEF, but not in HFrEF incidence prognosis.[54]

However, among patients diagnosed with HFpEF, significantly higher CRP levels were noted in those with a greater comorbidity burden, whereas CRP was within a normal range in 40% of individuals. These findings underscore the need to broaden the spectrum of biomarkers involved in the assessment of patients with HFpEF.[55] Even though current HF phenotyping relies primarily on the echocardiographic examination, a meta-analysis involving proteomic studies that compared HFpEF and HFrEF biomarker profiles suggested that they can be distinguished based on higher levels of IL-6 and lower levels of syndecan-1 (SDC-1) and NO in HFpEF patients, highlighting the difference in prevailing pathomechanisms underlying HF presentation.[56]

Table 1. Key findings on biomarkers and pathways of smoldering inflammation (SI) in heart failure (HF)

Biomarker/pathway	Role in HFpEF	Clinical relevance
CRP/hs-CRP	downstream of IL-6; marker of systemic inflammation	predicts HF onset and progression; hsCRP ≥ 2 mg/L identifies HFpEF patients at a higher risk of HF events/mortality
IL-6	central cytokine driving inflammation, fibrosis and endothelial dysfunction	strong predictor of new-onset HFpEF (PREVEND cohort); prognostic for mortality in HFpEF (LUCRIC)
TNF-α	pro-fibrotic, hypertrophic and pro-inflammatory mediator	associated with HF progression; significant in global HF cohorts, less specific in HFpEF
NLRP3 inflammasome	activates caspase-1, leading to IL-1β and IL-18 release; amplifies chronic inflammation	experimental/early clinical target linked to endothelial dysfunction and fibrosis
IL-1β	enhances NF-κB, upregulates IL-6, propagates inflammation	anakinra reduces CRP in HFpEF; canakinumab is effective in CVDs
sST2/IL-33 pathway	cardioprotective; sST2 acts as a decoy, blocking this effect	sST2 predicts rehospitalization and mortality; strong prognostic role in HFpEF
Gal-3	secreted by macrophages; induces fibroblast activation, collagen synthesis and ECM remodeling	predicts new-onset HFpEF, adverse outcomes and the severity of LVDD
GDF-15	marker of inflammation and oxidative stress	independent predictor of adverse outcomes, particularly strong in the HFpEF subgroup
Iron deficiency (sTfR, hepcidin)	causes impaired oxygen delivery, worsens exercise tolerance and outcomes	predicts mortality; IV iron supplementation improves prognosis
Congestion–inflammation crosstalk	congestion activates inflammation and contributes to lymphatic dysfunction	inflammation and congestion are partly independent pathways; therapeutic implications

CRP – C-reactive protein; CVD – cardiovascular disease; ECM – extracellular matrix; Gal-3 – galectin-3; GDF-15 – growth differentiation factor 15; HF – heart failure; HFpEF – heart failure with preserved ejection fraction; hs-CRP – highly sensitive C-reactive protein; IL – interleukin; IV – intravenous; LVDD – left ventricular diastolic dysfunction; NF-κB – nuclear factor kappa B; sST2 – soluble suppression of tumorgenicity 2; ST2L – suppression of tumorgenicity 2 ligand; sTfR – serum soluble transferrin receptor; TNF-α – tumor necrosis factor alpha.

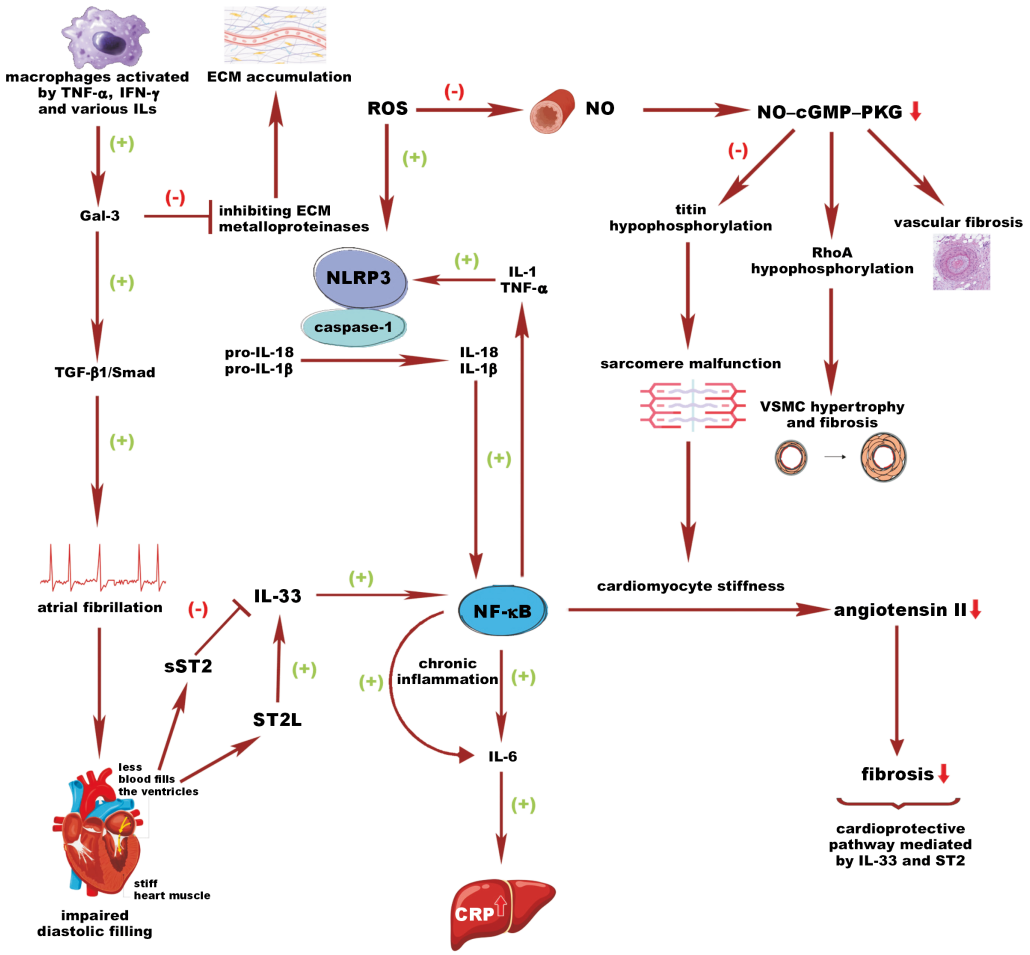

Fig. 1. Pathophysiological mechanisms of smoldering inflammation (SI) in the development and progression of heart failure (HF)

cGMP – soluble guanylate cyclase; CRP – C-reactive protein; ECM – extracellular matrix; Gal-3 – galectin-3; IFN-γ – interferon gamma; IL – interleukin; NF-κB – nuclear factor kappa B; NLRP3 – nucleotide oligomerization domain-like receptor family pyrin domain containing 3; NO – nitric oxide; PKG – protein kinase G; ROS – reactive oxygen species; sST2 – soluble suppression of tumorgenicity 2; ST2 – suppression of tumorgenicity 2; ST2L – suppression of tumorgenicity 2 ligand; TGF-β – transforming growth factor beta; TNF-α – tumor necrosis factor alpha.

Moreover, a meta-analysis on the role of CRP in HF progression confirmed its predictive value regarding cardiovascular and all-cause mortality. Of note, the significance of CRP regarding long-term adverse cardiovascular outcomes was inconclusive, depending on its presentation in the included studies. A significant association was found for categorical variables, while no such effect was observed for continuous variables.[6] Nevertheless, the LUCRIC study has analyzed the difference in the inflammatory biomarkers comparing patients with HFpEF and HFrEF, and exhibited the role of IL-6 in the mortality prognosis in a mean 9.9-year follow-up period among HFpEF patients.[5] Similar results were observed for hs-CRP, whereas they failed to be significant after full adjustment.[5] Of note, neither IL-6 nor hs-CRP predicted cardiovascular mortality in HFrEF patients, hence highlighting the role of subclinical inflammation in the HFpEF population.[5] However, recent results from the TOPCAT trial revealed that hs-CRP ≥ 2 mg/L is an appropriate tool to identify HFpEF patients at a higher risk of HF events and cardiovascular mortality.[57]

Other relevant biomarkers, whose role in the progression to clinically overt HF among patients with risk factors was evaluated in the retrospective analysis of the STOP-HF longitudinal study, are sST2 and Gal-3.[58] This study confirmed the role of Gal-3 in the predictive profile, along with B-type natriuretic peptide (BNP) and high-sensitivity troponin I, whereas it rejected sST2 and IL-6. The results in terms of IL-6 were contrary to those of larger, prospective studies.[58] The role of Gal-3 was further confirmed by a meta-analysis, which considered it an appropriate biomarker to indicate new-onset HFpEF, as well as a valuable predictor of adverse outcomes and severity of left ventricular diastolic dysfunction (LVDD) in HFpEF.[59] Furthermore, Gal-3 is involved in the activation of MMP.[46] Among patients with hypertension, MMP-9 and tissue inhibitor of metalloproteinase 1 (TIMP-1) indicated significantly greater degrees of asymptomatic LVDD.[46] Galectin-3 also induces the upregulation of TGF-β, whereas its role as a biomarker is modest.[46] In contrast to another TGF-β family molecule, substantially increased inflammation and oxidative stress, known as growth differentiation factor 15

(GDF-15), was shown to be an independent risk factor of adverse outcomes across the entire HF spectrum, with its incremental prognostic role in the HFpEF subgroup.[60] In another study, the GDF-15 level measured within 48 h of hospital admission in patients with HFpEF was a better predictor of 1-year rehospitalization than N-terminal pro-B-type natriuretic peptide (NT-proBNP).[61]

Nevertheless, while focusing on sST2 among individuals hospitalized due to HF, its baseline levels are associated with further rehospitalizations and all-cause mortality, independently of ejection fraction. Furthermore, sST2 combined with NT-proBNP, especially in HFpEF patients, is suggested to improve the predictive value of the test.[62] Another clinical trial analyzing the role of sST2 noted its role as a significant biomarker in both HF phenotypes, whereas in HFpEF, sST2 revealed a stronger association with patients' outcomes.[63] Other molecules involved in the pathogenesis of HFpEF are mechanistically crucial and play key roles as therapeutic targets, whereas their clinical predictive role is limited and currently of lower importance than the aforementioned parameters.

It is also important to note that SI and the activation of various inflammatory biomarkers or processes can contribute to congestion development, and vice versa.[64–67] Moreover, several mechanisms of both congestion and inflammation contribute to fibrosis and impaired sodium handling, which are part of the disease's pathophysiology. However, clinical trial data indicates that these pathways are independent. For example, interventions targeting the natriuretic peptide axis do not reduce inflammatory processes.[68] Importantly, the role of the lymphatic system has recently been demonstrated in both HF and decongestion, further linking the immune system to congestion development.[69–74]

Iron deficiency in patients with HF is also an important biomarker for predicting outcomes.[75–77] Patients with HF are more likely to develop an iron shortage, which can lead to poorer therapeutic results than in patients without such conditions. Jankowska et al. defined iron deficiency as a high (≥1.59 mg/L) level of serum soluble transferrin receptor (sTfR), indicating unmet cellular iron needs, and a low (<14.5 ng/mL) hepcidin level, reflecting low iron storage.[75] Patients with acute HF (AHF) who met both criteria had the highest mortality rate of 41%. In comparison, a mortality rate of 15% was noted in individuals with an isolated high sTfR level, and a rate of 7% was observed in those with an isolated low hepcidin level.[75] Another trial has connected lactate levels with sTfR. Biegus et al. assessed the relationship between elevated markers (>2 mmol/L and >1.59 mg/L, respectively) in patients with AHF and the occurrence of adverse outcomes.[76] It showed that with both markers present, the prognoses were poorer than in patients with one or none of the criteria met.[76] The solution to this issue may be iron supplementation. Ahmed et al. conducted a meta-analysis on intravenous (IV) iron supplementation in patients with HF.[77] The results indicated that such therapy can reduce CV mortality, 1-year all-cause mortality, first HF hospitalization, and even improve left ventricle ejection fraction (LVEF).[77] These studies underscore the importance of assessing iron status in patients with HF, especially those with AHF, as it may be considered a valuable predictive factor of future outcomes. Another reason for its importance is the possibility of reversing it by IV iron supplementation. Although the relationship between iron metabolism and inflammation is well-documented, with evidence pointing to the modulatory role of hepcidin in regulating iron availability, current state of knowledge does not allow us to understand the impact of inflammation on the effectiveness of IV iron therapy. Therefore, additional long-term studies are needed in this area.[78]

Comorbidities associated with HFpEF and inflammation

During the past decades, medical attention has concentrated on the management of HFrEF, which resulted in clinical success and a decline in mortality rates within this particular phenotype. The described progress can be attributed to the implementation of a well-established quadruple therapy, consisting of sodium–glucose cotransporter-2 (SGLT-2) inhibitors, mineralocorticoid receptor antagonists (MRAs), beta-blockers, and angiotensin converting enzyme (ACE) inhibitors.[2,79–81] Despite the improvement in HFrEF survivability, another issue has emerged. Currently, the predominant form of HF is HFpEF. This HF subtype is a surging threat, accounting for more than half of all HF hospitalizations.[2,82] It is a complex condition that creates various diagnostic and therapeutic difficulties due to its multifactorial and heterogeneous etiology, which is still not well understood.[2,14] Thereupon, specific attention should be given to established risk factors that contribute to the development of HFpEF, with a strong focus on the widespread comorbidities associated with this condition.[2,5,13–16,20–22,24,79,82–85] Distinguishing each coexisting disorder and a thorough evaluation are essential to assess whether the condition is a complication of HF or an independent disease that can be addressed through an individualized therapeutic strategy.[13] Accordingly, it is imperative to investigate the correlation between the onset of HFpEF and the prevalence of comorbidities. We hereby propose a focused examination of low-grade chronic inflammation, which not only contributes to HFpEF evolution but also worsens its course by the promotion of comorbidities (Fig. 2).[5,13,24,82,86]

Atrial fibrillation

Atrial fibrillation is a common form of arrhythmia, widely diagnosed in patients with HFpEF due to the presence

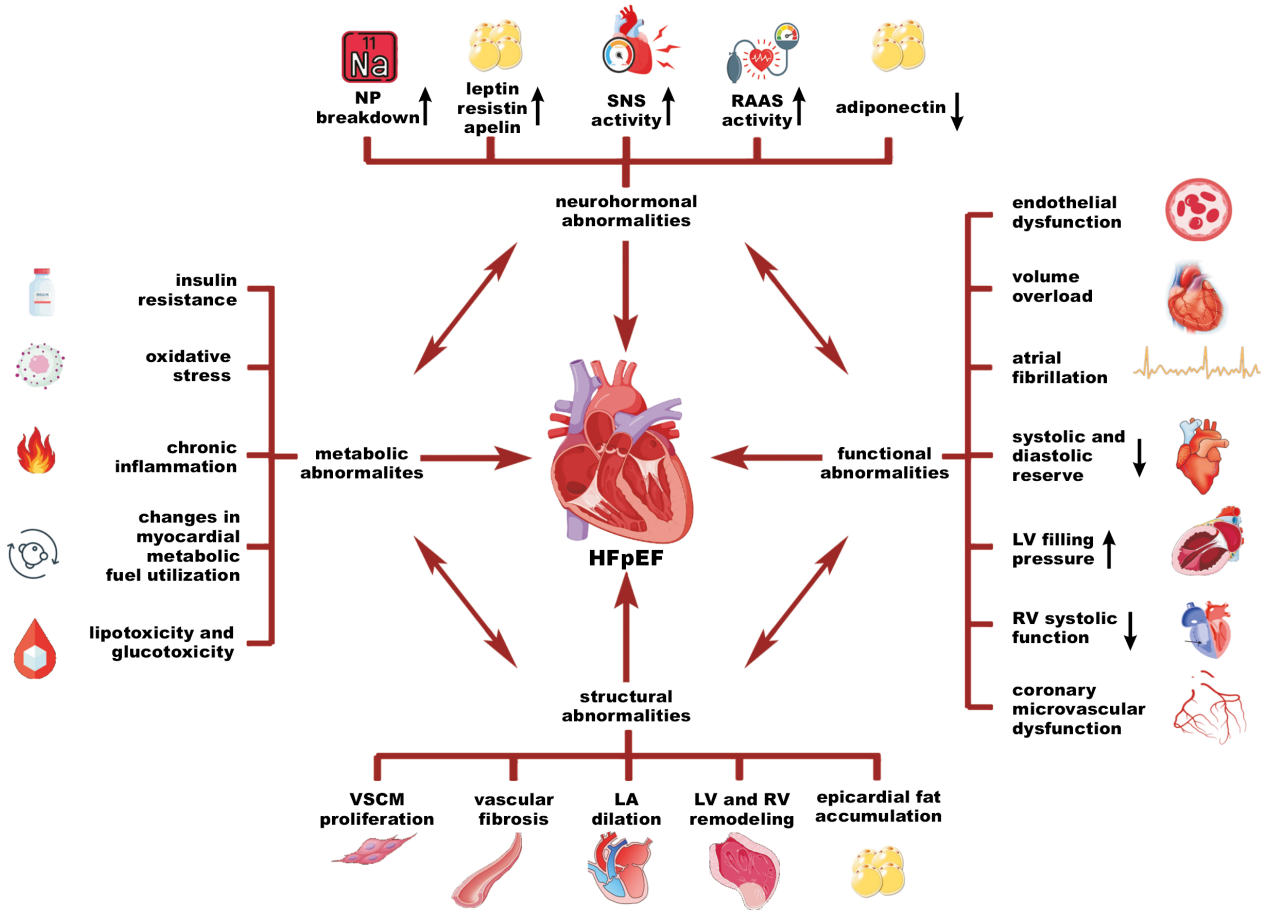

Fig. 2. Multifactorial pathogenesis of heart failure with preserved ejection fraction (HFpEF)

LA – left atrial; LV – left ventricular; NP – natriuretic peptide; RAAS – renin–angiotensin–aldosterone system; RV – right ventricular; SNS – sympathetic nervous system; VSCM – vascular smooth muscle cell.

of shared pathophysiological mechanisms. It has been associated with left atrial (LA) wall remodeling and initiated by increased LA pressure, as a consequence of impaired diastolic function of the left ventricle.[13,86,87] The etiological co-occurrence between these 2 conditions is associated with the activation of maladaptive processes, such as chronic inflammation, oxidative stress and endothelial dysfunction. These risk factors are induced by highly prevalent diseases, mostly hypertension, obesity, T2DM, and chronic kidney disease (CKD). The conditions are manageable in many cases, therefore, they represent an important aspect of curtailing HFpEF progression as well as implementing suitable treatment.[87,88] On a molecular level, inflammation plays a pivotal role in disease evolution since pro-inflammatory cytokines are responsible for activating fibroblasts that promote remodeling via collagen deposition within an ECM inside the LA myocardium. In patients suffering from HFpEF, AF might occur after HF diagnosis, concurrently, or even prior to HF identification, especially if HFpEF is subclinical or has been stable.[86] Regardless of the coincidence of these 2 conditions, AF has been identified as a significant risk factor for mortality when compared to HFpEF patients with sinus rhythm.[86] Therefore, it is important to perceive AF not only as a coexisting disease, but also as a substantial stratification factor that may influence therapeutic interventions, including the mitigation of risk factors, proper pharmacotherapy, and the performance of electrophysiological procedures.[2,86]

Obesity

Obesity is a condition characterized by excessive fat accumulation.[15] The global prevalence of this disease has increased drastically over the past few decades, reaching pandemic levels.[15,89] In 2024, it was estimated that 13% of the adult world population suffered from obesity, while 39% were overweight.[15] Accordingly, HF and obesity are two conditions that increasingly coexist each year, and impact each other via various pathophysiological interactions, including structural, functional, metabolic, and neurohormonal abnormalities.[13–16,79,82,90,91] Evidence suggests a strong interconnection between all phenotypes of HF and obesity; however, the association is remarkable and most significant in terms of HFpEF, where increased body mass is believed to be not only a comorbidity but, most importantly, a direct cause of HFpEF pathogenesis.[2,16,90]

It has been established that adipose tissue is an abundant source of biologically active molecules. Fat accumulation is associated with the dysregulation of various compounds, including leptin, resistin, adiponectin, and apelin.[92] These molecules have multidirectional effects, such as appetite regulation, vasodilatation, insulin sensitization, the suppression of inflammation, and the production of ROS.[92] The impaired function of these adipokines can contribute to the development of metabolic disorders, such as poor glucose tolerance, increased synthesis of fatty acids with pro-inflammatory potential, elevated production of reactive oxygen and nitrogen species, heightened sympathetic nervous system (SNS) activity, and, above all, the promotion of inflammation mediated by macrophages through the secretion of cytokines.[15,92,93] This particular situation is a starting point at which metabolic and neurohormonal abnormalities contribute to the development of functional and structural alterations that manifest in the course of HFpEF. It is important to acknowledge the chronic nature of this process, which may remain in the subclinical phase for many years. However, the presence of additional contributing factors, such as AF, can precipitate the development of full-blown HFpEF and result in a condition that may be difficult or even impossible to reverse.[13–16,79,90,93] The impact of inflammation on cardiac remodeling in the HFpEF–obesity subset is extremely complex, but some common structural observations include perivascular and interstitial fibrosis, VSMC proliferation, LV and RV remodeling, LA dilation, and epicardial fat accumulation.[15] Lastly, these findings contribute to functional impairment that is characterized by volume overload, coronary microvascular dysfunction, AF, a decline in systolic and diastolic reserve, and an increase in LV filling pressure.[15] On this account, obesity plays a crucial role in the pathogenesis of HFpEF, as well as in its management and therapy. Given its reversible nature and the potential for early intervention, especially in patients with an extreme risk of adverse cardiovascular events, it should receive proper attention. Pharmacotherapy plays an essential role in the treatment of obesity, primarily due to the ineffectiveness of lifestyle modification in many cases.

Type 2 diabetes mellitus

Type 2 diabetes mellitus is another example of comorbidity associated with inflammation-driven promotion of HFpEF. During periods of uncontrolled hyperglycemia, the spontaneous formation of advanced glycation end products (AGEs) has been observed.[79,93,94] These compounds can promote an inflammatory response by activating the receptors for advanced glycation end products (RAGEs), commonly expressed on the surface of endothelial cells, smooth muscle cells and macrophages.[95] The binding of AGEs to their receptor triggers a signaling cascade that activates the NF-κB pathway.[95] This, in turn, leads to the production and release of pro-inflammatory cytokines, such as TNF-α, IL-1β and IL-6,[95] which promote inflammation, cause vascular dysfunction and contribute to HF pathogenesis. A vicious cycle ensues: chronic hyperglycemia leads to increased accumulation of AGEs, resulting in a heightened inflammatory response and cardiovascular damage, which causes deterioration of glucose tolerance.[95] Chronic subclinical inflammation results in oxidative stress, endothelial injury, NO signaling deficiency, and altered calcium handling.[94,96] Subsequently, a compensatory response occurs in the form of neurohormonal activation, myocyte hypertrophy and fibrosis. These processes lead to ventricular remodeling, thereby contributing to the onset of HFpEF.[79,87,94,96] On account of sympathetic hyperactivity, symptoms such as tachycardia and/or arrhythmias may manifest.[96] Therefore, it is important to evaluate the role of T2DM-driven inflammatory changes in the progression of HF, given its reversible character and the potential to counteract the development of HFpEF.[2,14,87,97] The significance of SGLT-2 cotransporter inhibitors must be emphasized. The STRIDE trial substantiated the efficacy of semaglutide, demonstrating a 50% reduction in all-cause mortality.[27]

Treatment

Guideline-directed medical therapy (GDMT) has revolutionized the management of HFrEF, with high-level evidence supporting the use of ACE inhibitors and angiotensin receptor/neprilysin inhibitors (ARNIs), beta-blockers, MRAs, and, more recently, SGLT-2 inhibitors to reduce mortality.[98] Emerging studies suggest a potential therapeutic benefit of medication usage in the HFmrEF population.[98] In contrast, the treatment options for HFpEF remain limited, with SGLT-2 inhibitors being the only drugs that have demonstrated a clinical effect. Thus, there is a great unmet need for novel therapies that target the pathophysiological mechanisms of HFpEF. The new data concerning systemic inflammation and its potential origins in other systemic diseases may facilitate the identification of the phenotype of HF in patients and the provision of the most effective treatment.

Sodium–glucose cotransporter-2 inhibitors have emerged as promising therapeutic agents for the treatment of HF.[33] Their unique anti-inflammatory properties are crucial in the inflammation-based pathophysiology of HFpEF. Moreover, they lower the mortality rate in patients with T2DM.[99] In a trial on endothelial cells, it was demonstrated that SGLT-2 inhibitors reduce the expression of NF-κB and MMP-9, thereby lowering the inflammatory pathways.[100] Simultaneously, they increase the expression of SIRT6, which plays a role in DNA repair. The utilization of these pharmaceutical agents has been associated with a reduced likelihood of major adverse

cardiac events.[100] Other clinical trials have noted that SGLT-2 inhibitors lower the hs-CRP levels by over 54% after 1 year of usage in patients with T2DM.[101] In light of the EMPEROR-Preserved study findings, special attention should be placed on the implementation of empagliflozin in all HFpEF patients, regardless of the presence or absence of T2DM, given its established efficacy in mitigating HFpEF symptoms and improving prognoses.[25]

Several studies have demonstrated the efficacy of glucagon-like peptide 1 (GLP-1) receptor agonists, particularly semaglutide, in obese HFpEF patients. The administration of these agents resulted in weight loss, enhanced quality of life, and, most importantly, reduced mortality.[2,16,27,79,102,103] This establishes an important direction in the comprehensive treatment of patients with HF, emphasizing an approach in which obesity is treated as an independent co-occurring disease requiring treatment rather than placing the entire responsibility for alleviating obesity on the patient, which frequently proves unfeasible.

Another area of investigation aimed at finding a more effective method to phenotype patients is myeloperoxidase (MPO) inhibition. During the SATELLITE trial, AZD4831, the MPO inhibitor, has been administered to patients.[104] Afterward, the biomarker pathways most related to clinical outcomes in individuals with HFpEF were found to be downregulated. Nevertheless, the cohorts included in this trial were small; therefore, the results must be investigated in a larger group.[104]

The NLRP3 inflammasome is the next target of anti-inflammatory therapy. Many molecules, including colchicine, GDC-2394 and dapansutrile, are in the stage of clinical development.[26,104–106] Despite this, colchicine exhibits a strong anti-inflammatory action by targeting the NLRP3 inflammasome and inhibiting the production of pro-inflammatory cytokines, such as IL-1 and IL-6.[88,105] In HFpEF, this property has the potential to reduce SI and possibly regulate maladaptive myocardial remodeling, along with enhancing cardiac function.[88] Nevertheless, pro-inflammatory cytokines (IL-1β or IL-6), which are associated with the NLRP3 pathway, have undergone more advanced trials concerning their inhibitors. Anakinra and canakinumab, the inhibitors of IL-1β, have been found to reduce inflammation in patients with CVD. Anakinra has significantly decreased CRP levels in patients with HFpEF.[107] The IL-6 inhibitors, namely tocilizumab (ASSAIL-MI) and ziltivekimab (RESCUE) have reduced systemic inflammation, as evidenced by decreased CRP levels in patients with myocardial infarction and CKD, respectively.[108,109] Lastly, the recent CORTAHF trial has shown that the use of burst steroid therapy resulted in lower inflammation, more effective decongestion, and improvements in quality of life in the AHF population.[68,110–113] Specifically, this study has demonstrated that 7-day therapy with prednisone at a dose of 40 mg in patients with AHF and elevated hs-CRP led to a reduction in hs-CRP levels on day 7 of therapy, and additionally, on day 90, patients who received burst therapy showed a significantly lower risk of rehospitalization or HF decompensation.[68,110–113] The graphical summary of therapeutic modalities is demonstrated in Fig. 3 and summarized in Table 2.

Smoldering inflammation and oral connections in cardiovascular disease

Recent findings support a correlation between SI and oral diseases.[114–122] Numerous studies have demonstrated that chronic, low-grade inflammation accompanies periodontal disease and is associated with oral microbiome imbalances. These imbalances can contribute to a systemic inflammatory burden, directly increasing cardiovascular risk.[114–122] Smoldering inflammation may be a potential mechanistic link between oral health and CVDs, including HF.[119,120] Periodontal diseases, especially periodontitis, contribute to endothelial dysfunction, oxidative stress and vascular remodeling by mediating inflammation, thereby promoting CVD progression.[116] Additionally, oral microbiome dysbiosis, characterized by the entry of pathogenic bacteria and their metabolic products into the circulation, can trigger systemic immune pathway responses and induce SI of distant tissues, which amplifies the negative health outcomes associated with SI.[122] The translocation of pathological bacteria may occur during processes such as toothbrushing, flossing and chewing, resulting in minor mechanical injuries in the oral cavity, which are the gateway for bacterial dissemination throughout the vascular system.[115] Specific oral bacterial pathogens, such as *Porphyromonas gingivalis*, have

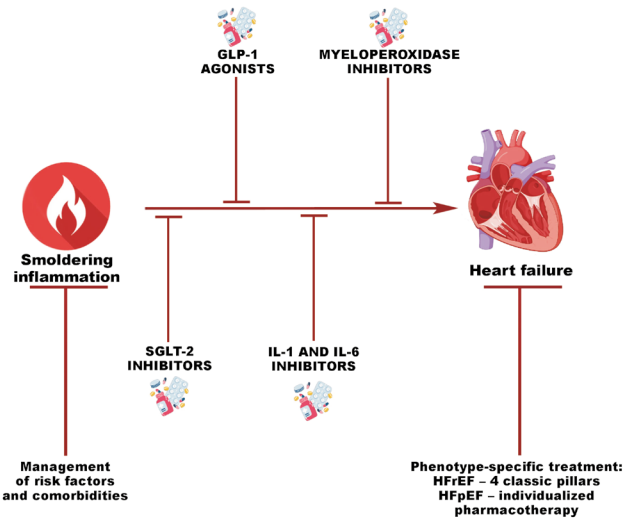

Fig. 3. Therapeutic modalities in heart failure (HF) with the potential to attenuate inflammatory responses

GLP-1 – glucagon-like peptide 1; HFrEF – heart failure with reduced ejection fraction; SGLT-2 – sodium–glucose cotransporter-2.

Table 2. Anti-inflammatory and related therapeutic strategies in heart failure (HF)

Therapy/drug class	Mechanism of action	Clinical relevance in HF
SGLT-2 inhibitors (e.g., empagliflozin, dapagliflozin)	indirect anti-inflammatory effects: ↓oxidative stress, ↓cytokine activation, improved endothelial function	reduction in HF hospitalization and CV mortality in HFpEF (EMPEROR-Preserved, DELIVER); anti-inflammatory effect
GLP-1 receptor agonists (e.g., semaglutide)	weight reduction, metabolic improvement, ↓systemic inflammation (IL-6, CRP)	improvement in exercise capacity and symptoms in patients with obesity and HFpEF; ongoing evaluation for CV outcomes
IL-1β inhibitors (anakinra, canakinumab)	blockade of IL-1β signaling → ↓downstream IL-6 and CRP	anakinra: improved CRP, NT-proBNP and symptoms in HFpEF pilot trials; canakinumab: reduced CV events (CANTOS), no HF-specific data
IL-6 pathway inhibitors (e.g., tocilizumab, ziltivekimab)	blockade of IL-6 signaling	phase 2 data (RESCUE trial) showed CRP reduction; ongoing outcome trials
MPO inhibitors (e.g., AZD5904)	reduction of oxidative stress and downstream inflammation	preclinical and early clinical development; potential for HFpEF therapy
Corticosteroids	broad suppression of inflammation	limited use; potential benefits in selected HFpEF patients with high SI; long-term safety concerns
Iron supplementation (IV ferric carboxymaltose)	reversion of iron deficiency, reduction in oxidative stress and inflammation	improvement in exercise capacity and a reduction in HF hospitalizations; benefits extend to HFpEF
Other emerging targets (e.g., NLRP3 inhibitors, inflammasome modulators)	blockade of IL-1β/IL-18 via the inflammasome pathway	promising preclinical data

GLP-1 – glucagon-like peptide 1; SGLT-2 – sodium–glucose cotransporter-2; MPO – myeloperoxidase; CV – cardiovascular; NLRP3 – nucleotide oligomerization domain-like receptor family pyrin domain containing 3; NT-proBNP – N-terminal pro-B-type natriuretic peptide.

been directly implicated in the initiation and progression of atherosclerotic lesions, further substantiating a definitive microbial contribution to cardiovascular risk.[115] What is more, overlapping risk factors, such as smoking, obesity and unhealthy diets, sustain persistent inflammatory pathways by establishing optimal conditions for the development of pathological microbiota that promote shared risk between the oral cavity and cardiovascular systems.[114] Clinical studies demonstrate that the treatment of oral health conditions (e.g., periodontal disease) may lead to a decreased systemic inflammatory burden and a reduced incidence or progression of CVD.[117,120,121] Therefore, the maintenance of proper oral health should be prioritized, especially among patients at risk of developing CVD.

Limitations

This review is subject to several limitations. The narrative style of the review, rather than a systematic or meta-analysis approach, raises the possibility of selection bias for the evaluated studies. The second limitation is the heterogeneity of HFpEF phenotypes and variations in study design, study population and biomarker assessment methodology that limit interpretations and generalizability of the findings. The third limitation pertains to the fact that, despite our efforts to provide a comprehensive overview of novel anti-inflammatory therapies, many of the interventions encompassed by this review are based on early-phase trials or relatively small cohorts. Therefore, the long-term efficacy and safety profiles of several interventions remain unknown. The final limitation regards the evolving nature of the field, which may have resulted in the omission of studies that were published at the time of the literature search. In summary, despite these limitations, this review provides context for SI in HFpEF and has identified important considerations for subsequent research.

Clinical implications and future research

Smoldering inflammation plays a crucial role in the pathogenesis of HFpEF, and it should not be overlooked during the diagnosis. The findings summarized in this article can convince clinicians to adopt a more holistic approach to patient care, rather than a narrow focus on one particular disease. Treating the underlying causes of inflammation can positively influence the course of HFpEF. The assessment of the levels of inflammatory markers is a crucial step in the diagnostic process for patients with various medical conditions. Subsequent research on inflammation in HFpEF may result in an update of the guidelines concerning its treatment.

Conclusions

Despite the presence of many known risk factors, recent research has examined the correlation between low-grade chronic inflammation, known as SI, and HF, from its onset to the end stage. A correlation between proinflammatory cytokines, such as IL-1β, IL-6, TNF-α, the NF-κB inflammatory pathway, and serum level of hs-CRP, and its impact on the remodeling of heart tissue has been proven. These factors, along with ROS and other proinflammatory molecules and pathways, lead to hypertrophy and fibrosis,

therefore impairing heart muscle function and resulting in HF. Other comorbidities related to inflammation, such as AF, T2DM and obesity, have also been associated with the onset of HF, mainly HFpEF. Therefore, treating these conditions with anti-inflammatory medications may result in the improvement during the course of HF. Moreover, trials on the efficacy of other inflammatory pathway inhibitors have provided promising outcomes in small clinical groups, suggesting the potential of these agents in HF treatment. The described pathophysiological mechanisms of SI demonstrate the complexity of the subject but also emphasize the importance of a thorough understanding of the topic in order to implement proper treatment. Smoldering inflammation begins as subclinical myocarditis and can remain unnoticed for a long time, finally progressing to post-inflammatory dilated cardiomyopathy. Therefore, anti-inflammatory interventions, when administered in conjunction with standard HF treatment, may contribute to the modulation of this progression. Further studies are necessary to optimize timing, dosage and patient selection for maximal benefit.

Ethics approval and consent to participate

Not applicable.

Data availability

Not applicable.

Consent for publication

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ORCID iDs

Michał Maksymilian Wilk https://orcid.org/0000-0002-3383-4357
Jakub Wilk https://orcid.org/0009-0000-8421-1670
Kamila Florek https://orcid.org/0009-0002-0256-2421
Wojciech Jan Zimoch https://orcid.org/0000-0003-4693-491X

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Review

Genomics in dental implantology: The role of genetic and epigenetic factors in dental implant failure – a narrative review

Shilpa Shrinivas Prabhu[B–E], Aparna Narayana[A,F], Gayathri Krishnamoorthy[D–F]

Department of Prosthodontics and Crown & Bridge, Manipal College of Dental Sciences, Manipal Academy of Higher Education (MAHE), 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

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Address for correspondence
Aparna Narayana
E-mail: aparna.narayan@manipal.edu

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Abstract

Dental implants are a widely used solution for tooth replacement, yet implant failures remain a challenge. Genetic predispositions and epigenetic modifications influence osseointegration and peri-implant health. The present review explores genetic mechanisms affecting implant healing and introduces implantogenomics – a personalized approach to implant therapy based on an individual's genetic profile.

A comprehensive review of literature from PubMed®, Scopus, EMBASE, and Web of Science (2008–2024) was conducted using Medical Subject Headings (MeSH) terms such as "genetic markers," "implantogenomics" and "epigenetics." After removing duplicates and screening for relevance, a total of 46 studies were included in the analysis.

Key genetic variants in bone metabolism (collagen type 1 alpha 1 (*COL1A1*), runt-related transcription factor 2 (*RUNX2*), vitamin D receptor (*VDR*)), immune response (interleukin-1 (*IL-1*), tumor necrosis factor alpha (*TNF-a*), *IL-6*), and osseointegration-related genes (osteoprotegerin (*OPG*), receptor activator of nuclear factor kappa B (*RANK*), receptor activator of nuclear factor kappa-B ligand (*RANKL*)) were identified as potential contributors to implant failure. Epigenetic modifications, including DNA methylation, histone changes and microRNAs (*miRNAs*), regulate bone remodeling and immune responses, and have an influence on implant integration.

Advances in genomics have paved the way for personalized implant therapy through genetic screening, optimizing outcomes and reducing the number of implant failures. Implantogenomics is aimed at tailoring treatments based on genetic profiles, while epigenetic therapies, such as gene modulation, enhance implant integration. Future research should focus on predictive biomarkers and precision-based strategies to improve implant longevity.

Genetic and epigenetic factors play a crucial role in the success of dental implants. Integrating genomic insights into clinical practice can enhance patient selection, predict implant success and improve treatment outcomes. Further research is necessary to establish predictive biomarkers and targeted interventions.

Keywords: genetic factors, epigenetics, implant failure, dental implant, genetic markers

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Highlights

- Although dental implants are the preferred option for tooth replacement, failures may still occur due to impaired osseointegration.
- Host-related factors, especially genetic predispositions, significantly influence implant outcomes, with some individuals at higher risk of failure.
- Advances in omics sciences and high-throughput methods enable the identification of molecular mechanisms influencing osseointegration, enabling early risk assessment.
- The emerging field of implantogenomics applies genetic insights to personalize treatment strategies and enhance long-term implant success.

Introduction

Over the past 30 years, dental implants have evolved and become the preferred treatment option for tooth replacement by dentists and patients. The survival rate of implant-supported restorations has increased from 94.6% to 97.1% over the past 2 decades.[1] Despite this high success rate, there has been a predominant increase in the incidence of dental implant failure. Previous studies have noted a dental implant failure rate ranging from 1% to 19%.[2] Early implant failures have been attributed to altered wound healing, preventing osseointegration, while late implant failures have been associated with extensive peri-implant bone loss after functional loading.[3] Understanding the cause of implant failures is essential for their prevention.

Knowledge regarding the factors influencing implant failure and meticulous observation of the implant after placement are crucial elements in the management of the complications such as inflammation, proliferation and progressive bone loss in and around the implant, compromised aesthetics, prosthesis failure, soft tissue dehiscence, implant fracture, and ultimately, implant failure.[4] Among all variables contributing to dental implant failure, the host factor has emerged as a contentious risk component.[2] Many researchers have sought to uncover the association between alleles and/or genotypes of genetic markers and the predisposition to implant failure. Analyzing these genetic elements associated with dental implant loss may offer insights into the factors contributing to the varied patient response to currently available treatment options.[5]

Clinicians are able to assess the risk of complications in patients with a negative host response before any elective surgical procedure, as such a response may lead to implant rejection, wherein the host body fails to integrate with the implant. Thus, it is evident that the incidence of implant failure is higher in a subset of individuals who demonstrate a definitive host characteristic, such as genetic factors that disturb the process of osseointegration.[6] This phenomenon of a small number of patients concentrating risk for implant loss has been termed "clusterization".[7] High-throughput methodologies are increasingly employed to gain a comprehensive understanding of cellular processes, enabling faster discoveries in health and disease research.[8]

The present review aims to summarize the genetic mechanisms underlying osseointegration healing and introduce implantogenomics, a concept that applies personalized medicine to tailor implant therapy for individual patients based on their unique genetic profile. The study will focus on the following key aspects: genetic factors influencing the prognosis of implant treatment; diagnostic tools for screening high-risk populations; omics profiling in osseointegration; and personalized dental implant therapy. The review will also emphasize the importance of integrating omics sciences with advanced bioengineering technologies to enhance bone formation and regulate osteogenesis by elucidating the genetic and epigenetic signaling cascades involved in dental implantology.

Material and methods

The included studies predominantly focused on genetic and epigenetic contributors to implant failure, with an emphasis on osseointegration. These comprised original research, clinical studies and relevant reviews that examined molecular mechanisms, gene polymorphisms, epigenetic modifications, and the application of omics technologies within the domain of dental implantology. Prominent electronic databases like PubMed®, Scopus, EMBASE, and Web of Science were used to retrieve articles published in the English language during the 16-year period from 2008 to 2024. The time restriction was implemented to preclude the introduction of inaccurate, questionable or outdated concepts while concurrently facilitating the comprehension of the contemporary perception of genetics and its relevance in implant failure. Studies were excluded if they focused solely on mechanical or prosthetic factors, without addressing genetic aspects, or if they lacked peer-reviewed content, including conference abstracts, editorials and opinion articles. A combination of Medical Subject Headings (MeSH)

terms such as "genetic markers", "implantogenomics", "genetic polymorphism", "genetic factors", "epigenetics", "peri-implantitis", "implant failure", "dental implant", "precision medicine", "epigenetic mechanisms", "DNA methylation", "histone modification", and "omics" were used with the Boolean operators to curate the data. Duplicates and methodologically weak studies were excluded during the screening process.

The total number of articles retrieved from 4 online databases was 158. During the screening phase, 40 articles were identified as duplicate entries and were hence removed from the study. In the eligibility phase, 118 records were reviewed, of which 72 were excluded due to deviation from the intended study objective. Finally, 46 articles were included in the review (Fig. 1).

Results and discussion

Peri-implantitis and periodontitis have shown similar clinicopathological features, involving soft tissue damage, infection and bone loss.[9] An inflammatory process is a notable problem in dental implant patients, as it spreads rapidly and more profoundly around an implant as compared to natural teeth. Therefore, more emphasis should be placed on genes associated with immune/inflammatory responses to foreign bodies.[6]

Wear of the implant surface results in debris-mediated implant loosening, which is one of the main causes of implant failure. This process is referred to as osteolysis. The particles shed from titanium implants trigger a more robust immune response from macrophages when compared to particles derived from supplementary substances used in implant restoration. The inflammatory and osteolytic process of peri-implantitis is driven by pro-inflammatory cytokines, involving interleukin-1 (*IL-1*) and tumor necrosis factor alpha (*TNF-α*), released by macrophages. The evidence indicates that titanium particles cause inflammation and osseo-disintegration only in certain individuals receiving implants, which underscores a critical role of the host factor in implant failure.[10]

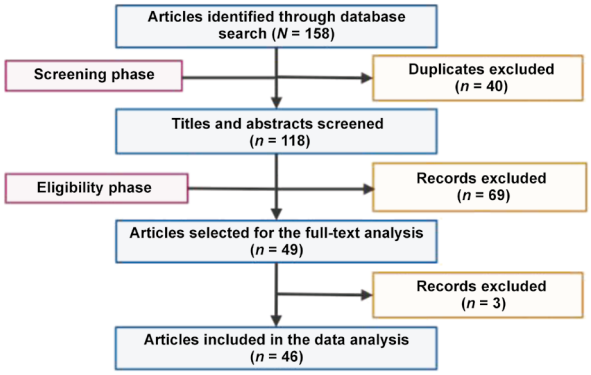

Fig. 1. Flowchart of the literature search process

Genetic factors influencing the prognosis of implant treatment

Genetic mediators that play a vital role in the immune/inflammatory reaction of the body can be categorized as follows (Fig. 2,3):

– ILs;
– bone morphogenetic proteins (BMPs), TNF, transforming growth factor (TGF) (*TNF-α* and *TGF-β*);
– matrix metalloproteinases (MMPs);
– bone metabolism biomarkers.[9,11]

Interleukins

The diagnostic markers of implant failure include *IL-1A –889 C/T* (rs1800587), *IL-1B +3954 C/T* (rs1143634), *IL-1RN +2018 T/C* (rs419598), and *TNF-α –308 G/A* (rs1800629) genotyping, in vitro *IL-1β*/*TNF-α* release assays, and lymphocyte transformation tests.[9] Other prognostic markers of peri-implantitis are cathepsin K, receptor activator of nuclear factor kappa-B ligand (*RANKL*) or osteoprotegerin (*OPG*), but further investigations and large clinical trials are necessary to confirm these findings.[12] The amalgamation of *IL-1 allele 2*

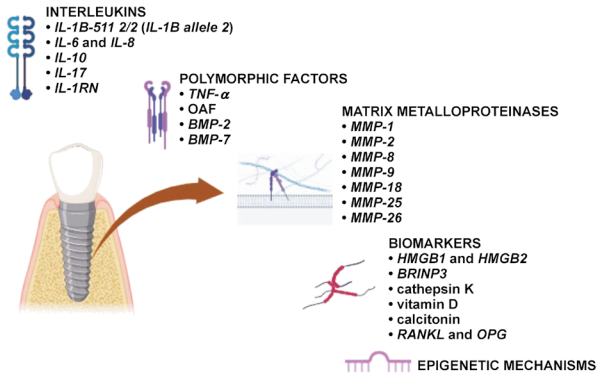

Fig. 2. Genetic mediators involved in the immune/inflammatory reaction

BMP – bone morphogenetic protein; HMGB – high-mobility group box; IL – interleukin; *IL-1RN* – gene encoding interleukin-1 receptor antagonist; MMP – matrix metalloproteinase; OAF – osteoclast-activating factor; *OPG* – osteoprotegerin; *RANKL* – receptor activator of nuclear factor kappa-B ligand; *TNF-α* – tumor necrosis factor alpha.

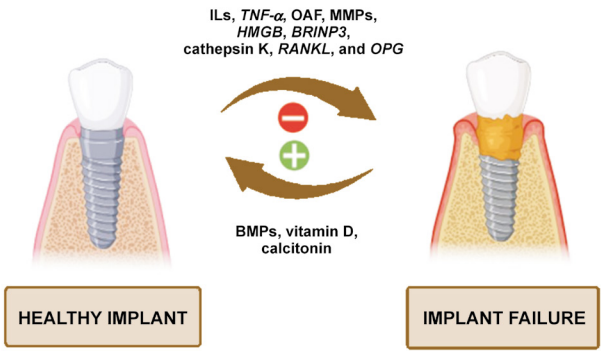

Fig. 3. Effect of genetic mediators on implant surfaces

(*IL-1A −889* and *IL-1B +3954*) in patients with inflamed periodontal or peri-implant tissues acts as a detrimental factor that exacerbates tissue destruction.[13] Table 1 summarizes the impact of different ILs on implant prognosis.

Another non-invasive means of inspecting the host's reaction in periodontal and peri-implant diseases is the analysis of gingival crevicular fluid (GCF) or peri-implant sulcular fluid (PISF).[16]

TNF and TGF

Single-nucleotide polymorphisms (SNPs) are minor DNA mutations that influence the process of osseointegration in implants. Inflammatory proteins play a crucial role in both the breakdown of the extracellular matrix (ECM) and the resorption of the alveolar bone.[5] Tumor necrosis factor alpha is a proinflammatory cytokine. It serves as the primary mediator in the immune response to Gram-negative bacteria, with *TNF-α* levels indicating the bacterial load and the severity of inflammation.[16] Bone morphogenetic proteins belong to the *TGF-β* superfamily and have been demonstrated to promote bone ingrowth, facilitate gap healing and enhance implant fixation in various animal studies.[18] BMP-2 and BMP-7 are termed human osteogenic proteins. Table 2 presents an overview of various polymorphic factors and their influence on implant treatment.

Matrix metalloproteinases and extracellular matrix remodeling mediators

The extracellular reaction may vary depending on the implant surface, with ECM exhibiting different morphological characteristics across various material surfaces.

Matrix metalloproteinases are a family of highly conserved endopeptidases. These ECM macromolecules contribute to cellular development and morphogenesis. They regulate growth factors, activate cell surface receptors and influence adhesion molecules. Matrix metalloproteinases are involved in various physiological processes, including inflammatory cell activity, wound healing, angiogenesis, and bone formation.[20,21]

The family of MMPs consists of 5 groups:

– collagenases: *MMP-1*, *MMP-8*, *MMP-13*, *MMP-18*;
– gelatinases: *MMP-2*, *MMP-9*;
– stromelysins: *MMP-3*, *MMP-10*, *MMP-11*;
– matrilysins: *MMP-7*, *MMP-26*;
– membrane-type (MT) MMPs:
  • 4 transmembrane MMPs: *MMP-14*; *MMP-15*; *MMP-16*; *MMP-24*,
  • 2 glycosylphosphatidylinositol-anchored MMPs: *MMP-17*; *MMP-25*.

The effects of MMPs on ECM remodeling are outlined in Table 3.

Bone metabolism biomarkers

Bone is a metabolically active tissue that undergoes continuous remodeling. This process is driven by the dynamic interaction of osteoblasts and osteoclasts, regulated by a complex network of molecular biomarkers. Table 4 summarizes the impact of these biomarkers on dental implant treatment.

Epigenetic mechanisms

Epigenetics refers to the study of heritable changes in the phenotype that occur without alterations to the

Table 1. Effects of different interleukins (ILs) on dental implant prognosis

Genotype		Effect
IL-1B-511 2/2 (*IL-1B allele 2*)		early marginal bone loss[12]
IL-6 and *IL-8*		higher expression in periodontitis sites and peri-implant inflammation[13]
IL-10		no association with implant failure[14]
IL-17		increased levels in gingivitis and periodontal disease; further investigation needed in peri-implantitis[15]
IL-1RN	gene coding for IL-1ra	multiple implant loss[16]
	allele 2	implant loss in a Caucasian population[17]
	allele 5	implant failure in a Portuguese Caucasian population[17]

IL-1ra – interleukin-1 receptor antagonist; *IL-1RN* – gene encoding *IL-1ra*.

Table 2. Polymorphic factors and their effects on dental implant treatment

Polymorphism	Effect
TNF-α	increases osteoclast activation upon implant placement[16]
OAF	high concentrations lead to increased bone loss and implant failure[16]
BMP-2	stimulates bone ingrowth, gap healing and implant fixation[18]
BMP-7	collagen solution causes a notable increase in BIC and reverse torque resistance following immediate application to implant sockets before insertion of implants[19]

BIC – bone implant contact; BMP – bone morphogenetic protein; *TNF-α* – tumor necrosis factor alpha; OAF – osteoclast-activating factor.

Table 3. Impact of matrix metalloproteinases (MMPs) on extracellular matrix (ECM) remodeling

Matrix metalloproteinase	Effect
MMP-1	• initiates bone resorption[22]
MMP-2 and *MMP-8*	• elevated levels in GCF and PISF • associated with bone destruction, cavitation, inflammation, and granulation tissue formation • high concentration seen in ECM of diseased implants[20]
MMP-9	• plays a critical role in the development of inflammatory periapical lesions and ECM degradation during the initiation and progression of apical periodontitis[20]
MMP-18	• diagnostic biomarker in peri-implant diagnostics • detected in PISF by Western blot analysis and associated with early implant failure[23]
MMP-25	• cleaves gelatin, type IV collagen and fibronectin • participates in cellular migration and intrusion of ECM and BM associated with peri-implantitis[21]
MMP-26	• cleaves fibrinogen and ECM components such as fibronectin, vitronectin, gelatin, and type IV collagen, contributing to peri-implant inflammation[21]

BM – basement membrane; GCF – gingival crevicular fluid; PISF – peri-implant sulcular fluid.

underlying DNA sequence. These changes involve modifications to the chromatin structure, which in turn regulate gene expression independently of base sequence variations.[29] Environmental factors such as toxins, microbes, stress, diet, and hormones can alter epigenetic patterns, thereby influencing gene activity and cell behavior. These modifications regulate gene expression by either promoting or silencing transcription, blocking mRNA formation or causing protein post-translational modification.[30] Key epigenetic mechanisms include DNA methylation, histone modifications and regulation by non-coding RNAs like microRNAs (*miRNAs*).[31] DNA methylation silences gene expression and regulates key bone-related genes. Histone modifications, like acetylation and methylation, also control gene activity. Histone deacetylases (HDACs) have been shown to influence bone health by regulating osteoblasts, osteoclasts and bone mass. MicroRNAs are short non-coding RNAs (18–22 nucleotides) that regulate gene expression by degrading or repressing target mRNAs. Increased levels of *miRNAs* suppress gene expression, while decreased levels enhance it. Several *miRNAs*, such as *miR-23a*, *miR-34c* and *miR-133a*, directly influence bone formation by targeting runt-related transcription factor 2 (*RUNX2*), a key transcription factor in the differentiation of osteoblasts.[32] Table 5 provides an overview of key genetic factors and their respective roles at the molecular and biochemical levels in implant integration and failure.

Screening of patients at high risk of implant failure

Some of the diagnostic interventions for the identification of individuals at high risk of implant failure include (Fig. 4):

1. Genome-wide association study: It examines the link between SNPs and traits, with a particular focus on major diseases, by comparing the DNA of individuals with different phenotypes related to a specific trait or disease. Participants are divided into 2 groups: those with the disease (cases); and similar sample without the disease (controls). This method, known as the phenotype-first approach, identifies SNPs in which one allele appears more frequently in the disease group. When a SNP demonstrates a significant association with a specific condition, it is considered to indicate a genomic region that could impact disease risk.[36]
2. Transcriptional profiling of osseointegration: It highlights the complexity of bone healing, a process that involves interwoven biological stages such as

Table 4. Impact of bone metabolism biomarkers on dental implant treatment

Biomarker		Effect
High-mobility group chromosomal protein	*HMGB1*	• promotes the release of cytokines in periodontitis and peri-implantitis
	HMGB2	• high levels in PI-PISF defend peri-implant tissues against inflammation[21]
BRINP3		• risk factor for the development of peri-implantitis in the absence of chronic periodontitis[24]
Cathepsin K		• determinant of peri-implant tissue health • increased levels in the crevicular fluid are associated with concomitant increase in peri-implant bone loss[25]
Vitamin D		• vitamin D deficiency critically impairs bone integration around implants[26]
Calcitonin		• improves bone maturation around titanium implants[27]
RANKL and *OPG*		• detected at high concentrations at peri-implantitis sites • associated with a risk of alveolar bone loss along the entire implant surface[28]

BRINP – bone morphogenetic proteins/retinoic acid inducible neural-specific protein; HMGB – high-mobility group box; PI-PISF – pro-inflammatory peri-implant sulcular fluid; *RANKL* – receptor activator of nuclear factor kappa-B ligand; *OPG* – osteoprotegerin.

Table 5. Key genetic factors and their influence on dental implants at the molecular and biochemical level

Genetic factor	Molecular/biochemical effect	Impact on dental implant
IL-1 (*IL-1A/B*) polymorphisms	Increased *IL-1* production promotes a stronger inflammatory response and activates osteoclasts.	higher risk of peri-implantitis, bone resorption and implant failure[12]
IL-6 variations	Elevated *IL-6* levels amplify inflammation and stimulate osteoclastogenesis.	increased risk of soft tissue inflammation and peri-implant bone loss[13]
TNF-α polymorphisms (e.g., *G-308A*)	Overproduction of *TNF-α* enhances osteoclast differentiation and bone resorption.	greater susceptibility to peri-implantitis and implant failure[16]
RANK/RANKL/OPG pathway genes	Imbalance in *RANKL/OPG* expression leads to excessive osteoclast activity.	accelerated bone loss around implants, compromised osseointegration[28]
VEGF polymorphisms	Altered *VEGF* expression affects angiogenesis and healing.	impaired vascularization leading to reduced bone healing and implant integration[33]
MMP gene variations	Increased *MMP* activity degrades ECM and connective tissue.	tissue breakdown around implants, higher risk of implant instability[20,22]
COL1A1 gene variations	Abnormal collagen production affects the quality of bone matrix.	compromised bone strength and impaired osseointegration[34]
RUNX2 regulation by *miRNAs*	Disrupted *RUNX2* function impairs osteoblast differentiation and bone formation.	delayed or defective bone healing around implants[35]

VEGF – vascular endothelial growth factor; *COL1A1* – collagen type 1 alpha 1; *RUNX2* – runt-related transcription factor 2.

inflammation, osteogenesis and angiogenesis. A comparison of gene expression profiles associated with wound healing and those observed at the post-implant site elucidates the natural delays between gene expression, protein translation and tissue maturation. The histological data indicates that the selected time points for transcriptional analysis effectively capture key early transcriptional events that are crucial in the process of osseointegration.[37]

3. Real-time polymerase chain reaction (PCR): Diagnostic qualitative PCR is used for a rapid detection of disease-specific nucleic acids, while quantitative PCR measures both the presence and the quantity of a particular DNA sequence in each sample. Both quantitative PCR and DNA microarrays are cutting-edge techniques for the analysis of gene expression, offering researchers a more profound understanding of molecular processes. This understanding supports the development of advanced therapeutic prosthetics for dental implant treatments and applications in tissue engineering biology.[38]
4. RNA sequencing: Next-generation sequencing (NGS) is used to identify and quantify RNA in a biological sample at a specific point in time, enabling the analysis of the dynamic cellular transcriptome.[39]
5. Genome-wide screening of implant failure by vitamin D deficiency: Genome-wide microarray analyses of implant osseointegration suggest that the unique microenvironment created by implant placement significantly influences multiple gene expression networks, potentially involving peripheral circadian rhythm pathways. Notable interactions between the *NPAS2* gene and cartilage matrix genes have led to a proposed model in which bone marrow mesenchymal cells, through a circadian rhythm-related mechanism, initiate ectopic synthesis of cartilage matrix molecules such as type X collagen without forming actual cartilage tissue at the implant site. Additionally, vitamin D deficiency disrupts these processes, impairing effective bone formation and implant integration.[40]
6. Epigenetic methylation assays: These techniques are used to examine changes in DNA methylation, an important regulatory mechanism that alters gene expression without modifying the underlying DNA sequence. Epigenetic methylation assays facilitate the assessment of the impact of environmental and external factors on gene activity, which contributes to diverse biological processes and disease development.[41]

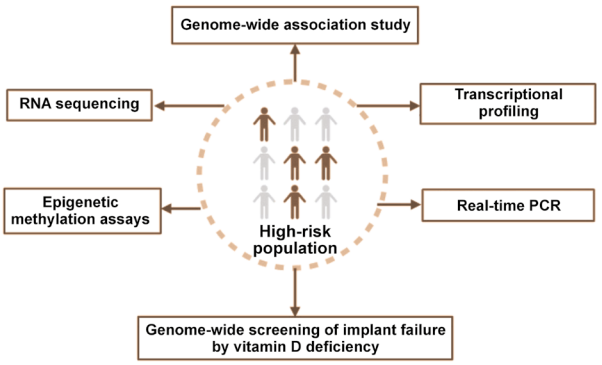

Fig. 4. Diagnostic tools for screening high-risk populations
PCR – polymerase chain reaction.

The presented molecular techniques are not currently part of routine clinical practice in implant dentistry. However, they are emerging tools with the potential for future applications as the field of precision diagnostics continues to evolve.

Clinically available tests for genetic screening related to dental implants or periodontal diseases include:

1. MyPerioID® IL-6: saliva-based genetic screening tool that identifies variations of the *IL-6* gene, a critical marker of inflammation. By detecting these genetic predispositions, the test enables the assessment of an individual's risk for developing severe periodontal disease[42];

2. TNF-α test: *TNF-α* (G-308A) gene polymorphism has been investigated for its potential association with implant failure and peri-implantitis, as it may influence inflammatory responses. However, research findings have been inconsistent, with some studies showing a possible link and others finding no significant association. Genetic testing for TNF-α variations has yet to be incorporated into standard dental implant planning, as clinical factors like patient health, oral hygiene, bone quality, and surgical technique remain the primary predictors of implant success[43];
3. IL-6 test: measures salivary IL-6 levels, serving as a biomarker for periodontitis, a major risk factor for dental implant failure. Elevated IL-6 levels indicate increased inflammation and help identify individuals with a higher risk for periodontitis or implant complications. The protocol entails the collection of a saliva sample, the analysis of IL-6 concentrations, and the assessment of the inflammatory status based on the obtained results. Although it is not a genetic test, the IL-6 test provides valuable insight into a patient's risk for implant failure due to inflammation and bone loss[44];
4. GenoType Periodontal Susceptibility Test (PST): a screening tool for *IL-1A* and *IL-1B* gene variations that have been linked to an increased risk of severe periodontitis. A positive result (PST+) indicates higher susceptibility to periodontal disease. While useful for assessing periodontal risk, it is not routinely used in dental implant planning, and evidence supporting its role in predicting peri-implantitis remains limited.[45]
5. PerioPredict: genetic risk assessment tool that is used to evaluate moderate to severe periodontal disease by analyzing *IL-1* gene variations, a key factor in inflammation. While it may offer insights for implant planning, it is not specific to dental implants and has shown mixed results in clinical studies. The clinical examination remains the primary method for assessing the periodontal risk. PerioPredict should be used as a complementary tool in conjunction with other clinical evaluations during treatment planning.[46]

Building upon the use of clinically available genetic screening tests, omics profiling is now being explored to gain deepe insights into the mechanisms underlying osseointegration.

Omics profiling in osseointegration

Omics is a branch of biology that encompasses fields like genomics, proteomics, transcriptomics, and metabolomics. The primary goal of omics sciences is to identify, characterize and quantify the diverse biological molecules that contribute to the structure, function and dynamics of cells, tissues or organisms.[47]

Omics technologies have been utilized in distinct preclinical studies to understand the early and late molecular events occurring during osseous formation.[48] Identifying the genes and proteins that affect osseointegration is essential in order to reduce the healing time associated with implant surgery and improve clinical outcomes in patients with local or systemic conditions that impair bone metabolism.[49]

Studies have shown that an early stage of osseous wound healing was associated with enhanced chemokine, NF-κB, *TNF-α* signaling pathway, and angiogenesis-related pathways. In the latter stages, an increased expression of mitogen-activated protein kinase (MAPK), Wnt pathways and proteins associated with ECM remodeling and bone mineralization was observed.[50–53]

A few in vitro studies were conducted to evaluate the impact of different implant surfaces on osteogenic markers, thereby influencing the rate of osseointegration. Moderately rough surfaces exhibited elevated levels of osteogenic markers when compared to polished surfaces.[54] The hydrophilicity of the implant surface further amplified osteogenesis by positively modulating osteogenesis- and angiogenesis-related pathways (e.g., vascular endothelial growth factor (*VEGF*), MAPK and BMP pathways).[55]

Personalized dental implant therapy

The integration of regenerative approaches to customize the implant therapy as per the patient's individual needs has given rise to a new concept termed implantogenomics or implantomics.[47]

Considerable efforts have been made to create bioactive surface coatings that emulate the biochemical composition and structural characteristics of human bone at the nanoscale. Taking insights from recent omics research, new experimental coatings are currently under development. These coatings are engineered to incorporate targeted drugs, agents, proteins, and growth factors that enhance implant stability by supporting the natural process of osseointegration.[56] Preclinical studies have shown that coating dental implants with ECM proteins can enhance peri-implant bone formation. De Barros et al. reported increased bone volume and mineralization with collagen type II/chondroitin sulfate coatings in a canine model.[57] Meng et al. reviewed 34 studies on biomolecular coatings for titanium dental implants, mostly in animal models, and found that growth factors, peptides and ECM proteins may support early stages of bone integration.[58] However, the authors noted inconsistent results and highlighted the need for clinical studies in humans.[58] Hasani-Sadrabadi et al. developed a layer-by-layer surface treatment for titanium implants incorporating *BMP-2*-mimicking peptides and gentamicin to enhance osseointegration and antibacterial activity.[59] Using a polydopamine coating to support nanolayer formation, the modified surfaces enabled sustained release of bioactive agents. In vitro and in vivo studies have demonstrated improved cytocompatibility, osteogenic differentiation and

peri-implant bone integration, suggesting promising applications in the fields of dental and orthopedic implantology.[59] Zhou et al. developed a 3,4-dihydroxyphenylalanine (DOPA)-based peptide coating (DOPA-P1@P2) for titanium implants to address aseptic loosening by promoting staged bone regeneration.[60] The coating sequentially modulated inflammation, angiogenesis and osteogenesis through specific bioactive peptides. In vivo, it significantly improved push-out strength, bone volume and bone-to-implant contact compared to TiO_2 controls, suggesting its strong potential for enhancing implant osseointegration.[60]

Due to ethical restrictions and an increased prevalence of implant failure, there has been a paucity of in vivo studies investigating the constituents of the host genetic susceptibility that influences biological complications in implant placement.[49] However, omics technology, which offers a comprehensive understanding of biomaterials, marks a major advance in biomedical science, which will significantly advance the growth of tailored and personalized medicine in implant dentistry.[61] Genetic screening offers potential for personalized dental implant therapy, as it enables the customization of implant design and treatment plans based on a patient's genetic profile. However, challenges such as the complexity of genetic data interpretation, the high cost of testing, and the need for further research to confirm the clinical relevance of genetic markers limit its routine use.

Conclusions

Implant failure is a serious concern in the prognosis of dental implants. Even though the mechanisms underlying implant loss are well-defined, they vary depending on a case. Determining the underlying direct or indirect cause of implant failure is of the utmost importance.

A single-nucleotide polymorphism of pro-inflammatory mediator genes might influence their expression intensity or amino acid sequence, thereby affecting the host inflammatory response. Some SNPs have been correlated with implant loss and determined as probable genetic risk factors for implant failure. Studies on the subject have contributed to the redefinition of prospective targets for successful screening, prevention and maintenance of dental implants. The application of insights from omics sciences has the potential to drive further advancements in personalized dental implant therapy, promoting long-term clinical success.

Ethics approval and consent to participate

Not applicable.

Data availability

Not applicable.

Consent for publication

Not applicable.

Use of AI and AI-assisted technologies

Not applicable.

ORCID iDs

Shilpa Shrinivas Prabhu https://orcid.org/0000-0002-8412-9500
Aparna Narayana https://orcid.org/0000-0002-3190-2379
Gayathri Krishnamoorthy https://orcid.org/0000-0001-6816-0754

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Review

Gallstones, obesity, insulin resistance, and obstructive sleep apnea – current knowledge on the topic: A literature review

Weronika Frosztęga[1,A,B,D,F], Maciej Sroczyński[2,A,B,D,F], Monika Michałek[1,A,C,F], Mieszko Więckiewicz[3,E,F], Katarzyna Madziarska[1,E,F], Helena Martynowicz[1,A,B,E,F]

[1] Clinical Department of Diabetology, Hypertension and Internal Diseases, Wroclaw Medical University, Poland
[2] University Center of General and Oncological Surgery, Clinical Department of General Surgery, Faculty of Medicine, Wroclaw Medical University, Poland
[3] Department of Experimental Dentistry, 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

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Address for correspondence
Weronika Frosztęga
E-mail: weronika.frosztega@gmail.com

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Abstract

Cholelithiasis is one of the most common gastrointestinal diseases, which often manifests asymptomatically. Statistically, up to 20% of the global population is affected by gallbladder diseases. The prevalence of these conditions rapidly increases with the patient's age. Obstructive sleep apnea (OSA) is a sleep-related breathing disorder that causes pharyngeal airway collapse, hypopnea and snoring. It is estimated that nearly half of the global population suffer from OSA. Cholelithiasis and OSA are separate medical conditions. However, they both affect a significant part of the general population, have tremendous impact on patients' overall health, and share common risk factors, pathophysiology and disease development.

Thus, the aim of this brief narrative review is to summarize and update the current knowledge on the link between gallstone disease and OSA regarding the prevalence of obesity and insulin resistance in patients with both OSA and cholelithiasis.

Keywords: obesity, insulin resistance, obstructive sleep apnea, gallstones, cholelithiasis

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Highlights

- Obesity is a risk factor for both obstructive sleep apnea (OSA) and gallstone disease.
- The association between OSA and gallstones remains poorly described in the current literature.
- Further research is needed to clarify the potential direct relationship between OSA and gallstone formation.

Introduction

Gallbladder diseases are among the most frequent gastrointestinal conditions, with a prevalence estimated at 20% in the general population. The prevalence increases with age, constituting a significant burden to healthcare system.[1,2] Gallbladder diseases include, among others, cholelithiasis, cholecystitis, choledocholithiasis, cholangitis, cysts, polyps, and malignancies.[3]

The diagnosis of gallstones requires the use of non-invasive imaging techniques, such as abdominal ultrasound, computed tomography (CT) of the abdomen and magnetic resonance cholangiopancreatography (MRCP). The invasive techniques, including endoscopic retrograde cholangiopancreatography (ERCP), are chosen as the preferred therapeutic method.[1,2]

The treatment of gallstones can be divided into preventive and minimally invasive procedures (e.g., endoscopic or laparoscopic) or urgent treatments that can be either minimally invasive or traditional, depending on patient's clinical status according to the established guidelines or algorithms.[4] Laparoscopic cholecystectomy, the invasive procedure, is estimated to be one of the most prevalent surgical interventions worldwide.[5] However, it is a standard to refrain from performing surgical procedures in asymptomatic patients, with a few rare exceptions.[6]

Obstructive sleep apnea (OSA) is a sleep-related breathing disorder caused by pharyngeal airway collapse. Airway collapse can result in obstructive apnea, hypopnea and snoring.[7] The incidence of OSA is constantly rising and is estimated to be between 3.0% and 49.7% in adults depending on sex, population characteristics and study methodology.[8] Benjafield et al. estimated that globally, almost 1 billion adults aged 30–69 years have mild to severe OSA, and 425 million adults aged 30–69 years have moderate to severe OSA.[9] The gold standard tool in OSA diagnosis is a whole-night polysomnography (PSG). The most common risk factors for the condition include increased weight, male sex and advanced age. Although cholelithiasis and OSA are separate medical disorders, they frequently coexist and affect a significant part of the general population. These conditions have a tremendous impact on patient's general health, and they often share common risk factors, pathophysiology and disease development.

Thus, the aim of this narrative review is to summarize and update the current understanding of the association between gallstones and OSA.

Material and methods

A comprehensive literature search was conducted using the PubMed®, Scopus and Web of Science databases. The search was limited to articles published between 1990 and 2024. The keywords and phrases used included "gallstones", "obstructive sleep apnea" and "insulin resistance". The articles in English concerning the relationship between OSA and gallstones were included. The case reports and non-peer-reviewed articles were excluded from the analysis. A total of 46 studies covering various aspects of gallbladder disease, OSA, insulin resistance, obesity, and their interrelations were reviewed. Table 1 presents a summary of the key findings from the literature.

Results

Development of gallstones

Cholelithiasis is a condition characterized by both clinical and biochemical abnormalities caused by disruptions in bile production and drainage. It can occur anywhere along the biliary tract, from the intrahepatic bile ducts to the opening of the bile ducts into the duodenum in the greater papilla.[10–14] Bile duct diseases have already been diagnosed 3,000 years ago. During archaeological research conducted in Egypt, stones in the gallbladder were found in the mummy of a priestess living in the 11th century BC. However, it was Soranus of Ephesus who first described the symptoms of mechanical jaundice in the 2nd century AD.[10]

Two main types of gallstones are distinguished: cholesterol stones (90%); and pigment (black, brown) stones (10%). According to the classification based on location, stones can be located in the gallbladder, common bile duct or intrahepatic bile ducts. Gallbladder stones are mostly cholesterol derivatives produced due to insufficient biliary cholesterol turnover. Turnover homeostasis can be disrupted as a consequence of hepatic hypersecretion of cholesterol, rapid phase transitions, intestinal factors, and gallbladder hypomobility. Pigment gallstones are

Table 1. Summary of key literature findings regarding the association between obstructive sleep apnea (OSA) and gallstones

Study	Journal	Focus area	Key findings	Limitations
Lammert et al. 2016[2]	Nature Reviews Disease Primers	risk factors and pathogenesis of gallstones	Obesity and insulin resistance contribute to gallstone formation via cholesterol supersaturation and bile stasis.	lack of prospective clinical validation
Cortés et al. 2020[19]	Obesity Reviews	metabolic links between gallstones, insulin resistance and obesity	Insulin resistance and obesity alter bile composition, promoting the formation of gallstones.	lack of direct intervention trials
Parra-Landazury et al. 2021[15]	Visceral Medicine	obesity and gallstone risk	Higher BMI correlates with an increased risk of gallstones; weight loss surgery can exacerbate short-term gallstone formation.	focus on obesity rather than insulin resistance or OSA
Liew et al. 2007[37]	Obesity Surgery	obesity and gallbladder disease	Obesity significantly increases the prevalence of gallstones; rapid weight loss raises the risk of gallstone formation.	lack of long-term follow-up data
Smelt 2010[41]	Clinica Chimica Acta	triglycerides and gallstone formation	High levels of triglycerides are linked to an increased risk of gallstone formation, reinforcing insulin resistance as a risk factor.	lack of RCTs
Mesarwi et al. 2013[24]	Endocrinology and Metabolism Clinics of North America	OSA and metabolic dysfunction	OSA triggers systemic inflammation, increasing insulin resistance and metabolic complications.	indirect connection to gallstone disease
Reichmuth et al. 2005[27]	American Journal of Respiratory and Critical Care Medicine	OSA and type 2 diabetes	OSA independently increases the risk of insulin resistance and diabetes.	lack of direct analysis of gallstone formation
Benjafield et al. 2019[9]	The Lancet Respiratory Medicine	global burden of OSA	OSA is underdiagnosed and strongly linked to obesity and metabolic syndrome.	lack of gallstone-specific data

BMI – body mass index; RCTs – randomized controlled trials.

produced as a result of bile stasis, bacterial infection, hepatic hypersecretion of bilirubin, and genetic factors.[11,15] There is a direct correlation between body fat and cholesterol synthesis. Excessive production of cholesterol results in its excretion into the bile, where it is concentrated. This process, in turn, leads to the formation of gallstones.[16]

Gallstones occur more frequently in women than in men. The main cause of cholelithiasis is metabolic syndrome. The acronym 4F, an abbreviation for "fat, female, fertile, and forty", is commonly used to enumerate the factors that contribute to the development of cholelithiasis.[16] Other risk factors include hyperinsulinemia, insulin resistance, type 2 diabetes, and obesity. Moreover, reduced physical activity and excess caloric intake play a pivotal role in the development of gallstones.[2]

Regarding the limited body of literature on this topic, the existing studies suggest a link between sleep disorders, obesity and gallstones. Seminal research conducted by Chen et al. assessed the cumulative incidence of gallstones in the OSA cohort compared with the non-OSA cohort.[17] Patients with OSA had an increased risk of gallstones (adjusted hazard ratio = 1.53, 95% confidence interval (95% *CI*) = 1.16–2.03) after adjustment for age, sex, hyperlipidemia, diabetes, hypertension, chronic obstructive pulmonary disease (COPD), stroke, and coronary artery disease (CAD).[17] However, a previous study was subject to limitations regarding a relatively small number of obesity cases in the OSA cohort.

Insulin resistance

Insulin resistance has been previously linked to metabolic disorders, including visceral obesity, dyslipidemia and endothelial dysfunction.[18] Gallbladder removal (cholecystectomy) has been associated with a frequent manifestation of insulin resistance. This observation has given rise to the theory that the gallbladder is responsible not only for storing and concentrating bile produced in liver but also for metabolic and hormonal regulation.[19] The relationship between insulin resistance and OSA has garnered increasing interest from the medical community. Excess fat accumulation in obese individuals results in disrupted fat tissue metabolism, a condition referred to as lipotoxicity.[20]

Lipotoxicity has been associated with impaired glucose and insulin metabolism, resulting in the disruption of metabolic pathways in both adipose tissue and peripheral organs. The condition has an influence on pancreatic, hepatic, muscular, and cardiac functions. Metabolic alterations related to lipotoxicity impact intracellular signaling and hormonal regulation. An extensive literature review showed that insulin resistance leading to diabetes mellitus and obesity is associated with dysregulation of metabolic pathways, including protein kinase C pathways that influence serine phosphorylation,[21] the c-Jun N-terminal kinase (JNK) pathway[21] and glucose transporter (GLUT)-4 receptor trafficking.[22]

GLUTs are transmembrane proteins that transport glucose across the cell membrane. The activation and translocation of these proteins is facilitated by insulin. Insulin resistance increases GLUT-4 receptors in adipocytes present in the heart, brain and skeletal muscles. Obesity, which is associated with visceral adiposity and insulin resistance, increases cardiometabolic risk. However, a large retrospective cohort study from Poland found that obesity is not an independent predictor of return of spontaneous circulation (ROSC) in patients experiencing out-of-hospital cardiac arrests (OHCAs).[23]

In a PSG-based study, Michalek-Zrabkowska et al. confirmed that OSA is a risk factor for insulin resistance.[8] Several studies remain in agreement with these results.[24,25] In general, insulin resistance is crucial in the early stages of type 2 diabetes mellitus development.[26] The association of type 2 diabetes with OSA, which has been widely discussed in recent literature, is an independent risk factor for sleep disordered breathing (SDB) and disease progression.[27] Moreover, insulin, acting as a growth factor, promotes the expression of genes involved in inflammation.[28] Excess body fat leads to chronic systemic inflammation and dysregulation of metabolic pathways, and can result in insulin resistance, pancreatic β-cell dysfunction and atherosclerosis.[29]

Obesity

Obesity is a major public health problem. According to the World Health Organization (WHO), approx. 39% of adults are overweight and 19% are obese (body mass index (BMI) ≥ 30 kg/m^2).[30] The condition is strongly associated with numerous disorders, including diabetes mellitus, gallstones and OSA.[31] Reduced physical activity and excess caloric intake are the main exogenous factors causing obesity. As a result, there is an elevated propensity for cholesterol synthesis in the liver, which results in an increased risk of gallstone development.[2] However, studies have shown that cholecystectomy can increase susceptibility to obesity.[16]

As previously mentioned, obesity is one of the risk factors the development of OSA. Increased neck circumference has been classified as an indicator of obesity and OSA. Fat deposits around the neck in obese patients can result in the narrowing of the upper airway,[32] potentially leading to oxygen desaturation.

In the diagnosis of OSA, questionnaires are often used to obtain possible diagnoses, with the STOP-Bang questionnaire being one of the most widely utilized screening tools. Furthermore, neck circumference and BMI are strongly related to OSA.[33] However, PSG and respiratory polygraphy remain the gold standard for the assessment of hypoxia and the severity of sleep fragmentation.

Gastric bypass surgery is a weight reduction procedure that is approved in specific cases. Moreover, glucagon-like peptide-1 (GLP-1) analogues are gaining importance in weight loss management. As a result of bariatric procedure and GLP-1 treatment, rapid weight reduction can be achieved. However, weight loss exceeding 1.5 kg per week has been observed to result in the formation of new gallstones in approx. 25% of patients.[34] Nonetheless, prophylactic cholecystectomy is not advised due to complications, unless symptomatic gallstones or intraoperative abnormal findings of the gallbladder are presented.[35,36]

The benefits of weight loss outweigh the risks of possible complications, including gallstone formation.[37] Weight reduction is considered an important component of treatment in patients with OSA[38] and other cardiovascular diseases, and is associated with a decrease in cardiovascular risk.

Discussion

The main symptoms of gallstones are abdominal pain and hepatic colic. There are 3 stages of cholecystitis: mild; moderate; and severe. Patients with mild symptoms are treated acutely when symptoms occur. The main recommendations focus on changing eating habits. Patients exhibiting moderate to severe symptoms are eligible for surgical interventions. However, the predominant risk factor continues to be obesity, which is also associated with cardiometabolic disorders and OSA.

Hyperinsulinemia, insulin resistance and dysregulation of metabolic pathways have a pro-inflammatory potential and can lead to the development of type 2 diabetes, obesity and systemic metabolic dysfunction.[39] Hence, the present review suggests that excess development of fatty tissue constitutes the potential link between OSA and gallbladder diseases.

The main question refers to the potential impact of OSA on the risk of gallstone formation. Firstly, OSA can activate enzymes involved in triglyceride synthesis, including sterol-regulatory element-binding protein 1 (SREBP-1) and stearoyl-coenzyme A desaturase-1 (SCD-1).[40] Triglyceridemia is related to obesity and insulin resistance, which can lead to impaired gallbladder motility.[41] It is noteworthy that hypertension, which occurs concurrently with OSA in most cases, inhibits gallbladder emptying through an increase in sympathetic tone, resulting in an elevated risk of gallstones.[33] Moreover, OSA has been demonstrated to cause systemic inflammation via intermittent hypoxia, sleep fragmentation and insulin resistance.[42] Respiratory events are followed by arousals in OSA patients, thus resulting in sleep fragmentation. Sleep fragmentation is defined as repetitive short interruptions of sleep.[43] In PSG, it is expressed by the arousal index (AI) that measures the number of arousals per hour. Sleep fragmentation can lead to several metabolic and physiological disturbances, including hyperinsulinemia, dysregulation of the lipid profile, increased sympathetic nervous system activity, and hypertension. These factors may contribute to an elevated risk of gallstone formation. In summary, both intermittent hypoxia and sleep fragmentation may increase the risk of cholelithiasis in patients with OSA.

Adipose tissue produces key signaling molecules that significantly influence the development of various diseases, many of which are closely linked to a patient's psychological state. Notably, both OSA and gallstones, conditions associated with adipose tissue dysfunction, can substantially impact quality of life (QOL).[44,45]

To assess QOL in patients with gallstones, numerous scales have been designed. Previous studies have attempted

to evaluate patient's subjective QOL after cholecystectomy, with most reporting notable improvements post-surgery.[2]

Sleep quality, a fundamental component of overall well-being, is strongly associated with QOL. Altered sleep patterns impair daily functioning and can exacerbate underlying health conditions. In this context, the somatic symptoms associated with chronic cholelithiasis, such as persistent abdominal pain, can contribute to a decline in sleep quality, further decreasing patient's QOL. This observation highlights a potential feedback loop where gallstone-related symptoms negatively affect sleep, which, in turn, may lead to the deterioration in the perception of health and well-being.

Sleep quality is a subjective and objective form of patient's sleep evaluation. Numerous conditions can disrupt the overall sleep architecture, including chronic pain associated with somatic disorders, OSA, psychiatric conditions, infections, and musculoskeletal disorders. While PSG remains the gold standard tool for sleep evaluation, self-reported questionnaires are widely used and accepted in both clinical practice and research. Several tools such as the Sleep Apnea Quality of Life Index (SAQLI), the Functional Outcomes of Sleep Questionnaire (FOSQ), and the Medical Outcomes Study 36-Item Short-Form Health Survey (SF-36) are approved for the evaluation of OSA-related QOL. As anticipated, QOL in patients with OSA is inversely related to the severity of apnea symptoms.[46] However, continuous positive airway pressure (CPAP) therapy, the gold standard therapeutic device for moderate to severe OSA, has been observed to enhance patients' QOL.[44]

The evidence suggests that gallstone disease and OSA independently, and potentially synergistically, contribute to diminished QOL. This effect may be mediated by similar underlying mechanisms, including chronic inflammation, increased sympathetic activity, and metabolic dysregulation. The question of whether CPAP reduces the risk of gallstone formation remains open due a paucity of research on the topic. In the present study, we aimed to investigate and evaluate the association between OSA and gallstones. To the best of our knowledge, only 1 observational study previously raised concerns about the presence of gallstones in OSA patients.[17] However, this study was published recently, and the awareness of the comorbidity of these 2 medical conditions is still an area of increasing attention.

Conclusions

Obesity is a well-established and commonly recognized risk factor for both OSA and gallstone disease. However, other metabolic disturbances, including hypertension, hyperlipidemia and insulin resistance may impair gallbladder motility and promote gallstone development through increased sympathetic nervous system activity, systemic inflammation, and altered cholesterol and triglyceride biosynthesis.

In conclusion, the association between OSA and gallstones is poorly described in the literature and remains unclear. Further research is required to:

– explore the biological mechanisms linking OSA to gallstones, particularly the role of metabolic, inflammatory and hormonal pathways;
– investigate the long-term effects of obesity and insulin resistance on gallstone formation in individuals with OSA;
– examine the impact of OSA treatment, such as CPAP, on the risk or progression of gallstones;
– conduct large-scale, longitudinal studies to better understand the temporal relationship between these conditions and their interaction over time.

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.

Use of AI and AI-assisted technologies

Not applicable.

ORCID iDs

Weronika Frosztęga https://orcid.org/0000-0002-3924-5545
Maciej Sroczyński https://orcid.org/0000-0002-4291-5714
Monika Michałek https://orcid.org/0000-0002-9740-011X
Mieszko Więckiewicz https://orcid.org/0000-0003-4953-7143
Katarzyna Madziarska https://orcid.org/0000-0002-3624-3691
Helena Martynowicz https://orcid.org/0000-0003-1283-8460

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Review

Anterior Stafne bone defect: Literature review and a case series

Dominika Kopciuch[1,B–D], Marzena Dominiak[2,A,E,F], Ingrid Różyło-Kalinowska[3,A,E,F], Paweł Kubasiewicz-Ross[2,B–D]

[1] Nova-Dent Dental and Aesthetic Medicine Clinic, Strzegom, Poland
[2] Department of Dental Surgery, Faculty of Dentistry, Wroclaw Medical University, Poland
[3] Department of Dental and Maxillofacial Radiodiagnostics, Medical University of Lublin, 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

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Address for correspondence
Paweł Kubasiewicz-Ross
E-mail: pawelkubasiewicz@wp.pl

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Abstract

The study objective was to review the literature and to present 3 cases of the anterior Stafne bone defect (SBD). The electronic databases – MEDLINE via PubMed and Google Scholar – were searched by 2 independent authors, who retrieved 20 articles concerning this pathology. The Stafne bone defect is an asymptomatic bone lesion, diagnosed mostly incidentally through radiological imaging, typically located in the lateral section of the mandible. The anterior SBD is exceedingly rarely observed. So far, less than 40 cases have been described. The hypothesis of the formation of a bone cavity in connection with the sublingual salivary gland has not been confirmed in the literature, considering other tissue structures present within the lesion, including lymphoid or adipose tissues. The anterior variant of SBD can be mistaken for other lesions, considering its atypical location and lower incidence rate. In most cases, it does not require any treatment and the 'wait-and-see' strategy is adopted. In the present study, 2 cases of two-chamber and 1 case of single-chamber anterior SBDs were presented. Their course was asymptomatic; however, in 2 cases, increased tension of the suprahyoid muscles on physical examination was reported. The cone-beam computed tomography (CBCT) imaging was employed in each case. There was no need for biopsy, and the monitoring of the lesion was established in each reported case.

Keywords: review, case series, anterior Stafne bone defect

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Highlights

- The anterior Stafne bone defect (SBD) is a rare, asymptomatic condition, typically identified as an incidental finding through three-dimensional (3D) imaging.
- The condition is often overlooked in clinical practice, as the incidence of the anterior SBD tends to be underestimated due to the predominance of two-dimensional (2D) imaging in dentistry.
- Although the exact etiology of the anterior SBD remains unclear, some studies suggest that increased suprahyoidal muscle tone may play a role in the development of this condition.

Introduction

The Stafne bone defect (SBD), first reported in 1942 by Edward Stafne, has also been described in the literature as the Stafne bone cavity or lingual mandibular depression. It is a rare, asymptomatic bone cavity characterized as a single-focus bone lesion, with the most predominant location being the lateral aspect of the mandible, typically below the inferior alveolar nerve and artery. Less frequently, SBD affects the anterior aspect of the mandible and its ramus, leading to differential diagnostic concerns. Regardless of the location, it is most frequently detected between the 5th and 7th decade of life, and shows a predilection for male sex. The accidental detection of SBD in panoramic radiography occurs in 0.08%–0.7% of cases.[1–7] The etiopathogenesis of SBD is not fully understood. However, the hypothesis of the sublingual salivary gland and, in case of the ramus location, the deep lobe of the parotid gland ingrowth, or incomplete Meckel cartilage calcification during ossification are most commonly presented.[7–10] The World Health Organization (WHO) classifies SBD in the same group as pseudocysts.[10]

The anterior SBD was first reported in 1957 by Richard and Ziskind.[9] It is located in the mental section of the mandibular body, in the area of incisors, canines and first molars, and is even a rarer finding. In contrast to the posterior variant of the lesion, it may be difficult to diagnose. The anterior SBDs concern the bone area above the mylohyoid muscle attachment and occur beneath the root apices. They may be seen superimposed over the roots or at the sites of previous extractions. Therefore, they may be misdiagnosed as other radiolucencies.[2,11]

Over time, there have been several attempts to establish a complementary classification of SBD. One of the first was the classification by Ariji et al., which distinguished 3 types of SBD, depending on its contents: types F, S and G, in which the cavity is filled with fat, soft tissue or submandibular gland tissue, respectively.[12]

The classification introduced in 1993 by Shigematsu et al. was based on radiological evaluation and the location of SBD.[13] The classification includes 4 types of bone cavity. The first 3 variants concern the lateral location of SBD, distinguishing the location relative to the mandibular canal. The latter variant refers to the anterior location of SBD. Type I, referred to as a pit, covers the lower mandible margin, below the mandibular canal. Type II, known as intermediate, is located above the lower mandibular margin, albeit below the mandibular canal. Type III, known as a deviation, is deviated from the mandibular canal. Type IV, referred to as the anterior variant, is above the insertion of the mylohyoid muscle, in the mental area of the mandible.[13] According to this classification, the most common SBD variant is type II. Types III and IV may be observed in the area of dental apices, making an erroneous diagnosis of an odontogenic inflammatory cyst probable.

The newer classification from 2020 focuses on the lingual-buccal extent of the lesion.[14] The lesion can either be a lingual impression and thinning of the cancellous bone (type I), with complete resorption of the bone and no involvement of the buccal cortical plate (type II), or it can additionally cause a buccal bulging of the thinned buccal cortex (type III). In extremely rare, severe cases, a complete loss of the basal bone in the affected area can be classified as type IV.[14]

An important aspect in types III and IV is a differential diagnosis, which should exclude salivary gland tumors, odontogenic tumors, such as ameloblastoma (mainly unicystic ameloblastoma (UA)), odontogenic keratocyst (OKC), central giant-cell lesion (CGCL), odontogenic myxoma (OM), and ossifying fibroma (OF). Among the cysts of the jaw, pseudocysts should be eliminated: solitary bone cyst (SBC); and aneurysmal bone cyst (ABC).[1,15–21]

The current report is a review of the literature on the anterior SBD; it presents 3 cases of the anterior SBD. To the best of our knowledge, this is the first review concerning the anterior SBD. The locations of SBDs were defined based on the radiographic classification from 2020.[14]

Methods

Information on sources and search strategies

A search of electronic databases was conducted using MEDLINE via PubMed and Google Scholar. The electronic search was carried out in March 2024 by 2 authors (D.K. and P.K.-R.) with the use of advanced search options. The search terms included all combinations of the

following keywords: 'Stafne bone defect' OR 'Stafne bone cavity' OR 'lingual mandibular depression'. The resulting references were exported and duplicates were removed where identified. All the information concerning the anterior variant of SBD was retrieved and collected.

Eligibility criteria

Articles in English describing clinical studies, case reports, case series, or clinical trials on SBD were included in the study. Considering case studies, only reports meeting the CARE (CAse REports) criteria – a clear description of the patient's demographic characteristics, the patient's medical history and its presentation as a timeline, current clinical condition, the description of diagnostic tests, a clear description of the treatment provided, if needed, information on the post-intervention clinical condition, and the identification of possible complications – were included in the study.[22] Bibliographic reviews, systematic reviews, editorial reviews, meeting/congress abstracts, experimental studies, in vitro or ex vivo studies, studies older than 25 years, and articles in which it was not possible to access the full texts were excluded.

Selection process

The titles/abstracts of all the articles retrieved through the electronic search were read independently by 2 authors (D.K. and P.K.-R.). After the full texts were evaluated, references that met the eligibility criteria were also included (Fig. 1). Differing opinions between the reviewers with respect to inclusion or exclusion were resolved after discussion with the third author (M.D.).

Data extraction

From each article, the following data was exported and analyzed, if available: the authors' names and the year of publication; the patients' age and sex; the clinical manifestation of the lesion; the radiological featuring of the lesion; the radiological diagnosis strategy; possible surgical intervention; the suggested contents of the defect; the specification of the follow-up strategy; and the biological behavior of the lesion.

In the case of studies on SBD in different locations, the articles were screened and all the data referring to the anterior variant was also extracted from the study where possible. In the end, 20 articles were included in the review (Table 1).

Risk of bias and certainty assessment

Due to the fact that the vast majority of the hits that met the above criteria were case studies, and consequently had extremely small sample sizes, we were not able to assess the risk of bias, and certainty or confidence.

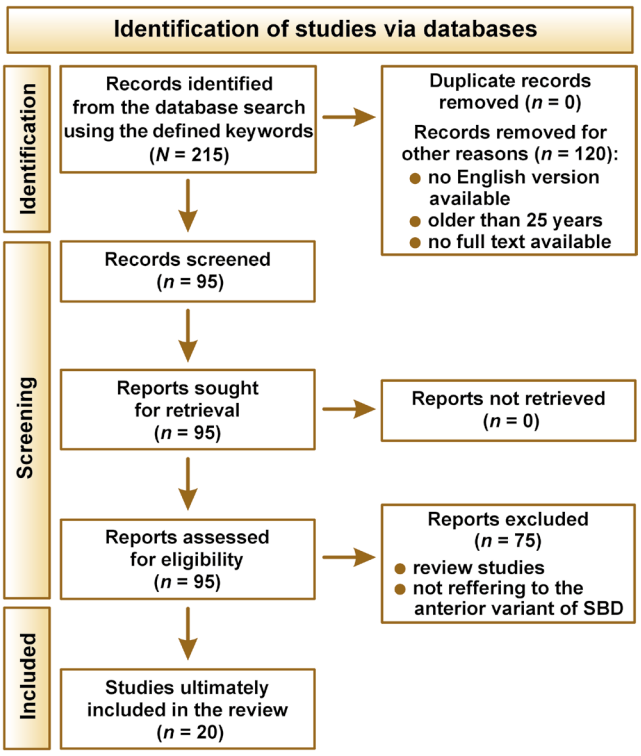

Fig. 1. Flow chart of the study selection process
SBD – Stafne bone defect.

Case presentation

Case 1

A female patient, aged 26 years, visited the dental clinic to start orthodontic therapy. During anamnesis, the patient claimed no constitutional diseases or disturbing focal symptoms within the oral cavity. The patient was subjected to orthodontic treatment. In the extraoral examination, Angle class III (overbite defects) and Steiner skeletal class III (morphological protrusive occlusion) were diagnosed, accompanied by the protrusion of the lower lip and the smoothing of the mentolabial groove. An orthodontic defect from the cross-bite group was also present, occurring on teeth 12, 42 and 43. The patient underwent treatment with the multi-loop edgewise archwire (MEAW) technique, which enabled control over the occlusion plane in 3 dimensions. The intraoral examination revealed a tense oral cavity floor and increased suprahyoidal muscle tone. The mental muscle did not feature increased muscular tone by palpation. The frenulum of the tongue was located in the correct position and no deformations of the tongue were present. Although the panoramic radiograph did not reveal any pathology, the cone-beam computed tomography (CBCT) performed for orthodontic treatment detected a bilateral, single-chamber, well-localized bone defect in the anterior part of the mandible. The shape of the bone defect was elliptical; it extended from the sublingual side in the area of the mental spine. The defect

Table 1. Results of data extraction from articles that met the eligibility criteria

Study	Patients' age [years] and sex	Clinical data	Additional examinations	Radiological manifestation of the lesion	Surgical intervention	Suggested contents	Changes at the follow-up
Hayashi et al.[7] 2020	68/M	asymptomatic	MRI within 3 months	unilateral, extending from the lingual side to the buccal side and, at the most recessed point, contiguous to the buccal cortical bone, no continuity between the apex of the adjacent tooth and the cavity, a high-density surrounding line	yes, for other reasons	salivary gland tissue	no
Taysi et al.[11] 2014	56/M	asymptomatic	CBCT	unilateral, the premolar region, the lesion eroded the lingual cortical bone and caused a buccal expansion, leaving a thin layer of bone	biopsy	mixed salivary gland tissue	not mentioned
Friedrich et al.[14] 2020	12/F	asymptomatic	CBCT	a bilateral enlargement of bone depression in sagittal and transverse directions	no	salivary gland tissue	no
Turkoglu and Orhan[23] 2010	52/M	mistaken for a periapical cyst	CT	a unilateral cyst-like cavity on the lingual side	no	salivary gland tissue	not mentioned
He et al.[24] 2019	37/F	asymptomatic	MRI	a unilateral, oval radiolucent image	no	adipose tissue	no changes at 2 years
Krafft et al.[25] 2010	46/M	asymptomatic	–	a unilateral, oval radiolucent image	exploration	connective tissue, fatty tissue, striated muscle, bony fragments, and salivary gland tissue	progression at 7 years
Watanabe et al.[26] 2021	10/M	asymptomatic	CT, MRI	oval-shaped depression (6 mm × 5 mm × 3 mm in size) at the lingual apex	exploration	glandular tissue	regression at 1 year
Hisatomi et al.[27] 2019	2 cases	asymptomatic	CT	unilateral, oval, with thin sclerosis on the borders	not mentioned	not mentioned	not mentioned
Asgary and Emadi[28] 2020	40/M	mistaken for a non-odontogenic cyst	CBCT	a unilateral, oval cyst-like cavity	no	sublingual gland tissue	not mentioned
Katı et al.[29] 2022	83/M	asymptomatic	CBCT, MRI	unilateral, oval, accompanied by the posterior variant	no	sublingual gland tissue	not mentioned
Kim et al.[30] 2014	44/F	asymptomatic	OPG	bilateral, oval	biopsy	salivary gland tissue with mixed serous and mucinous cells	not mentioned
Sisman et al.[31] 2010	62/F	mistaken for a residual cyst	CT	unilateral, oval	no	not mentioned	not mentioned
Deyhimi et al.[32] 2016	45/M	mistaken for a periapical lesion	OPG	well-defined, unilocular radiolucency below the apices of the left lateral incisor and the left canine	a periapical lesion suspected, no vitality test was done	sublingual gland inflammatory cells, muscle tissue, fat tissue, blood vessels, and nerve bundles	no changes at 3 months
Friedrich et al.[33] 2012	11/F	class III occlusion	MRI, USG	an oval osteolytic lesion superimposed on the apical parts of mandibular incisors, no sclerotic margin	no	sublingual gland tissue	not mentioned
Ozaki et al.[34] 2015	76/M	asymptomatic	CBCT, MRI	unilateral, oval, accompanied by the posterior variant	exploration to rule out salivary gland tumor	sublingual gland tissue with the infiltration of lymphocytes – chronic sialoadenitis	not mentioned
Bornstein et al.[35] 2009	47/M	asymptomatic	CBCT, MRI	unilateral, mimicking a periapical lesion	no	salivary gland tissue	not mentioned
Bornstein et al.[35] 2009	62/M	before implant therapy	CBCT, MRI	unilateral, mimicking a periapical lesion	no	salivary gland tissue	not mentioned
Vieira Aguiar et al.[36] 2011	60/M	before implant therapy	CT	bilateral, oval, accompanied by the posterior variant	no	salivary gland tissue	not mentioned
Altwaim and Al-Sadhan[37] 2019	17/M	before third molar procedure	OPG, CBCT	oval-shaped depression, with a width of 2.1 cm on the right side and 2.9 cm on the left side	no	not mentioned	not mentioned
de Courten et al.[38] 2002	42/M	mimicking a residual cyst	OPG	radiolucency located on the left side of the mandible, in the region of an absent second premolar and a first molar, above the alveolar canal	biopsy	sublingual gland tissue	not mentioned
Tomrukcu and Kose[39] 2020	45/M	asymptomatic	OPG, CBCT, MRI	a well-defined, unilocular lesion, not related to the tooth roots	no	not mentioned	not-mentioned

M – male; F – female; MRI – magnetic resonance imaging; CBCT – cone-beam computed tomography; CT – computed tomography; OPG – orthopantomography; USG – ultrasonography.

did not come into any contact with the tooth periapical tissues and did not imitate a dental cyst. After the dimensional scope of the bone lesion was established during the CBCT examination, a height of 17.0 mm and a width of 4.0 mm were determined for the defect (Fig. 2). The young age of the patient and the asymptomatic panoramic radiograph did not indicate susceptibility to major bone defects in the presented mandibular area, yet such a defect was present. The alveolar process measured 9.0 mm in its broadest point, while in the area of the defect it was considerably smaller (3.5 mm). The percentage of general bone loss was 38.88%. The bone cavity was diagnosed as the anterior SBD, with no indications for surgical intervention, taking into account the current bone resorption. According to the abovementioned classification of SBD,[14] it was categorized as type II. The patient was referred for a further magnetic resonance imaging (MRI) examination and the routine control of the lesion every 6 months for the next 5 years.

Case 2

A female patient, aged 34 years, visited the dental clinic to consult a bone defect in the anterior section of the lower jaw. The patient's medical history did not reveal any systemic diseases or dental ailments. In the physical examination, the patient had a symmetrical and proportional face. In the upper and lower arches, there were no interdental losses of Angle class I, there was no transposition, and abnormal tooth inclination was observed. The sublingual frenulum was not shortened or thin. No tension or tenderness of the submucosa could be determined in the anterior SBD area. The propylaeum depth was normal. The tongue was the appropriate color, had no defects in the resting position and showed standard mobility. In the area of the suprahyoid muscles, no reduced or increased muscle tone could be found. The first examination in routine clinical practice involved panoramic imaging, which did not show a bone lesion. The additional CBCT examination, for orthodontic purposes, revealed immense bone cavities on both sides of the mandible. The change was well-localized and had a circular shape. No contact or displacement of the teeth in the area was reported. The severe bone defect was measured via CBCT in the axial section. The bone lesion height was 16.8 mm and its width was 6.2 mm on the right side. On the left side, the bone cavity was characterized by smaller dimensions, amounting to 10.6 mm in height and 4.0 mm in width (Fig. 3). In its broadest point, the alveolar process beyond the lesion measured 10.1 mm. The amount of bone loss on the right side was 16.83% and 26.59% on the left side. The lesion was qualified as type II SBD (Fig. 4). Due to the relatively large bone defect, the patient was instructed on the need of an uninterrupted follow-up every 6 months for the next 5 years. In addition, the patient was referred for an MRI examination.

Case 3

A female patient, aged 26 years, undergoing orthodontic treatment visited the dental clinic for consultation. According to the medical history, the patient had no chronic diseases. Upon physical examination, Angle class II was diagnosed, and a prominent chin with severe mental muscle tension and activity, as well as the orange peel symptom, were present. The patient did not have interdental or lateral pterygoid deficiencies. The oral cavity floor was significantly tense within the area of the bone lesion.

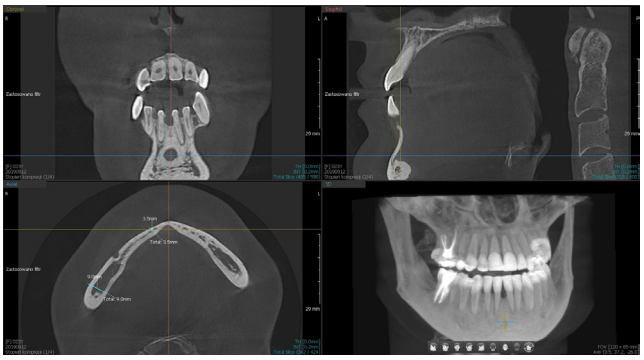

Fig. 2. Case 1: Images collected from the cone-beam computed tomography (CBCT) examination

Visible osseous resorption in the anterior section of the mandible from the lingual side. Bone on the vestibular side is completely retained.

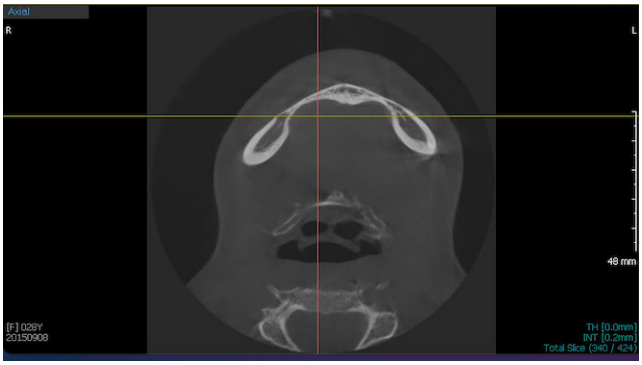

Fig. 3. Case 2: Bone depression in the anterior section visualized in the axial plane (CBCT)

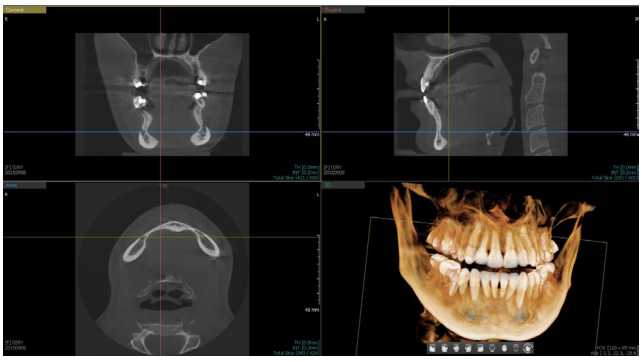

Fig. 4. Case 2: Imaging of well-isolated, bilateral bone defects in the area of the mental spine of the mandible (CBCT)

No hypertrophy or abbreviation of the fibrous sublingual frenulum was found, while the lateral frenula were absent. According to the Placek classification, type II insertion of the upper lip frenulum was diagnosed. The pull syndrome test of the lower lip was performed, which did not reveal symptoms of blanching or tearing. The patient was referred for a CBCT examination for further diagnostics. Cone-beam computed tomography was recommended to assess the volume and morphology of bone tissue in the maxilla and the mandible, to detect potential tooth resorption, and to observe the direction and extent of bone structure displacement. Incidentally, a bilateral pathological bone defect was visualized on the right and left in the anterior region of the mandible (Fig. 5). The tooth viability examination in the anterior section demonstrated a positive reaction. Following the CBCT analysis, the presence of the anterior SBD was confirmed. The lesion had an ovoid shape, with an isolated milieu (Fig. 6). The height of the right defect was 16.3 mm and its width was 7.4 mm. On the left side, bone loss was minimally minor, calculated as having a height of 16.2 mm and a width of 6.2 mm. The amount of bone loss on the more affected side was 21.05%. The lesion was categorized as type III. In the additional examinations, an abnormal level of vitamin D3 was determined (18.9 ng/mL). Surgical treatment was not required for the current condition and bone penetration was abandoned. The patient was referred for a further MRI examination. The routine control of the lesion every 6 months for the next 5 years was recommended. In addition, vitamin D3 supplementation was prescribed to correct its concentration in the system.

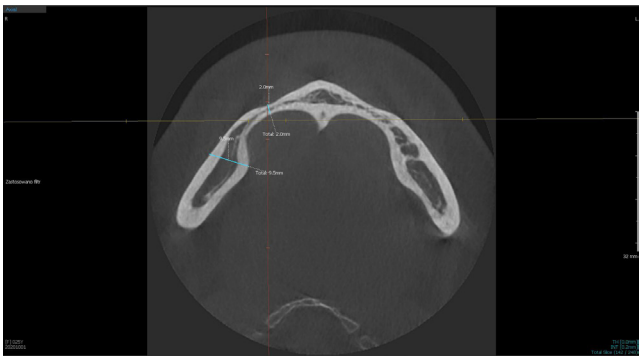

Fig. 5. Case 3: Axial view in the CBCT examination illustrating the area transparent for X-rays as bi-chamber erosion on the right side and the left sublingual surface of the mandible

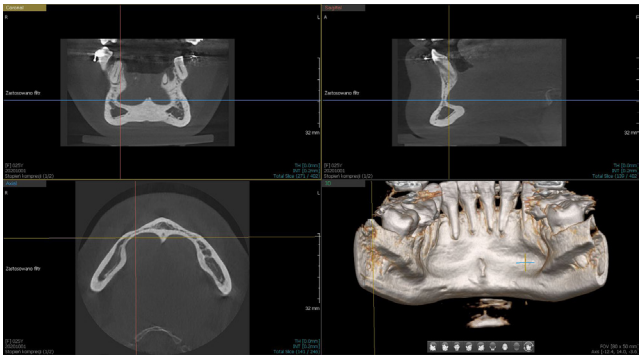

Fig. 6. Case 3: Images of the cross-sections of the anterior Stafne bone defect (SBD) in the frontal, sagittal and axial planes in the CBCT examination, and the three-dimensional (3D) mandible model

Discussion and literature review

Epidemiology and patient characteristics

The results of our study show that the anterior SBD is an extremely rare condition, as only 22 cases were ultimately included in the review on the basis of the eligibility criteria (Table 1). This finding is in line with other reports estimating the total number of the anterior SBD cases previously described in the literature at several dozen.[11,39]

It is a well-established fact that SBD is more prevalent in the Asian population. The results of our review study also suggest that the anterior variant of SBD demonstrates a higher morbidity rate in the Asian and Caucasian populations, as the majority of cases in the review came from those geographic regions. The Anterior SBD is most commonly reported in middle-aged people, with a strong predilection for male sex (Table 1).

Clinical manifestation

Similarly to SBD, in the vast majority of cases, the anterior SBD remains asymptomatic and challenging to detect on physical examination. Only in 5 of the reviewed cases did the anterior SBD mimic a jaw cyst (mostly periapical) and was initially diagnosed as such.[23,28,31,32,38] In the rest of the cases, the anterior SBD was an accidental finding. In contrast to jaw cysts, the anterior SBD eggshell crackling syndrome is usually absent on clinical examination. Observing that sign might be a criterion for differentiating the anterior SBD from an odontogenic inflammatory cyst.[31,32,38]

Radiological examination and featuring

Most of the anterior SBD cases were initially diagnosed based on the panoramic X-ray. However, authors commonly agree with a strong suggestion that in such cases, diagnosis should include three-dimensional (3D) imaging. Cone-beam computed tomography is proposed as the first choice for the additional examination. Only 3 authors of the articles under review judged the panoramic X-ray to be satisfactory at providing a definite diagnosis of the anterior SBD[30,32,38]; however, 2 of them decided to additionally carry out biopsy[30,38] and in one case, the initial diagnosis turned out to be erroneous.[38]

A CBCT examination usually demonstrates a rounded bone cavity on the lingual side of the mandible, circular

or oval, well-defined depression, reminiscent of cyst-like depression. The size of the defects ranges from 5 mm to 20 mm.[10–13,15,32,36,37]

Shimizu et al. divided the types of SBD radiological featuring into 2 main kinds.[40] Typical cases, as in our case series, show continuity from the base of the mandible and make the diagnosis of this defect easy. On the other hand, non-typical cases show an obscure margin and do not show a connection to the mandibular border. In those cases, the additional examinations are more likely to be needed.[40]

Some authors suggest performing an MRI examination in the borderline cases of the anterior SBD. Magnetic resonance imaging is not based on ionizing radiation. Furthermore, it is characterized by high resolution and is suitable for visualizing the tissues filling the anterior bone cavity, especially the lingual region of the mandible.[8,15] It is more likely to exclude the pathological processes originating from soft structures in the vicinity of the lesion and, in many cases, it can exclude surgical intervention. An MRI examination was recommended in 9 of the cases under review[7,24,26,29,33–35,39] and it largely allowed surgical intervention to be avoided, as in only one of them was biopsy needed.[34]

Although we considered the radiological features of our cases to be typical, we referred each patient for a further MRI examination. The reasons for that were the negligible invasiveness of this examination and the extremely rare location of SBD in the anterior aspect of the mandible. Thus, each such case might be generally considered an atypical SBD.

Treatment options

Although the majority of the anterior SBD cases do not need any surgical intervention, in the case of abnormalities in bone structures near the lesion or the tissues of the oral cavity floor, it is necessary to surgically explore the cavity and collect material for a histopathological examination. In the cases under review, biopsy was carried out in less than half of them.[7,11,25,26,30,32,34,39] Such a procedure is quite difficult due to the proximity of the anatomical structures of the oral cavity floor, the sublingual nerve and artery, as well as the submandibular and sublingual glands. Therefore, it requires general anesthesia and a full-thickness flap from the lingual surface of the mandibular body.[1,2]

Lesion contents

In the great majority of the cases under review, the suggested contents of the bone cavity based on the imaging examinations was the sublingual salivary gland.[14,23,28,29,33,35,36] Furthermore, when a biopsy was carried out, the microscopic examination predominantly confirmed sublingual gland tissue.[7,11,26,34,38] Occasionally, the salivary gland structure was accompanied by other physiological tissue structure from the direct vicinity of the cavity.[25,30,32] This might be explained by the biopsy technique and a small operational field, which can lead to the collection of other tissues surrounding the lesion. This finding is in line with other reports on SBD and is an explanation for the "glandular" hypothesis of the pathogenesis of the anterior SBD.[1,40] According to this hypothesis, during their development, the submandibular or sublingual glands compress the lingual part of the mandible, which is followed by the resorption of the cortical bone, and ultimately develop a defect occupied with glandular tissue. The main limitation of this hypothesis is the fact the vast majority of the anterior SBD cases are reported in the 5th and 7th decades of life, but are relatively rarely reported in the first 2 decades. Being a congenital lesion, it should be represented more frequently in the earlier stages of development.

There are contrary reports to the congenital hypothesis, suggesting other contents and etiology of the anterior SBD. The dehiscence of the muscles (mainly the mylohyoid muscles) and the fascia of the oral floor is one hypothesis for the formation of an anterior bone defect containing muscle tissue.[14] The abnormal mobility and non-physiological dehiscence of the myofascial complex lead to disorders in the correct positioning and functioning of bone structures. They may also impact the growth and development of osseous tissue, particularly in the period of skeletal growth.[41,42] The salivary gland ingrowth would be the secondary process in such situations. It is worth noting that increased tension of selected muscles in the oral region was reported on physical examination in 2 of the 3 cases presented by us.

Clinical significance

In 4 cases, the anterior variant was diagnosed in edentulous patients,[31,35,38] which could make the differential diagnosis of a residual cysts based on two-dimensional (2D) imaging challenging.[32,38,43,44] As mentioned above, the anterior SBD is a rarely detected condition and should be strictly separated from other pathologies, such as sialadenosis, ABC, bone marrow defects, giant-cell granuloma (GCG), or residual cysts. The anterior SBD and residual cysts are asymptomatic. Both have well-defined sclerotic bone margins, which is conducive to misdiagnosis, whereas the radiographic borders of other pathologies would be more undefined. Small to moderate-sized ABC and CGCL are similar to an asymptomatic anterior SBD. However, on radiological imaging, both the abovementioned conditions show more locally aggressive growth, including the resorption of the adjacent anatomical structures, as well as the swelling of the salivary gland in association with acinar hypertrophy and ductal atrophy. Sialdenosis, on the contrary, presents as non-tender swelling that is often bilateral and symmetric. Sialadenosis is usually associated

with systemic metabolic conditions, which is not typical for the anterior SBD.[1,14,15,19–21,24,45]

The clinical significance of the lesion is acknowledged during implant treatment planning, as the presence of a bone cavity and the possibility of perforating the cortical bone are associated with the possible risk of near-fatal complications.[46–49] Implant placement in the frontal aspect of the mandible is generally considered a safe procedure, with a relatively low risk of complications.[50–52] However, intraoperative bleeding in the floor of the mouth can result in a sublingual hematoma obscuring the airway and leading to a life-threatening emergency.

Our experience shows that contrary to SBD, which is easily detected on 2D imaging, the anterior SBD may not be diagnosed through the panoramic X-ray. The assistance of CBCT is generally needed for that purpose. Considering that 2D imaging is still much more popular than CBCT in dentistry – in fact, it is considered the first-line diagnostic tool in many dental specializations, while 3D imaging is standard mostly before implantation and certain other surgical interventions – it can be assumed that the morbidity of the anterior SBD might be underestimated.

Conclusions

Two cases of two-chamber and 1 case of single-chamber anterior SBDs were presented, which is a very uncommon diagnosis. All cases were observed in female patients at a relatively young age. Bone resorption varied, so it remains unknown what influence it has on the morbidity and size of the anterior SBD. However, in our cases, the hyperactivity of selected muscles in the vicinity of the lesions, as well as vitamin D3 deficiency, were reported. It should be strongly emphasized that case series study results do not allow definite conclusions in this matter. Further studies should be conducted to determine the possible cause-and-effect relationship with regard to this issue.

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.

Use of AI and AI-assisted technologies

Not applicable.

ORCID iDs

Dominika Kopciuch https://orcid.org/0000-0002-8615-315X
Marzena Dominiak https://orcid.org/0000-0001-8943-0549
Ingrid Różyło-Kalinowska https://orcid.org/0000-0001-5162-1382
Paweł Kubasiewicz-Ross https://orcid.org/0000-0001-7305-7161

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