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Effects of adding graphene oxide to composite resin: An in vitro study

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ABSTRACT

Composite resin has been extensively used as a direct restorative material to restore posterior teeth due to its low cost and more complete preservation of the tooth than conventional resin. Nanohybrid resins are a popular alternative due to their apparently better mechanical strength and aesthetic properties compared to conventional resins. However, their clinical performance is still under evaluation. Adding small amounts of graphene oxide (GO) could improve the physicochemical properties of these resins. ***Aim:*** *The purpose of the current study was to evaluate the effects on the morphological and physicochemical properties of nanohybrid composite resins when GO is added to them.* ***Materials and Method:*** *Three kinds of composite resin (Universal Nanohybrid Restorer 3M Filtek Z250 XT) were evaluated: G0, without GO; G1, composite resin with 0.01 % GO; and G2, composite resin with 0.02 % GO. Surface morphology and physicochemical characterization were analyzed for all three groups. Data were submitted to analysis of variance, followed by post hoc Tukey's tests at a significance level of $p < 0.05$.* ***Results:*** *The addition of GO did not modify the surface of the composite resin compared to the control. The peak of the GO spectrum obtained by FTIR was very small and almost imperceptible due to the close combination of graphene molecules with the rest of the material. Flexural strength increased significantly in group G1, but decreased in G2 compared to the control group. Both GO concentrations resulted in a significant increase in resistance to wear compared to the control group.* ***Conclusions:*** *The addition of graphene oxide did not modify the surface morphology of the nanohybrid compound resin evaluated. However, it did produce changes in mechanical properties: resistance to wear increased significantly at both concentrations studied, while flexural strength changed in a concentration-dependent manner. These results suggest that low concentrations of GO may contribute to improving certain physicochemical properties of nanohybrid compound resins.*

Keywords: *composite resin - graphene oxide - dental restoration wear - flexural strength*

Efecto de la adición de óxido de grafeno a resina compuesta: estudio *in vitro*

RESUMEN

La resina compuesta se ha utilizado extensamente como material de restauración directa en el sector posterior debido a su bajo costo y una mayor preservación de la estructura dentaria. Las resinas nanohíbridas representan una alternativa atractiva por sus aparentemente mejores propiedades estéticas y resistencia con respecto a las resinas convencionales, sin embargo, su desempeño clínico continua en evaluación. En este sentido, la incorporación de cantidades pequeñas de óxido de grafeno (GO) podrían mejorar las propiedades fisicoquímicas de estas resinas. ***Objetivo:*** *El propósito de esta investigación fue evaluar los efectos de la incorporación del GO sobre las propiedades morfológicas superficiales y fisicoquímicas de una resina compuesta nanohíbrida.* ***Materiales and Métodos:*** *La resina compuesta (Restaurador Nanohíbrido Universal 3M Filtek Z250 XT) se evaluó en tres grupos: G0, sin GO; G1, resina compuesta con GO al 0,01 %; G2, resina compuesta con GO al 0,02 %. Se realizó la caracterización morfológica y fisicoquímica de la superficie en todos los grupos. Los datos se analizaron mediante análisis de varianza, seguido de pruebas post hoc de Tukey con un nivel de significancia de $p < 0.05$.* ***Resultados:*** *La incorporación de GO no modificó la superficie de la resina compuesta en comparación con el grupo control. El pico del espectro de GO obtenido por FTIR fue muy pequeño y casi imperceptible debido a la estrecha combinación de las moléculas de grafeno con el resto del material. En el grupo G1 se observó un aumento significativo de la resistencia a la flexión, mientras que en el grupo G2 se observó una disminución en comparación con el grupo control. La incorporación de ambas concentraciones de GO resultó en un aumento significativo de la resistencia al desgaste en comparación con el grupo control.* ***Conclusiones:*** *La incorporación de óxido de grafeno no modificó la morfología superficial de la resina compuesta nanohíbrida evaluada. Sin embargo, produjo cambios en sus propiedades mecánicas, aumentando significativamente la resistencia al desgaste en ambas concentraciones estudiadas y modificando la resistencia a la flexión de manera dependiente de la concentración. Estos resultados sugieren que bajas concentraciones de GO pueden mejorar determinadas propiedades fisicoquímicas de las resinas compuestas nanohíbridas.*

Palabras claves: *resina compuesta - óxido de grafeno - restauración dental - resistencia a la flexión.*

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INTRODUCTION

From a materials science perspective, many dental materials are classifiable as "composites", including glass ionomer (polyalkenoate) cements and some impression materials. However, majority usage within dentistry conventionally confines the term "composite" to materials that have a polymeric (organic) matrix and a particulate inorganic glass phase, possibly with the addition of fibers, which are known as resin composites or polymeric composites[1], or by other more specific terms such as dimethacrylate composites[2]. Composite resin as a direct restorative material has been extensively used to restore posterior teeth due to its low cost and more complete preservation of the tooth than conventional resins, as well as good clinical performance[1]. Nanohybrid composite resins are used for posterior restorations. In a complex class II cavity with cervical walls in dentin, the marginal adaptation is more challenging in relation to the cervical wall in enamel due to the bond to dentin being more complex[3]. In addition, composite shrinkage stresses associated with the masticatory loads can decrease the bond strength between the tooth and the composite resin[4].

Nanohybrid resin composites contain conventional particles mixed with nanometric fillers, so their performance is similar to that of nanofilled or micro-hybrid resins. The addition of well dispersed inorganic particles to a resin matrix is extremely effective for improving the performance of polymeric composite material, providing high initial polishing, better polish and gloss retention, and resistance to wear[5].

Restorative materials have evolved to conform to increasingly greater demands and higher standards, often strictly related to aesthetic aspects. Thus, composite resins have become the material of choice for the restoration of posterior teeth[6].

The most significant change in commercial composite resins in recent years has been modification in mineral filler content. The size of the fillers added to the matrix of new composite resins has been reduced, leading to nano-filled composite resins with improved properties. Several studies have reported that nanocomposite resins (nanohybrids or nano-filled) have better surface polish and lower abrasion resistance, and found that nano-filled composite resins wear out by detachment of particles or individual parts of nanoclusters, rather than by detachment of larger particles, resulting in a relatively smooth wear surface[5]. The Academy of Dental Materials designates discoloration, modulus of elasticity, fracture resistance, fatigue, occlusion resistance, and abrasion and friction wear measurements as the most important characteristics for material performance evaluation[6,7].

Graphene oxide (GO), an oxidized form of graphene, is a carbon nanomaterial formed of single-atom layers. Graphene is a two-dimensional layer of sp^2 hybridized carbon atoms organized in a hexagonal framework. Its unique properties and prominent features include high electrical conductivity, optimal mechanical properties, large surface area, low thermal expansion coefficient, and very high aspect ratio[8].

Graphene oxide consists of graphene sheets to which oxygen-containing functional groups are attached. It is produced by the chemical oxidation of cheaply available graphite. As it readily disperses in water and other polar solvents, it is a good alternative for applications that require hydrophilicity, unlike graphene[9-10]. Generally, GO is believed to have a graphene backbone where hydroxyl and epoxide groups are on the basal plane, and predominantly carboxyl and hydroxyl groups are on the edges. There is also inter-platelet hydrogen bonding through the alcohol and epoxide functional groups, which helps to maintain the stacked structure of GO[10-12].

The aim of this study is to observe the effects on the morphological, physicochemical and mechanical properties of composite resin when GO is added to it in two different concentrations.

MATERIALS AND METHOD

This *in vitro* study with the was registered with the research committee of the School of Stomatology, Benemérita Universidad Autónoma de Puebla (registration code 202320). It is important to mention that prior to this analysis, a pilot test was carried out in which concentrations of 2%, 1% and 0.1% GO were added to the resin and tested. These concentrations were discarded because they caused a drastic change in color and a decrease in hardness in the flexural test.

Specimen preparation

In this *in vitro* study, GO (synthesized from graphite using the modified Hummers method[13], provided by

INMETRO, Brazil) was mixed with composite resin (Universal Nanohybrid Restorer 3M TM Filtek TMZ250 XT) at concentrations of 0.01 wt % and 0.02 wt %. To prepare the chemical mixture, the GO was weighed and mixed manually for 15 min under red light with 1g composite resin until the mixture was homogenous (process modified from Alghyamah et al.[9]). The following study groups were prepared: control group (without GO), G1 containing 0.01 wt % GO, and G2 containing 0.02 wt % GO. It is worth noting that the addition of these concentrations did not modify the macroscopic appearance of the resin. The following specimens were prepared with bulk fill composite (Universal Nanohybrid Restorer 3M TM Filtek TMZ250 XT): 15 disks (5 mm in diameter and 2 mm in height) to measure wear resistance, and 15 rods (22 mm x 2 mm x 2 mm) to measure flexural strength.

The specimens were light-cured, first at the center and then at the ends, using an IVOCLAR halogen light lamp coupled to a Blue phase N MC system (100-240 V). The specimens were polished with 500 silicon carbide discs to remove the oxygen-inhibited resin layer resulting from storage in distilled water at 37 °C for 48 hours. A digital vernier caliper was used to re-evaluate their sizes.

Morphological characterization

The morphological characterization of the modified composites containing GO was done via Scanning Electron Microscopy (SEM) using a JEOL model JSM-6610LV[14]. Specimens were attached to a pin with double-sided carbon tape, and gold-sputtered (DENTON VACUUM, DESK V). SEM voltage ranged from 15 kV to 30 kV, and magnification ranged from 500x to 1000x.

Fourier Transform InfraRed Spectroscopy (FTIR)

FTIR is used to define the vibrational deformations of chemical bonds. GO-incorporated composite resins were characterized by attenuated total reflectance-Fourier transform infrared spectroscopy (BRUKER, model VERTEX 70) in the range of 4000 a 400 cm^{-1} according to a previously reported method[14]. Representative images and results are shown for independent experiments performed in triplicate.

Flexural test

Flexural strength was measured on 5 rod-shaped specimens (22 mm x 2 mm x 2 mm) per group. After storage in distilled water at 37°C for 48 hours, the specimens were polished using 500-grit silicon carbide discs to remove the oxygen-inhibited layer. The flexural test was performed with an Instron universal destructive tensile testing machine (Model 4411, Instron Corp., Cantan, MA, USA), for 3-point flexural tests at a span length of 14 mm. The flexural strength and elastic modulus were measured at a 1.0 mm/min crosshead speed (n = 5). During the test, the rods were supported at two points. The load was applied to the surface in front of the support points at a point equally distant from the first two points. Compression events were observed on the rod before it fractured[15,16]. One-way analysis of variance was used to determine the statistical differences of flexural strength among groups with Graph pad version 7 package.

Wear resistance test

Resistance to wear was measured on 5 disk-shaped specimens (5 mm in diameter × 2 mm thickness) per group. The specimens were light-cured with a bluephase C8 (Ivoclar Vivadent) light-curing unit for 20 s, and stored in distilled water at room temperature for 24 h for maximum water sorption[17,18].

Before abrading, the samples were thoroughly cleaned and dried. The initial weight of each sample was recorded on a precision electronic analytical balance (Ohaus, accurate to 0.0001 g). After being sanded, specimens were abraded using a Strong 210/108 micromotor and contra-angle at 2500 rpm for 30 seconds. A constant load of 500 mg was applied to the disc using CPT microbrushes (used in the dental prophylaxis procedure and manufactured with steel bristle heads and nylon plastic). At the end of each abrasion cycle, the samples were thoroughly cleaned, dried, and weighed again to determine the amount of abrasion (adapted from Ziaei et al.[8]).

Statistical analysis

The statistics consultant determined the sample size based on similar articles. The data are expressed as the mean ± SD of five independent experiments, unless otherwise noted. Statistical significance was determined by one-way analysis of variance with a post hoc test (Tukey) using Graph Pad (version 7.0). A p-value < 0.05 was considered statistically significant.

RESULTS

The purpose of this study was to observe the effects of adding minute amounts of GO to composite resins, specifically on surface morphology and mechanical characteristics such as flexural strength and resistance to wear, to study the properties of GO described by other authors. All the tests were performed *in vitro*, which does not simulate all the conditions of the oral cavity. Due to the conditions in our laboratory, it was difficult to compare the results obtained with previous studies.

Surface morphology analysis with scanning electron microscopy (SEM)

The surface morphology and the distribution of GO in the composite specimens were investigated by SEM. Homogeneous surfaces were observed during morphological analysis. Before the abrasion process, examination of specimen roughness clearly showed that they all shared the same properties, and no agglomerations were observed (Fig. 1).

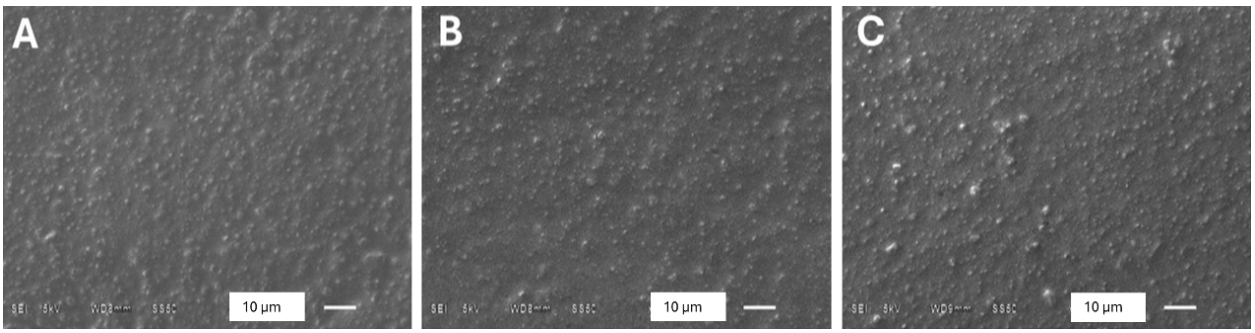

Fig. 1: Morphological characteristics of composite modified with GO. Micrographs show that the GO is uniformly distributed over the entire surface of the composite specimen in A) control, B) G1, and C) G2. Magnification 1000x.

Structural analysis by FT-IR

The FTIR spectra of the composite modified with GO were evaluated. The goal of FTIR analyses was to assess and compare the chemical structure of two series of resin-based samples. Peaks at 1000 and 1226 cm^{-1} that can be attributed to C-O vibrations, and peaks at 1026, 1150, 1630 and 1750 cm^{-1} reflecting C-OH, C-C and C=O groups can be observed in all samples, indicating that the addition of GO to G1 and G2 had no impact on the overall chemistry of the resins (Fig. 2).

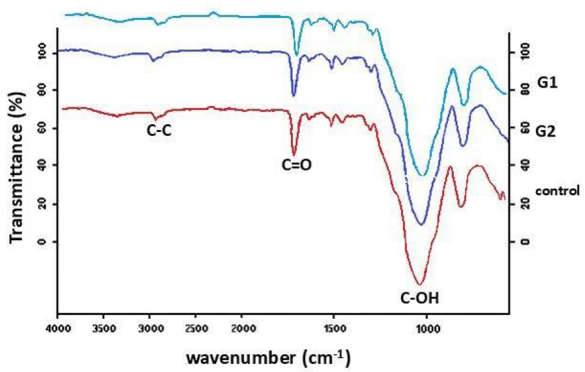

Fig. 2: FTIR spectra of the different groups: control, G1= 0.01% GO and G2= 0.02% GO.

Flexural test

One-way ANOVA showed $p<0.05$ for comparison of both G1 and G2 against the control group. Interestingly, there was a greater statistically significant difference for group G1 compared to group G2 ($p<0.001$) (Fig. 3A).

Wear resistance test

The lowest mass loss was observed in both group G1 (one-way ANOVA, $p<0.0001$) and G2 (one-way ANOVA, $p<0.0001$) compared to the control. It is important to highlight that G2 lost less mass than G1 (one-way ANOVA, $p<0.0001$) (Fig. 3B).

DISCUSSION

This study compared Filtek Z250 3M composite resins modified with 2 concentrations of GO. The aim was to investigate possible changes in morphology, chemical composition, and properties such as bending and wear.

SEM showed that there was no modification of surface morphology in specimens containing GO compared to the control (unmodified resin), therefore the addition of GO does not compromise the texture of the composite resin. This agrees with Guazzo et al.[19], who reported that GO does not modify the

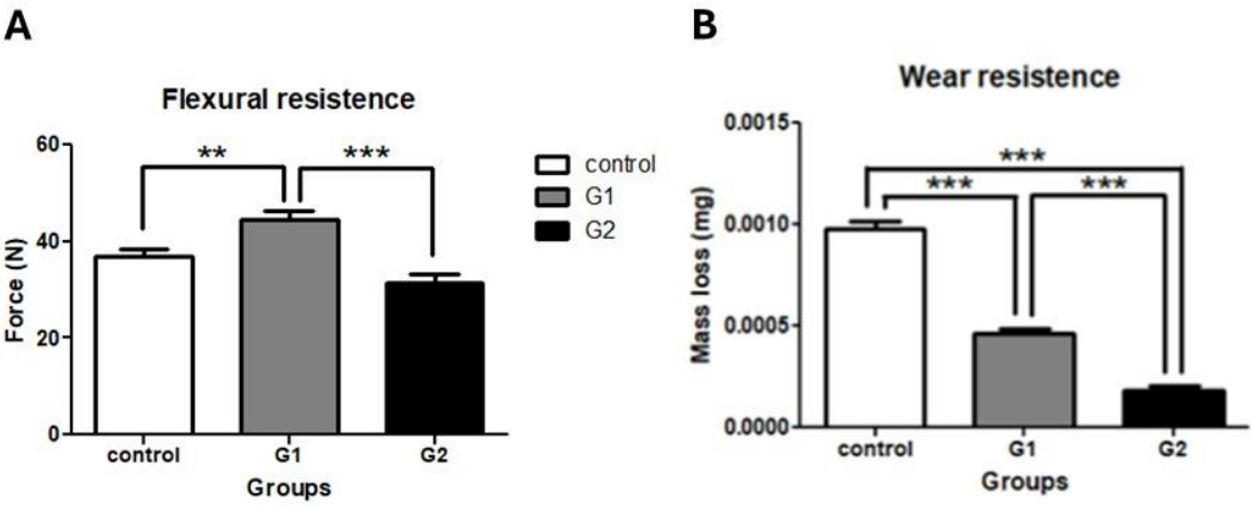

*Fig. 3: A) Flexural strength test showing statistically significant differences between groups G1 and G2. B) Wear resistance test compared to the control (n=5, one-way ANOVA, ** p<0.05 *** p<0.0001).*

texture of other materials. Thus, graphene-based materials may provide new, improved options or substitute materials currently used in dentistry.

FTIR analysis did not show different functional groups in the composite and modified resins, so it may be assumed that the addition of such small concentrations of GO did not alter the chemistry of the resin. Various papers report that the functional groups C-OH, C-C and C=O are present both in the materials containing graphene and in the composite resins (e.g., Agarwallaa et al.)[20]. Our result also agrees with Lee et al.[21], who published spectra of the GO with minimal signals corresponding to its functional groups, which are very small and almost imperceptible due to the close combination of graphene molecules with the rest of the material.

Flexural strength tests provide information about the ability of a material to flex before breaking. Group G1 had significantly greater compressive strength than the control group ($p< 0.05$). Increased resistance in the presence of GO agrees with Lee et al.[21], who added nano-graphene oxide (nGO) to PMMA-based acrylic resin, and found that it increased resistance by 0.5% compared to the control group. However, group G2 had significantly lower resistance ($p<0.001$), suggesting that the presence of a greater amount of GO could weaken the composite resin and decrease its resistance. It would thus be advisable to perform further tests using different percentages of GO, different techniques for adding GO to the resin, and different solvents to integrate it into the resin.

The wear test measured mass loss from specimens subjected to brushing. The presence of GO in the composite resin increased its resistance to wear with brushing, suggesting that when used as a restoration material, it would undergo less wear from daily toothbrushing. This agrees with Turssi et al[22], who reported that larger filler particles, the distance between the filler particles, and the amount of filling by weight are related to greater wear resistance of the resin. In addition, the resistance capacity that GO provides to other materials such as acrylic resins, adhesives, implant coatings, scaffolds, etc. has already been very well reported. Similarly, Dubey et al.[23] and Bitounis et al.[24] tested wear in various materials for tissue engineering and modified acrylic resins, concluding that GO provides the materials with greater resistance to bending and wear. The wear mechanism of a dental material consists of a series of intricate steps that can lead to loss of shape, increased roughness, pigmentation, and plaque buildup[25]. Therefore, to understand its limitations and indications, it is necessary to examine the restorative materials. In this approach, *in vitro* brush abrasion testing can be used to compare the abrasion resistance of various types of materials and determine which type of material would be best for use as a restorative treatment.

CONCLUSION

The addition of GO to Filtek Z250 XT composite resin resulted in a homogeneous distribution of the nanomaterial, without evidence of particle

agglomeration, as observed by SEM analysis. Furthermore, FTIR analysis indicated that the addition of GO did not significantly alter the overall chemical structure of the resin matrix.

The addition of low concentrations of GO influenced the mechanical performance of the composite resin. Both GO concentrations significantly improved wear resistance compared to the control group. Regarding flexural strength, the addition of 0.01 wt % GO resulted in a significant improvement, whereas 0.02 wt % GO led to a reduction in this property. These findings suggest that the effect of GO on the mechanical behavior of the composite is concentration-dependent.

Among the tested formulations, the composite containing 0.01 wt % GO exhibited the most favorable balance between flexural strength and wear resistance, indicating that low concentrations of GO may provide a promising strategy for enhancing selected properties of nanohybrid composite resins.

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CONFLICT OF INTEREST

The authors declare no potential conflicts of interest regarding the research, authorship, and/or publication of this article.

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Design and validation of an instrument to assess awake bruxism in adults

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ABSTRACT

The definition of bruxism has evolved over the past few years. Currently, two clinical presentations are recognized: Sleep Bruxism (SB) and Awake Bruxism (AB). Although various bruxism assessment strategies have been developed to guide clinical practice, no specific, valid, reliable tool has been designed specifically for patients with AB. ***Aim:*** *To design and validate a questionnaire to assess the presence, severity and symptomatology of AB. Secondary objectives included exploring the clinical and psychiatric manifestations of AB.* ***Materials and Method:*** *A prospective case-control study was conducted on 14 AB cases and 20 healthy controls. The presence or absence of the condition was confirmed by electromyographic study. A specific questionnaire for AB (AB-Q) was developed, and AB psychometric properties were evaluated. The AB-Q consists of 24 items grouped into four domains. The reliability, sensitivity and specificity of the AB-Q were assessed.* ***Results:*** *Significant differences were observed between groups: EMG-confirmed bruxism episodes were recorded in all 14 AB patients and none of the controls. The AB-Q showed high internal consistency (Cronbach's alpha: 0.924) and strong discriminative capacity, with a sensitivity of 100% and a specificity of 90%. A significant association was found between bruxism and the presence of anxiety and depressive disorders, as well as obsessive-compulsive traits.* ***Conclusion:*** *The AB-Q questionnaire proved to be a useful tool with acceptable clinimetric properties for assessing the presence and severity of bruxism.*

Keywords: *bruxism - diagnosis - validation - questionnaire*

Diseño y validación de un cuestionario para evaluar el bruxismo en vigilia en adultos.

RESUMEN

La definición de bruxismo ha evolucionado en los últimos años. Actualmente se reconocen dos presentaciones clínicas: Bruxismo del Sueño (BS) y Bruxismo en vigilia (BV); Si bien se han desarrollado estrategias de evaluación del bruxismo que guían la práctica clínica, no existen herramientas específicas, válidas y fiables desarrolladas para pacientes con AB. ***Objetivos:*** *Diseñar y validar un cuestionario para evaluar la presencia, gravedad y sintomatología del BV. Los objetivos secundarios fueron explorar las manifestaciones clínicas y psiquiátricas del BV.* ***Materiales y Método:*** *Se realizó un estudio prospectivo sobre 14 casos de AB y 20 controles sanos. La presencia o no de enfermedad fue confirmada mediante estudio electromiográfico. Se desarrolló un cuestionario específico de BV (BV-Q) y se determinaron sus características psicométricas. El BV-Q contiene 24 ítems agrupados en 4 dominios. Se determinaron la fiabilidad, la sensibilidad y la especificidad del BV-Q.* ***Resultados:*** *Se encontraron diferencias significativas entre los grupos: se registraron episodios de bruxismo confirmados mediante EMG en 14 pacientes del grupo de BV y en ninguno de los controles. El cuestionario BV-Q mostró una alta consistencia interna (alfa de Cronbach: 0,924) y una sólida capacidad discriminativa, con una sensibilidad del 100 % y una especificidad del 90 %. Se observó una asociación significativa entre el bruxismo de Vigilia y la presencia de trastornos de ansiedad y depresión, así como de rasgos de (TOC).* ***Conclusión:*** *El cuestionario BV-Q demostró ser una herramienta útil con propiedades clinimétricas aceptables para evaluar la presencia y la gravedad del bruxismo.* ***Palabras clave:*** *bruxismo - diagnóstico - cuestionario - validación.*

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INTRODUCTION

The term bruxism has been used for decades to identify mandibular activities associated with tooth grinding sounds that occur during sleep[1]. However, this conception has changed, and two clinical presentations are currently recognized: Sleep Bruxism (SB) and Awake Bruxism (AB)[2]. The paradigm shift proposing that bruxism has two distinct clinical presentations (AB/SB) requires a thorough review of the accumulated knowledge regarding the conception of a single entity. In support of this idea, a consensus of experts in 2018 recommended separate definitions for the two entities (SB and AB)[3].

Identification methods for SB have been further developed[4], while the assessment and measurement of AB still pose a challenge. Multimodal assessment strategies with a comprehensive approach to guide clinical practice[5] have been published, providing a major contribution to the assessment of bruxism[6]. However, the instruments included for the assessment of AB were neither specifically designed to measure it nor tested in patient series. There is still a lack of precision in the assessment guidelines, which directly affects their clinical management.

Dental correlates of bruxism range from muscular and joint symptoms (fatigue and/or pain in the masticatory muscles and temporomandibular joint) to conditions of the dental tissue (tooth wear) and the soft tissues in the oral cavity, with negative health consequences[7]. Nevertheless, some authors suggest that bruxism could have a positive effect on the health of patients with sleep apnea, as it restores upper airway permeability[8].

Various psychological traits have been described in association with AB[9-10], while SB is described as a complex activity with multiple neurological implications[11-13]. However, the overlap of symptoms in the two conditions has not been thoroughly studied.

The international consensus on the assessment of bruxism emphasized the need to improve self-assessment, based on the validity and reliability of the methods used for this purpose. Therefore, the primary objective of this study was to design and validate an AB questionnaire (AB-Q) to assess the presence and severity of AB and its symptoms. Secondary objectives included evaluating the clinical and psychiatric characteristics associated with awake bruxism.

MATERIALS AND METHOD

We conducted a prospective case-control study, divided into two parts: the development of a questionnaire to detect subjects with AB, followed by the validation of the tool through statistical analysis of the responses of cases and controls.

The study patients were selected from the Emergency Department and Patient Counseling at the School of Dentistry, University of Buenos Aires (FOUBA). The study was conducted at the Department of Oral and Maxillofacial Surgery at FOUBA, and at the Movement Disorders and Clinical Neurophysiology Sections of the Raúl Carrea Neurological Research Institute (FLENI). The study was approved by the Ethics Committees of both institutions. After receiving a full explanation of the study methodology, all participants provided written informed consent.

The study initially included 34 patients: 17 clinical AB cases and 17 controls, randomly selected, with similar sex and age as the cases. Only subjects who met the following demographic criteria were selected to participate in the study: 1) adults between 18 and 80 years of age of either sex; 2) complete, stable dental occlusion; 3) individuals with permanent dentition and correct alignment of the dental arches; 4) capable of providing written consent to participate in the study. Exclusion criteria were: 1) patients diagnosed with sleep bruxism according to the criteria of the International Classification of Sleep Disorders III (2014)[14] (since the aim of the study was to evaluate the effectiveness of the questionnaire for detecting AB, the SB item was added to ensure the exclusion of cases of nocturnal bruxism); 2) patients undergoing orthodontic treatment; 3) patients with periodontal disease; 4) patients with cerebral or cardiac pacemakers.

To obtain two characterized groups for this initial validation phase, sample AB consisted of patients with a presumptive diagnosis of bruxism. The definitive diagnosis was reached through electromyographic studies. Clinical preselection was based on the recommendations of the International Consensus on the Evaluation of Bruxism[2]: a) self-perceived awake bruxism described as repetitive or sustained dental contact and/or bracing or thrusting of the jaw during wakefulness[3], with "frequent" being defined as occurring 3 to 5 times per week; and b) presence of lingual indentations and/or linea alba, and/or jaw muscle pain and/or fatigue during the day. These specific features were considered signs

of active AB. The questionnaire also considered mandibular muscle pain and/or fatigue during the day, characterized by spontaneous muscular pain that increases with masticatory function, primarily located in the masseter and temporal regions; fatigue described as secondary discomfort typically manifested during masticatory function, affecting masticatory muscles[15].

Questionnaire development

Consensus meetings were held with experts in dentistry, neurology and movement disorders to define the format and general structure of the questionnaire to assess self-perceived AB and its clinical characteristics. Item selection was based on clinical experience and literature review. The following were defined: the period for self-perception of bruxism, the methodology for reporting symptoms, the items to be assessed, and their grouping into different sections of the questionnaire. A pilot questionnaire containing 27 items was developed and administered to case-control patients. Participants rated the frequency of symptom occurrence for each question using a 7-point Likert scale, from 0 ("No") to 7 ("Always, every day of the week"). The collected data were analyzed, and the questionnaire was reformulated based on the 24 items with the highest internal validity and reliability, as determined by Cronbach's alpha ($r > 0.90$). The final version of the questionnaire consists of 4 domains: 1) Self-perceived bruxism, with 9 questions assessing self-perceived bruxism, premonitory aura, and sleep bruxism in the past 3 weeks. Higher scores indicate more severe levels of bruxism. 2) Symptomatology domain, including 6 items assessing the severity of pain/fatigue, functional limitation of the masticatory muscles, tension-type headache, and sustained mandibular activity without dental contact (bracing or thrusting). Higher scores indicate greater symptom severity. 3) Comorbidity domain, with two items that identify the presence and severity of body-focused repetitive behaviors (BFRBs) such as lip, cheek, and tongue biting. Higher scores indicate more severe levels of BFRBs. 4) Concentration task domain, consisting of 6 items that identify daily activities that may induce bruxism (e.g., driving, reading, using a computer or watching television, physical activity, social interactions, or work-related stressors). The total scores from sections 1, 2 and 3 of the questionnaire were used to calculate reliability and validity. The new scale is called the "Awake Bruxism Questionnaire" and consists of 24 items (Table 1).

Electromyographic studies

A standardized working protocol was developed following the recommendations of Shiffman[16]. The assessment protocol was administered over a period of 90 to 110 minutes, during which participants remained alone, connected to the recording equipment (Fig. 1), while they received instructions from a prerecorded voice. The standardized task protocol was divided into 4 blocks: (a) baseline registration and administration of Beck Anxiety Inventory (BAI)[17], Beck Depression Inventory (BDI)[18] Premonitory Urgency Tic Scale (PUTS)[19], and Revised Repetitive Behavior Scale (RBS-R)[20] and Yale-Brown Obsessive-Compulsive Scale (Y-BOCS)[21]; b) video viewing on tablet – two 10-minute videos on current events were pre-selected; c) playing Candy Crush; d) free use of cell phone. Each block was separated by 5 minutes of inactivity. Chewing gum or candies was not allowed during the protocol execution. Continuous electromyographic (EMG) monitoring was performed using monopolar surface electrodes with digital bipolar conversion, placed on the masticatory muscles (bilateral masseter and temporal muscles) and a muscle involved in mouth opening (digastric muscle). Electromyographic activity was sampled at 640 Hz and later analyzed offline. Before starting the recording, a maximum voluntary contraction was obtained, and its activity was used as a parameter for comparing the electromyographic intensity of the episodes recorded during the study. Additionally, the patient was instructed to perform different functional activities (yawning, smiling, and swallowing saliva) to compare the activation patterns observed during the study with those of specific activities, facilitated by simultaneous video analysis. Root mean square (RMS) was calculated for every EMG burst, as a measure of signal intensity. Every masticatory muscular activity with RMS exceeding 20% of the maximum voluntary contraction was considered as a possible bruxism episode from an EMG point of view. These episodes were later correlated with the video recordings, to determine whether they were indeed episodes of bruxism, or a reflection of other types of oromandibular activity (such as yawning, swallowing, etc.). The electromyographic tracings

Table 1. Awake Bruxism Questionnaire

.UBA Universidad de Buenos Aires

.UBA ODONTOLOGÍA Facultad de Odontología

Full name: .....

Date: .....

Evaluator: .....

The following questions are about sensations and symptoms you may have perceived in relation to your jaw function in daily life during the past two weeks. Please circle the number of the best answer to each question.

1. Do you clench your teeth when you sleep? If so, how often?
- **0.** No.
- **1.** I don't know.
- **2.** I have done it, but I don't do it now.
- **3.** 1 to 3 days a month.
- **4.** Sometimes (1 or 2 times per week).
- **5.** Frequently (3 to 5 times per week).
- **6.** Always (every day of the week).

2. Has anyone observed or told you that you clench your teeth while asleep? If so, how often?
- **0.** No.
- **1.** I don't know.
- **2.** I have done it, but I don't do it now.
- **3.** 1 to 3 days a month.
- **4.** Sometimes (1 or 2 times per week).
- **5.** Frequently (3 to 5 times per week).
- **6.** Always (every day of the week).

3. Do you grind or clench your teeth when you sleep? If so, how often?
- **0.** No.
- **1.** I don't know.
- **2.** I have done it, but I don't do it now.
- **3.** 1 to 3 days a month.
- **4.** Sometimes (1 or 2 times per week).
- **5.** Frequently (3 to 5 times per week).
- **6.** Always (every day of the week).

4. Has anyone heard you grinding or clenching your teeth while asleep? If so, how often?
- **0.** No.
- **1.** I don't know.
- **2.** I have done it, but I don't do it now.
- **3.** 1 to 3 days a month.
- **4.** Sometimes (1 or 2 times per week).
- **5.** Frequently (3 to 5 times per week).
- **6.** Always (every day of the week).

5. Do you clench your teeth while awake? If so, how often?
- **0.** No.
- **1.** I don't know.
- **2.** I have done it, but I don't do it now.
- **3.** 1 to 3 days a month.
- **4.** Sometimes (1 or 2 times per week).
- **5.** Frequently (3 to 5 times per week).
- **6.** Always (every day of the week).

6. Has anyone observed or told you that you clench your teeth while awake? If so, how often?
- **0.** No.
- **1.** I don't know.
- **2.** I have done it, but I don't do it now.
- **3.** 1 to 3 days a month.

Table 1. (cont.)

- **4.** Sometimes (1 or 2 times per week).
- **5.** Frequently (3 to 5 times per week).
- **6.** Always (every day of the week).

7. Do you grind your teeth while awake? If so, how often?
- **0.** No.
- **1.** I don't know.
- **2.** I have done it, but I don't do it now.
- **3.** 1 to 3 days a month.
- **4.** Sometimes (1 or 2 times per week).
- **5.** Frequently (3 to 5 times per week).
- **6.** Always (every day of the week).

8. Has anyone heard you grinding or clenching your teeth while awake? If so, how often?
- **0.** No.
- **1.** I don't know.
- **2.** I have done it, but I don't do it now.
- **3.** 1 to 3 days a month.
- **4.** Sometimes (1 or 2 times per week).
- **5.** Frequently (3 to 5 times per week).
- **6.** Always (every day of the week).

9. Do you feel the need to clench/grind your teeth? If so, how often?
- **0.** No.
- **1.** I don't know.
- **2.** I have done it, but I don't do it now.
- **3.** 1 to 3 days a month.
- **4.** Sometimes (1 or 2 times per week).
- **5.** Frequently (3 to 5 times per week).
- **6.** Always (every day of the week).

10. Do you hold or tense your jaw muscles without tooth contact while awake? If so, how often?
- **0.** No.
- **1.** I don't know.
- **2.** I have done it, but I don't do it now.
- **3.** 1 to 3 days a month.
- **4.** Sometimes (1 or 2 times per week).
- **5.** Frequently (3 to 5 times per week).
- **6.** Always (every day of the week).

11. Do you feel pain in your facial/jaw muscles during the day? If so, how often?
- **0.** No.
- **1.** I don't know.
- **2.** I have done it, but I don't do it now.
- **3.** 1 to 3 days a month.
- **4.** Sometimes (1 or 2 times per week).
- **5.** Frequently (3 to 5 times per week).
- **6.** Always (every day of the week).

12. Do you feel fatigue/muscle tiredness when chewing? If so, how often?
- **0.** No.
- **1.** I don't know.
- **2.** I have done it, but I don't do it now.
- **3.** 1 to 3 days a month.
- **4.** Sometimes (1 or 2 times per week).
- **5.** Frequently (3 to 5 times per week).
- **6.** Always (every day of the week).

13. Do you feel fatigue in your facial/jaw muscles at the end of the day? If so, how often?
- **0.** No.
- **1.** I don't know.
- **2.** I have done it, but I don't do it now.
- **3.** 1 to 3 days a month.
- **4.** Sometimes (1 or 2 times per week).
- **5.** Frequently (3 to 5 times per week).
- **6.** Always (every day of the week).

Table 1. (cont.)

14. Do you press your tongue hard against your teeth while awake? If so, how often?
- **0.** No.
- **1.** I don't know.
- **2.** I have done it, but I don't do it now.
- **3.** 1 to 3 days a month.
- **4.** Sometimes (1 or 2 times per week).
- **5.** Frequently (3 to 5 times per week).
- **6.** Always (every day of the week).

15. Do you chew or bite your cheeks, tongue, or lips? If so, how often?
- **0.** No.
- **1.** I don't know.
- **2.** I have done it, but I don't do it now.
- **3.** 1 to 3 days a month.
- **4.** Sometimes (1 or 2 times per week).
- **5.** Frequently (3 to 5 times per week).
- **6.** Always (every day of the week).

16. Do you feel your teeth are rougher or sharper? If so, how often?
- **0.** No.
- **1.** I don't know.
- **2.** I have done it, but I don't do it now.
- **3.** 1 to 3 days a month.
- **4.** Sometimes (1 or 2 times per week).
- **5.** Frequently (3 to 5 times per week).
- **6.** Always (every day of the week).

17. Do you experience headaches during the day? If so, how often?
- **0.** No.
- **1.** I don't know.
- **2.** I have done it, but I don't do it now.
- **3.** 1 to 3 days a month.
- **4.** Sometimes (1 or 2 times per week).
- **5.** Frequently (3 to 5 times per week).
- **6.** Always (every day of the week).

Please mark any of the following situations in which you have found yourself clenching/grinding your teeth while awake:

18. While driving.? If so, how often?
- **0.** No.
- **1.** I don't know.
- **2.** I have done it, but I don't do it now.
- **3.** 1 to 3 days a month.
- **4.** Sometimes (1 or 2 times per week).
- **5.** Frequently (3 to 5 times per week).
- **6.** Always (every day of the week).

19. While reading? If so, how often?
- **0.** No.
- **1.** I don't know.
- **2.** I have done it, but I don't do it now.
- **3.** 1 to 3 days a month.
- **4.** Sometimes (1 or 2 times per week).
- **5.** Frequently (3 to 5 times per week).
- **6.** Always (every day of the week).

20. While using the computer or watching TV? If so, how often?
- **0.** No.
- **1.** I don't know.
- **2.** I have done it, but I don't do it now.
- **3.** 1 to 3 days a month.
- **4.** Sometimes (1 or 2 times per week).
- **5.** Frequently (3 to 5 times per week).
- **6.** Always (every day of the week).

Table 1. (cont.)

21. While performing physical activity? If so, how often?
- **0.** No.
- **1.** I don't know.
- **2.** I have done it, but I don't do it now.
- **3.** 1 to 3 days a month.
- 4. Sometimes (1 or 2 times per week).
- 5. Frequently (3 to 5 times per week).
- 6. Always (every day of the week).

22. During a social activity? If so, how often?
- **0. No.**
- **1.** I don't know.
- **2.** I have done it, but I don't do it now.
- **3.** 1 to 3 days a month.
- **4.** Sometimes (1 or 2 times per week).
- **5.** Frequently (3 to 5 times per week).
- **6.** Always (every day of the week).

23. During stressful work activities? If so, how often?
- **0.** No.
- **1.** I don't know.
- **2.** I have done it, but I don't do it now.
- **3.** 1 to 3 days a month.
- **4.** Sometimes (1 or 2 times per week).
- **5.** Frequently (3 to 5 times per week).
- **6.** Always (every day of the week).

24. In any other situation, please describe:

.....
.....

Total score:

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were classified based on their characteristics and duration as: a) Continuous EMG activity, with a duration longer than 2 seconds. b) Repetitive EMG activity, with a duration between 0.25 and 2 seconds. c) Combined or Mixed EMG activity (combinations of both).

Statistical Analysis

Statistical analysis was performed using IBM SPSS Statistics version 28.0 and Amos structural equation modeling package. Frequencies and percentages were calculated for item analysis and group comparisons, using binomial proportion tests and Bonferroni post hoc tests. Scores were compared using medians and interquartile ranges from the 25th to 75th percentiles. The Mann-Whitney U test was applied for statistical analysis. The assignment of items to each section was validated using exploratory factor analysis with principal component extraction (PCA) and subsequent Varimax rotation. The internal consistency of the instrument and each section was evaluated using Cronbach's alpha coefficient. The discriminatory ability of the groups and the instrument was evaluated using ROC curves, and the area under the curve was contrasted using Hanley & McNeil's approximation method. The identified cutoff points were also evaluated by calculating sensitivity and specificity with a 95% confidence interval. A significance level of $p < 0.05$ was selected.

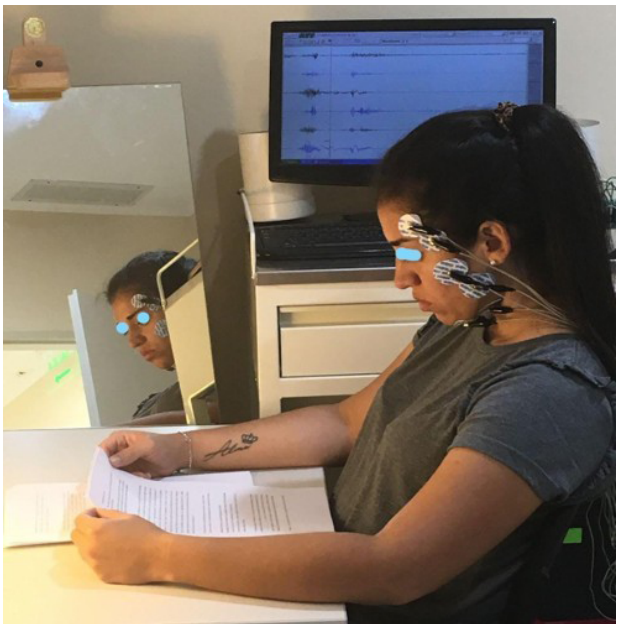

Fig. 1: Electromyographic evaluation. Throughout the study, the patient was seated in a chair at a desk. The equipment was placed 10 cm away from the patient, on a rolling module. A mirror was positioned at an angle of 45 degrees to the right of the patient, to reflect facial actions, which were captured by a camera located in the room.

Table 2. Clinical and demographic findings.

	AB (n = 14)	Controls (n = 20)	p Value
Men (%)	7 (50%)	9 (45%)	0.7
Age (y)	34.6 ± 2.8	35.7 ± 3.0	0.5
IAB	5.5±1.6	1.5 ± 0.6	**0.03**
Anxiety	5 (35.7%)	0 (0%)	0.04
IDB	7.0 ± 1.4	2.9 ± 0.8	**0.02**
Depression	3 (21.4%)	0 (0%)	0.2
PUTS	9.8 ± 0.8	9.0 ± 0.0	0.3
Tics	1 (5.9%)	0 (0%)	1.0
Y-BOCS	12 (85.7%)	5(25%)	**0.02**
RBS-R-I	0.2 ± 0.9	0.2 ± 0.9	1.0
RBS-R-II	0.1 ± 0.1	0 (0%)	0.3
RBS-R-III	0.7 ± 0.3	0.8 ± 0.3	0.8
RBS-R-IV	0.3 ± .01	0.5 ± 0.3	0.5
RBS-R-V	0.7 ± 0.3	0.6 ± 0.3	0.8
RBS-R-VI	0.2 ± 0.1	0.1 ± 0.1	0.4
RBS-R-Total	2.1 ± 0.8	1.8 ± 0.6	0.7
Stereotypes	0 (0%)	0 (0%)	1.0

Abbreviations: AB= Awake Bruxism. IAB=Beck's Inv. Anxiety Disorder; IDB=Beck's Inv. Depression Disorder; PUTS=Premonitory Urge for Tic Scale; Y-BOCS=Yale-Brown Obsessive Compulsive Scale; RBS-R=Repetitive Behavior Scale-Revised.

RESULTS

Following electromyographic evaluation, participants were reclassified according to the EMG findings. The final study groups consisted of 14 participants with EMG-confirmed awake bruxism (50% men- median age 34 years; interquartile range: 28-45 years), and 20 participants without EMG evidence of awake bruxism. (45% men - median age 35 years; interquartile range: 27-45 years) (Table 2).

Psychometric characteristics of the questionnaire

The questionnaire showed high internal consistency (Cronbach's alpha = 0.924). The analysis of the self-perceived bruxism and symptomatology domains showed high reliability values in each section (Table 3).

Questionnaire responses and clinical correlates

Bruxism episodes, confirmed by EMG and video analysis, were identified in all 14 patients in the bruxism group. These findings were not present in any of the healthy controls. Mandibular activities consistent with bruxism episodes involving dental contact, but without audible grinding sounds, were observed in 82.4% of bruxism cases. The average duration of these events was 4.8 seconds.

According to the questionnaire scores, 10 patients (58.8%) experienced facial/jaw muscle pain at the end of the day ($p < 0.001$), 11 patients (64.7%) experienced muscle fatigue upon mastication ($p < 0.001$), and 10 patients (58.8%) ($p < 0.001$) maintained a tense jaw without tooth contact and pressed their tongue firmly against their teeth while awake. The activities most frequently associated with bruxism episodes were stressful work-related tasks (88.2%) ($p < 0.001$), reading (70.6%) ($p < 0.001$), and computer use (58.8%) ($p < 0.001$).

Based on the BAI and BDI evaluations, 5 patients (35.7%) had anxiety (p=0.03), and 3 patients (21.4%) had depression (p=0.03). Twelve patients (85.5%) exhibited OCB traits (Table 2).

Discriminant Validity of the Questionnaire

A ROC curve was constructed using the scores. The AUC of the ROC curve, which assesses the ability of the total score of the scale to discriminate between Bruxism and healthy controls, was 94.12% (95% CI). The established cutoff value of 2 showed a sensitivity of 100% and a specificity of 90% in discriminating Bruxism from controls (Fig. 2).

Table 3. Psychometric properties for the AB-Q total score and domains.

	AB-Q **Total score**	AB-Q domain **Self-reported**	AB-Q domain **Symptoms**	AB-Q domain **Comorbidities**	AB-Q domain **Tasks Concentration**
Internal consistency (Cronbach's alpha).	0.924	0.849	0.924	0.920	0.720

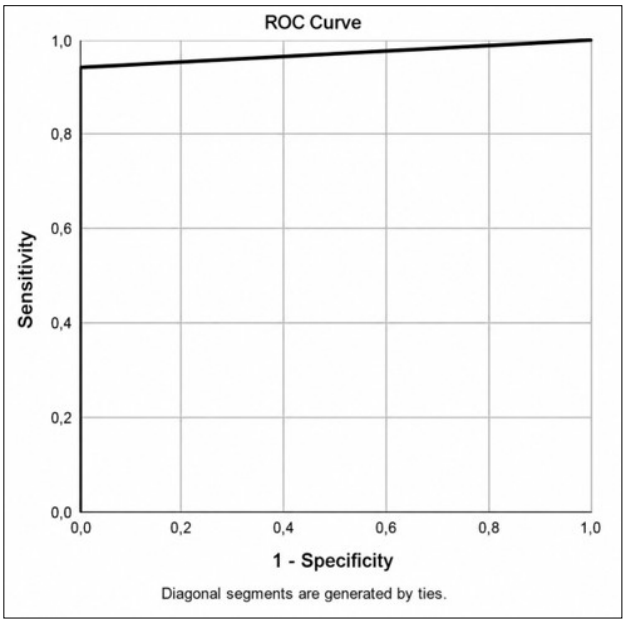

Fig. 2: ROC curve total scale score.

DISCUSSION

Awake bruxism was formally recognized in 2013 as one of the clinical manifestations of bruxism. Five years later, Lobbezoo et al.[3] consolidated the differences between SB and AB and proposed separate definitions. AB is defined as: "a masticatory muscle activity during wakefulness that is characterized by repetitive or sustained tooth contact and/or by bracing or thrusting of the mandible and is not a movement disorder"[3].The results of the present study confirm that AB is a type of repetitive activity that can be voluntarily suppressed and may share similarities with other body-focused repetitive behaviors (BFRBs), such as onychophagia, dermatophagia, and dermatillomania, which cause physical damage and have been reported to be associated with stress, anxiety and frustration. These behaviors can be considered a spectrum ranging from common habits to pathological states that can significantly impact quality of life. BFRBs are currently classified within the broader category of obsessive-compulsive disorders (ODC)[26]. Interestingly, OCD is also a commonly reported comorbidity in tic patients, particularly those with Tourette syndrome (TS) and Leg Stereotypy Syndrome (LLS)[27]. From a phenomenological perspective, we consider that the observed similarities between Tics, BFRBs, LLS and AB suggest the existence of shared pathophysiological processes. Further studies are needed to confirm these possible sensory associations.

The AB-Q we developed demonstrated strong psychometric properties, including high internal consistency, good acceptability and validity, high discriminative capacity, and diagnostic accuracy. Furthermore, during its development, we were able to identify specific activities related to concentration tasks associated with bruxism episodes. Among the limitations of our study, we should note that the sample consisted of a small group of patients with bruxism and may not be representative of bruxism in the general population. At the time of recruitment, electrophysiological confirmation was not available. Therefore, the initial selection of participants was based on the available clinical criteria derived from the literature and expert clinical judgment. Electromyography was subsequently used, which confirmed the clinical selection criteria in 82.4% of cases.

CONCLUSIONS

This study provided an initial approach to validating the developed questionnaire. The selection of AB cases was intentionally used to analyze the relationships between responses and clinical and electromyographic manifestations in two well-characterized groups. Future studies should evaluate the questionnaire in unselected populations representative of routine clinical practice.

The AB-Q is an assessment tool designed for self-administration (non-instrumental assessment). It is quick to answer and easy to use, making it a useful instrument for assessing AB in adults, both in clinical and research settings, as there are currently no tailored questionnaires specifically designed for the assessment of AB.

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CONFLICT OF INTEREST
The authors declare no potential conflicts of interest regarding the research, authorship, and/or publication of this article.

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Bibliometric analysis of dental research in Peruvian universities, 2013–2023

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ABSTRACT

Scientific production in dentistry has grown in Peru over the past decade; however, institutional differences in publication impact, thematic areas, and collaboration patterns remain unclear. ***Aim:*** *To compare the Scopus-indexed dental scientific output of five Peruvian universities between 2013 and 2023.* ***Materials and Method:*** *A cross-sectional bibliometric study was conducted using Scopus to identify dental publications affiliated with the five most productive Peruvian universities from 2013 to 2023. Eligibility criteria included dental-related topics and full variable availability. After screening 825 records, 584 were included. Extracted variables included publication year, type, thematic area, citations, scientific productivity, number of authors, and collaboration patterns. Descriptive indicators were computed; normality was assessed using Shapiro–Wilk. Group differences were tested with Chi-square and Kruskal–Wallis tests, with Bonferroni-adjusted post hoc analyses. Statistical significance was set at $p<0.05$.* ***Results:*** *The greatest increase in scientific output occurred between 2019 and 2023, peaking in 2021. Universidad Científica del Sur produced the highest number of publications (34.25%), while Universidad Peruana Cayetano Heredia obtained the highest citation impact. Public Oral Health was the most frequently represented area. Significant differences were found in thematic specialization ($p=0.016$), citation averages ($p<0.001$), number of authors ($p=0.007$), and collaboration types ($p<0.001$). National collaborations predominated, while international partnerships remained limited.* ***Conclusions:*** *Peruvian dental research indexed in Scopus has increased substantially in recent years. Citation impact and thematic specialization differ among universities, reflecting heterogeneous research development. The predominance of national collaboration highlights the need to reinforce international cooperative networks. These findings support the formulation of differentiated strategies to strengthen institutional research capacity and global visibility.*

Keywords: *bibliometrics - dental research - universities*

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Análisis bibliométrico de la investigación en odontología en universidades peruanas, 2013-2023

RESUMEN

La producción científica en odontología ha aumentado en Perú durante la última década; sin embargo, persisten diferencias institucionales en impacto, áreas temáticas y patrones de colaboración. ***Objetivo:*** *Comparar la producción científica odontológica indexada en Scopus de cinco universidades peruanas durante 2013–2023.* ***Materiales y Método:*** *Se realizó un estudio bibliométrico transversal usando Scopus para identificar publicaciones odontológicas afiliadas a las cinco universidades peruanas más productivas entre 2013 y 2023. Tras identificar 825 registros, se incluyeron 584 que cumplieron los criterios de elegibilidad. Se extrajeron datos sobre año de publicación, tipo, área temática, citas, productividad científica, número de autores y colaboración. Se aplicaron estadísticas descriptivas; la normalidad se evaluó con Shapiro–Wilk. Para comparar grupos se usaron Chi-cuadrado y Kruskal–Wallis, aplicando post hoc con corrección de Bonferroni. Se consideró significancia $p<0.05$.* ***Resultados:*** *El mayor incremento de producción científica se observó entre 2019 y 2023, con un pico en 2021. La Universidad Científica del Sur generó la mayor cantidad de publicaciones (34.25%), mientras que la Universidad Peruana Cayetano Heredia obtuvo el mayor impacto de citas. La Salud Pública Oral fue el área predominante. Hubo diferencias significativas en especialización temática ($p=0.016$), citas promedio ($p<0.001$), número de autores ($p=0.007$) y tipos de colaboración ($p<0.001$). Predominó la colaboración nacional, mientras que la internacional fue limitada.* ***Conclusiones:*** *La producción científica odontológica peruana indexada en Scopus ha aumentado notablemente. El impacto de citas y la especialización temática difieren entre universidades, reflejando un desarrollo heterogéneo. El predominio de la colaboración nacional evidencia la necesidad de fortalecer redes internacionales. Estos hallazgos respaldan estrategias diferenciadas para consolidar la capacidad investigativa y la visibilidad global.*

Palabras clave: *bibliometría - investigación dental - universidades*

INTRODUCTION

Scientific production is a continuous process aimed at disseminating knowledge through formal academic publication channels. Bibliometrics provides a quantitative approach that enables the evaluation of scientific development, the identification of research areas, and the detection of emerging trends, offering essential information for guiding research policies[1,2]. In dentistry, bibliometric studies have revealed patterns of international collaboration and a concentration of research in preventive oral health, alongside marked disparities between high-income and developing regions[3]. Globally, Scopus and Web of Science are among the main databases used to assess scientific output, providing broad journal coverage and standardized metrics such as the Scimago Journal Rank[1]. Although most dental research worldwide originates in high-income countries, Peru has made increasing efforts to strengthen research within dental schools[4]. Recent evidence shows a rise in Peruvian scientific production indexed in Scopus across the health sciences, with a notable growth in dental publications between 2017 and 2023[5]. This trend has been reinforced by the recent inclusion of Peruvian dental journals in international indexes, contributing to a mean annual national scientific growth of 33% in recent years[6]. Despite this progress, the overall level of scientific output in the health sciences remains low compared with other countries[7]. Currently, only four Peruvian dental journals are indexed in major international databases, reflecting limited but growing national visibility and emphasizing the need to strengthen research capacity[4]. Parallel to this, national initiatives such as those led by the National Council for Science, Technology and Technological Innovation (CONCYTEC) have contributed to gradually improving research quality and productivity, although previous bibliometric studies highlight persistent gaps in international collaboration and underdeveloped emerging research areas[8,9]. Given this scenario, it is essential to understand how Peruvian universities have contributed to the scientific development of dentistry over time. Therefore, the present study aims to compare the dental scientific output indexed in Scopus from Peruvian universities during 2013–2023, under the hypothesis that significant differences exist among universities in terms of publication volume, thematic areas, citation impact, and collaboration patterns.

MATERIALS AND METHOD

This cross-sectional study analyzed the scientific production in dentistry from Peruvian universities indexed in Scopus between 2013 and 2023. The initial population consisted of 825 records identified using an advanced search in the Scopus database (Scopus, Elsevier, Amsterdam, Netherlands). The inclusion criteria were a) publications indexed in Scopus between 2013–2023; b) exclusively dental-related topics; and c) primary affiliation to one of the five Peruvian universities with the highest scientific output. Records with incomplete information for any study variables were excluded.

Because the aim was to characterize the full population of publications produced by these universities, no sample size calculation was required. All eligible records were considered, resulting in a final dataset of 584 records, obtained through convenience sampling after applying the eligibility criteria.

Data extraction and classification were performed by a single researcher trained in bibliometric analysis, following a standardized protocol with explicit operational definitions. The variables analyzed were university affiliation, year of publication, type of publication, thematic area, number of citations, scientific productivity, number of authors per publication, collaboration status, and collaboration regions.

The "type of publication" variable included the categories: Letter to the Editor/Editorial, Literature Review, Case Report, Original Article, and Systematic Review, as indicated by Scopus. The "thematic area" variable included: Dental Public Health, Endodontics, Oral and Maxillofacial Pathology, Oral and Maxillofacial Surgery, Orthodontics and Dentofacial, Orthopedics, Pediatric Dentistry, Periodontics, Prosthodontics and Restorative Dentistry, Oral Implantology, and Oral and Maxillofacial Radiology, assigned after manual review of the publications.

Scientific productivity was defined as the mean number of citations per publication. Collaboration status was classified as Simple, Multiple, or No collaboration. Collaboration regions included Peru, South America, Central America, North America, Asia, Africa, Oceania, and Europe, with additional categories added when more than one region was involved.

The search procedure consisted of accessing the Scopus website, selecting "Advanced document search," and entering the following query: AFFILCOUNTRY (peru) AND PUBYEAR > 2012 AND PUBYEAR < 2024 AND (LIMIT-TO (SUBJAREA, "DENT")).

The results were then filtered by "Affiliation" to include only the top five Peruvian universities according to publication volume. Each record was manually reviewed to confirm that it met the eligibility criteria.

Statistical analyses were performed using Stata Now 19 SE (StataCorp LLC, College Station, TX, USA). Descriptive statistics were calculated for all variables. Normality of quantitative variables was assessed using the Shapiro–Wilk test. Since most indicators did not follow a normal distribution, non-parametric tests were used. The Chi-square test was applied for categorical variables, and the Kruskal–Wallis test for continuous variables, followed by Mann–Whitney U post hoc comparisons with Bonferroni adjustment when required. A 95% confidence level and a significance level of $p<0.05$ were used. Microsoft Excel 365 (Microsoft Corporation, Redmond, WA, USA) was used to organize and present the final tables.

The study protocol was approved by the Research Regulatory Affairs Office of Universidad Peruana Cayetano Heredia (ethical file: CAR-DUARI-O-225-25). Confidentiality was ensured by anonymizing identifying data. The manuscript follows the STROBE guidelines for cross-sectional observational studies.

RESULTS

A total 825 records were identified in the initial search. After applying the eligibility criteria related to institutional affiliation and data completeness, 584 Peruvian dental publications indexed in Scopus between 2013 and 2023 were included in the analysis. The university with the highest contribution was Universidad Científica del Sur (UCSUR), accounting for 34.25% of all publications, followed by Universidad Peruana Cayetano Heredia (UPCH), Universidad Nacional Mayor de San Marcos (UNMSM), Universidad Nacional Federico Villarreal (UNFV), and Universidad de San Martín de Porres (USMP) (Table 1).

Regarding temporal distribution, scientific output showed a marked increase beginning in 2019. From 2013 to 2018, annual production remained below 22 documents, whereas the highest peaks were observed in 2021 and 2023. This upward trend was consistent across most institutions, particularly UCSUR, which maintained the highest volume during the years of greatest productivity. No statistically significant association was found between the year of publication and university affiliation ($p=0.286$) (Table 1).

Original articles were the most frequent publication type (72.43%), particularly from UCSUR and UPCH. Literature reviews and systematic reviews followed in prevalence. Distinct institutional patterns were observed; for example, UPCH contributed nearly one third of all systematic reviews, whereas UNMSM produced the highest proportion of editorials. However, no significant association was observed between publication type and university affiliation ($p=0.777$) (Table 1).

Analysis by thematic area showed that Public Oral Health was the most frequently represented field, comprising one quarter of the total output. High contributions were also observed in Periodontics and Oral Medicine and Pathology. Institutional specialization was evident: UCSUR produced the majority of publications in Implantology and Orthodontics and Dentofacial Orthopedics. A statistically significant association was found between thematic area and university affiliation ($p=0.016$) (Table 1).

The mean number of citations per publication was 6.32 ± 14.29 (median: 2.00), with significant differences across universities ($p<0.001$). UPCH achieved the highest citation impact, followed by USMP and UCSUR, while UNMSM and UNFV showed the lowest means. For scientific productivity, defined as the mean number of citations relative to total scientific production, UPCH demonstrated the strongest balance between impact and volume, whereas UCSUR led in total production, but with moderate impact (Fig. 1, Table 2).

Mean number of authors per publication was 5.75 ± 0.27, differing significantly among institutions ($p=0.007$), with USMP having the highest, and UCSUR and UNMSM the lowest values (Table 2).

Collaboration patterns revealed that multiple collaboration was the most common structure (58.73%), followed by simple collaboration and no collaboration. UCSUR generated the largest share of publications with multiple collaboration, whereas UNFV had the lowest proportion of papers without

Table 1. Scientific production in dentistry from Peruvian universities in Scopus during 2013–2023, by year of publication, type of publication and thematic area

	n	%	Affiliation university										p*
			Universidad Científica del Sur		Universidad Nacional Mayor de San Marcos		Universidad Peruana Cayetano Heredia		Universidad de San Martín de Porres		Universidad Nacional Federico Villarreal		
			n	%	n	%	n	%	n	%	n	%	
Total	584	100.00	200	34.25	114	19.52	125	21.40	72	12.33	73	12.50	
Year of publication													
2013	13	2.23	2	15.38	1	7.69	5	38.46	5	38.46	0	0.00	0.286
2014	15	2.57	4	26.67	0	0.00	4	26.67	7	46.67	0	0.00	
2015	11	1.88	3	27.27	0	0.00	7	63.64	1	9.09	0	0.00	
2016	14	2.40	4	28.57	0	0.00	9	64.29	1	7.14	0	0.00	
2017	19	3.25	2	10.53	2	10.53	10	52.63	5	26.32	0	0.00	
2018	22	3.77	8	36.36	2	9.09	6	27.27	6	27.27	0	0.00	
2019	59	10.10	19	32.20	13	22.03	14	23.73	10	16.95	3	5.08	
2020	95	16.27	34	35.79	28	29.47	10	10.53	8	8.42	15	15.79	
2021	127	21.75	47	37.01	30	23.62	19	14.96	13	10.24	18	14.17	
2022	106	18.15	43	40.57	23	21.70	19	17.92	7	6.60	14	13.21	
2023	103	17.64	34	33.01	15	14.56	22	21.36	9	8.74	23	22.33	
Type of publication													
Letter to the editor / Editorial	36	6.16	10	27.78	13	36.11	5	13.89	2	5.56	6	16.67	0.777
Literature review	52	8.90	19	36.54	11	21.15	1	1.92	10	19.23	11	21.15	
Case report	34	5.82	14	41.18	12	35.29	6	17.65	2	5.88	0	0.00	
Original article	423	72.43	145	34.28	75	17.73	101	23.88	46	10.87	56	13.24	
Systematic review	39	6.68	12	30.77	3	7.69	12	30.77	12	30.77	0	0.00	
Thematic area													
Dental Public Health	146	25.00	38	26.03	30	20.55	40	27.40	18	12.33	20	13.70	0.016
Endodontics	24	4.11	8	33.33	1	4.17	8	33.33	2	8.33	5	20.83	
Oral and Maxillofacial Pathology	81	13.87	16	19.75	21	25.93	14	17.28	12	14.81	18	22.22	
Oral and Maxillofacial Surgery	26	4.45	11	42.31	7	26.92	2	7.69	4	15.38	2	7.69	
Orthodontics and Dentofacial Orthopedics	62	10.62	34	54.84	16	25.81	9	14.52	1	1.61	2	3.23	
Pediatric Dentistry	23	3.94	7	30.43	8	34.78	4	17.39	3	13.04	1	4.35	
Periodontics	105	17.98	40	38.10	16	15.24	26	24.76	16	15.24	7	6.67	
Prosthodontics and Restorative Dentistry	70	11.99	22	31.43	8	11.43	15	21.43	11	15.71	14	20.00	
Oral Implantology	10	1.71	7	70.00	1	10.00	0	0.00	0	0.00	2	20.00	
Oral and Maxillofacial Radiology	37	6.34	17	45.95	6	16.22	7	18.92	5	13.51	2	5.41	

n: Absolute frequency. %: Relative frequency. p: Statistical significance.
*Chi-square test corrected by Yates

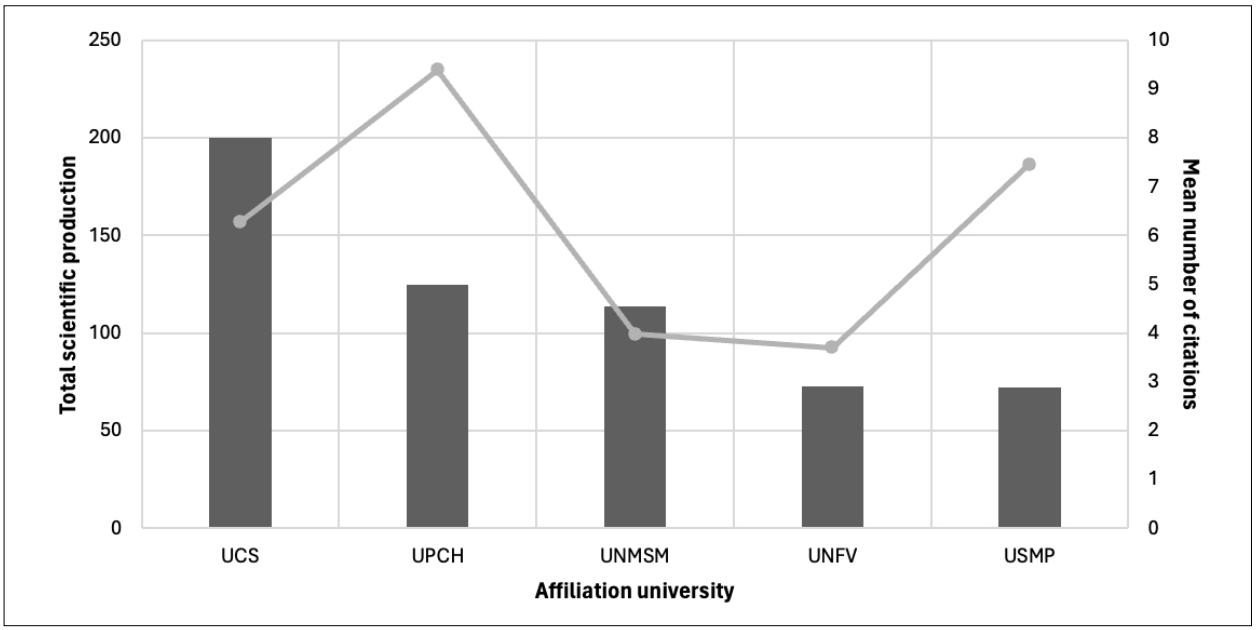

Fig. 1: Scientific productivity of Peruvian universities in dentistry: Mean number of citations relative to total scientific production, 2013–2023

Table 2. Scientific production in dentistry from Peruvian universities in Scopus during 2013–2023, by number of citations and number of authors per publication

	n	%	Number of citations					Number of authors				
			X	SD	Mdn	IQR	p*	X	SD	Mdn	IQR	p*
Total	584	100.00	6.32	14.29	2.00	6.00		5.75	0.27	5.00	2.00	
University affiliation												
UCSUR	200	34.25	6.28	11.84	2.00ab	7.00		5.12	2.21	5.00a	3.00	
UNMSM	114	19.52	3.98	9.46	1.00ac	3.00		4.91	1.88	5.00b	3.00	
UPCH	125	21.40	9.40	21.67	4.00cde	9.00	<0.001	5.69	4.91	5.00c	3.00	0.007
USMP	72	12.33	7.46	15.04	1.00d	7.50		8.82	16.37	5.00	4.00	
UNFV	73	12.50	3.70	7.76	1.00be	4.00		5.84	1.91	6.00abc	1.00	

n: Absolute frequency. %: Relative frequency. p: Statistical significance.
X: Mean. SD: Standard Deviation. Mdn: Median. IQR: Interquartile Range. p: Statistical significance.
*Kruskal-Wallis test with Mann-Whitney U post hoc comparisons; groups sharing the same letter notation indicate statistically significant differences ($p < 0.05$).

collaboration. A significant association was found between collaboration type and university affiliation ($p<0.001$) (Table 3).

Regarding collaboration regions, partnerships with Peruvian institutions were the most frequent (45.45%), with UNMSM leading this category. Collaborations with South American institutions accounted for roughly one fifth of all cases, mainly driven by UCSUR. Europe and North America represented smaller proportions, led primarily by UPCH and UCSUR, respectively. No significant association was found between collaboration region and university affiliation ($p=0.237$) (Table 3).

DISCUSSION

Scientific production at Peruvian schools of dentistry increased notably between 2013 and 2023, particularly in recent years. The 584 Scopus-indexed publications identified reflect a sustained effort to strengthen university research, which may be associated with institutional strategies aimed at improving international visibility, academic productivity requirements, and growing emphasis on research as a core component of professional training[9,10].

Before the enactment of University Law No. 30220 in 2014, research was not considered essential

Table 3. Scientific production in dentistry from Peruvian universities in Scopus during 2013–2023, by collaboration patterns

	n	%	Affiliation university										p
			Universidad Científica del Sur		Universidad Nacional Mayor de San Marcos		Universidad Peruana Cayetano Heredia		Universidad de San Martín de Porres		Universidad Nacional Federico Villarreal		
			n	%	n	%	n	%	n	%	n	%	
Total	584	100.00	200	34.25	114	19.52	125	21.40	72	12.33	73	12.50	
Collaboration status													
Single-institution	152	26.03	44	28.95	44	28.95	32	21.05	10	6.58	22	14.47	
Multi-institutional	343	58.73	122	35.57	49	14.29	77	22.45	45	13.12	50	14.58	<0.001*
No collaboration	89	15.24	34	38.20	21	23.60	16	17.98	17	19.10	1	1.12	
Collaboration regions													
Peruvian University + Peruvian Institutions	225	45.45	46	20.44	70	31.11	19	8.44	25	11.11	65	28.89	
Peruvian University + South American Institutions	110	22.22	60	54.55	9	8.18	31	28.18	7	6.36	3	2.73	
Peruvian University + Central American Institutions	7	1.41	4	57.14	3	42.86	0	0.00	0	0.00	0	0.00	
Peruvian University + North American Institutions	26	5.25	13	50.00	2	7.69	8	30.77	2	7.69	1	3.85	
Peruvian University + Asian Institutions	7	1.41	3	42.86	0	0.00	1	14.29	2	28.57	1	14.29	
Peruvian University + African Institutions	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	
Peruvian University + Oceanian Institutions	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	
Peruvian University + European Institutions	29	5.86	9	31.03	0	0.00	15	51.72	5	17.24	0	0.00	
Peruvian University + Peruvian & European Institutions	1	0.20	0	0.00	0	0.00	0	0.00	0	0.00	1	100.00	
Peruvian University + South American & Central American Institutions	9	1.82	4	44.44	0	0.00	4	44.44	1	11.11	0	0.00	
Peruvian University + South American, Central American & North American Institutions	3	0.61	0	0.00	0	0.00	3	100.00	0	0.00	0	0.00	
Peruvian University + South American, Central American, North American, Asian, African, Oceanian & European Institutions	2	0.40	0	0.00	0	0.00	1	50.00	1	50.00	0	0.00	0.237**
Peruvian University + South American, Central American, North American & European Institutions	3	0.61	0	0.00	0	0.00	2	66.67	1	33.33	0	0.00	
Peruvian University + South American & North American Institutions	34	6.87	19	55.88	5	14.71	8	23.53	2	5.88	0	0.00	
Peruvian University + South American, North American, Asian, African & European Institutions	3	0.61	0	0.00	0	0.00	1	33.33	2	66.67	0	0.00	
Peruvian University + South American, North American, Asian & European Institutions	1	0.20	0	0.00	0	0.00	0	0.00	1	100.00	0	0.00	
Peruvian University + South American, North American & European Institutions	3	0.61	1	33.33	1	33.33	1	33.33	0	0.00	0	0.00	
Peruvian University + South American, Asian, African & European Institutions	2	0.40	0	0.00	0	0.00	0	0.00	2	100.00	0	0.00	
Peruvian University + South American, Asian & European Institutions	2	0.40	1	50.00	0	0.00	1	50.00	0	0.00	0	0.00	
Peruvian University + South American & European Institutions	10	2.02	3	30.00	1	10.00	4	40.00	1	10.00	1	10.00	

(continues in next page)

Table 3. (cont.)

	n	%	Affiliation university										p
			Universidad Científica del Sur		Universidad Nacional Mayor de San Marcos		Universidad Peruana Cayetano Heredia		Universidad de San Martín de Porres		Universidad Nacional Federico Villarreal		
			n	%	n	%	n	%	n	%	n	%	
Peruvian University + North American & Asian Institutions	1	0.20	0	0.00	1	100.00	0	0.00	0	0.00	0	0.00	
Peruvian University + North American, Asian, Oceanian & European Institutions	1	0.20	0	0.00	0	0.00	0	0.00	1	100.00	0	0.00	
Peruvian University + North American & African Institutions	2	0.40	0	0.00	0	0.00	2	100.00	0	0.00	0	0.00	
Peruvian University + North American & European Institutions	10	2.02	3	30.00	0	0.00	6	60.00	1	10.00	0	0.00	
Peruvian University + Asian & European Institutions	4	0.81	0	0.00	1	25.00	2	50.00	1	25.00	0	0.00	

n: Absolute frequency. %: Relative frequency. p: Statistical significance.
*Chi-square test
**Chi-square test corrected by Yates

or mandatory within Peruvian universities. This legislation established research as a central responsibility and encouraged the active participation of faculty, students, and graduates in scientific activities and collaboration networks at national and international levels[11,12]. This regulatory change marked a turning point in the Peruvian higher education system by reinforcing the role of research and promoting the development and retention of qualified researchers[9].

The results of this study show that dental scientific output did not increase immediately following the implementation of the law. The most pronounced growth occurred between 2019 and 2023, peaking in 2021. This suggests that, although the law provided a favorable framework, the full impact materialized gradually as universities expanded incentives, research units, and support structures. The COVID-19 pandemic also accelerated scientific output, as international analyses have shown an increase in publications related to biosafety and oral public health during 2020–2022[13].

Institutional differences during the study period contrast with previous findings. While Mayta-Tovalino et al. reported UPCH as the most productive institution during 2014–2019, the present study identified UCSUR as the leading contributor during 2013–2023. These discrepancies may stem from differences in the study periods, evolving institutional strategies, funding, and faculty research profiles. Likewise, the institution with the lowest output differed between studies, which reinforces the importance of long-term, equitable strategies to enhance dental research capacity across all universities[10].

The observed upward trend, with a peak in 2021, may be partly explained by post-pandemic dynamics, strengthening of research offices, and implementation of incentive policies. Similar irregular growth patterns have been described in other Latin American countries, where productivity often fluctuates due to economic, administrative, and institutional factors[14].

The predominance of original articles aligns with previous Peruvian studies reporting that empirical research represents more than 70% of dental publications[9]. The lack of significant association between publication type and institutional affiliation suggests that research output is not strongly influenced by editorial structures or university-specific preferences.

Regarding thematic areas, Public Oral Health was the most frequent category, whereas Implantology was the least frequent. This distribution may reflect the national emphasis on prevention, resource limitations for advanced clinical research, and the socially oriented mission of Peruvian dental schools[15]. The significant association between thematic area and university affiliation indicates institutional specialization patterns, consistent with regional findings[9,16].

In terms of citation impact, UPCH showed the highest mean number of citations per publication, consistent with its longstanding research trajectory, international collaborations, and publication in high-impact journals. This pattern aligns with evidence indicating that institutional reputation and international cooperation are associated with increased visibility and citation rates[17,18]. Conversely, the lower means observed for some institutions may relate to reduced international exposure, publication language barriers, or weaker collaborative networks. The higher mean number of authors observed in USMP may reflect extensive interinstitutional collaboration or internal co-authorship policies. This is consistent with the perspective of Põder, who notes that large author groups often reflect interdisciplinary collaboration rather than higher impact[19].

In the analysis of collaboration networks, multiple collaboration was predominant, although most partnerships were national. This suggests opportunities to strengthen international collaboration, which has been recognized as a key factor in enhancing scientific performance[17]. The limited partnerships with certain regions, such as Africa and Oceania, indicate unexplored opportunities for broader global engagement.

This study has several limitations. As it focuses on the five Peruvian universities with the highest productivity, the results cannot be extrapolated to all institutions in the country. The exclusive reliance on Scopus may exclude valuable research published in local or regional journals not indexed in this database. Furthermore, the cross-sectional design prevents causal inference, and bibliometric indicators do not assess methodological quality, originality, or social impact.

Nevertheless, these findings provide meaningful insights into the evolution of dental research in Peru. Future work should integrate qualitative analyses to explore underlying dynamics of scientific production. Comparative regional studies could contextualize Peruvian productivity within broader Latin American trends. Additionally, expanding the concept of scientific production beyond high-impact journals—considering theses, technical reports, and books—would contribute to a more holistic understanding of the national research ecosystem.

CONCLUSION

This bibliometric analysis demonstrates a positive evolution of Peruvian dental scientific production between 2013 and 2023, characterized by sustained growth and increasing institutional consolidation. The marked rise in publications after 2019 suggests a delayed yet effective response to national research policies and institutional strengthening efforts. The shifting leadership among universities highlights the dynamic nature of research development across institutions.

The predominance of Public Oral Health research reflects alignment with national health priorities, while differences in citation impact indicate varying levels of research maturity and visibility. Collaboration patterns point to the need for stronger international partnerships, particularly with underrepresented regions, to enhance global integration and scientific impact.

Overall, these findings underscore the need for differentiated strategies to support equitable research capacity, foster strategic international collaboration, and encourage publication in high-impact outlets. The conclusions apply to the institutions analyzed and should be interpreted within the context of the study's scope and limitations.

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CONFLICT OF INTEREST

The authors declare no potential conflicts of interest regarding the research, authorship and/or publication of this article.

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Radiographic study of mandibular alterations resulting from a ketogenic diet in growing rats. Its orthodontic significance

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ABSTRACT

Aim: *This study analyzes the development of mandibular condyles and its likely impact on the direction of mandibular displacement during maxillofacial development in a ketogenic diet model in growing rats.* ***Materials and Method:*** *Twenty male Wistar rats, weaned at 21 days, were assigned to one of the following groups: control (fed a regular hard diet ad libitum) and experimental (fed a ketogenic hard diet, ad libitum). After 30 days of diet, ketonemia was determined and animals were euthanized. The mandibles were resected, hemisected at the symphysis and fixed in 10% formalin. Remaining soft tissues were removed. Metallic landmarks were placed in the mandibular foramens of one hemimandible of each rat. The hemimandibles were radiographed and cephalometric study was performed on paper tracings of the projected image of the radiographs. Data obtained were analyzed with Student's "t" Test.* ***Results:*** *Food consumed was lower in the experimental group. Body weight was 179±1 g in controls, and 128±11 g in the experimental group, $p<0.05$. Ketonemia was 0.29±0.042mmol/l in controls and 1.89±0.7 mmol/l in the experimental group, $p<0.05$. Condylar process length and angular process convexity were significantly lower in the experimental group.* ***Conclusions:*** *These results suggest that the ketogenic diet alters the development of the condylar process and the angle of the mandible in rats, promoting a dolichofacial growth pattern.*

Keywords: *ketone bodies - bone development - mandible - craniofacial development.*

Estudio radiográfico de alteraciones mandibulares resultantes de una dieta cetogénica en ratas en crecimiento. Su importancia ortodóncica.

RESUMEN

Objetivo: *Este trabajo experimental analiza el desarrollo del cóndilo mandibular, y su posible impacto en la dirección del desplazamiento de la mandibula en el desarrollo maxilofacial en un modelo de dieta cetogénica en ratas en crecimiento.* ***Materiales y Método:*** *Veinte ratas Wistar macho destetadas a los veintiún días de edad se asignaron a dos grupos: control (dieta normal dura ad libitum) y experimental (dieta cetogénica dura ad libitum). Tras 30 días de dieta, se determinó la cetonemia y se sacrificaron los animales. Luego de resecar las mandíbulas, se separaron en la sínfisis, se fijaron en formol y se eliminaron tejidos blandos. Referencias metálicas fueron colocadas en los orificios mandibulares y a continuación se radiografiaron. El estudio cefalométrico se realizó en trazados de la imagen proyectada de las radiografías. Los datos se analizaron con la prueba "t" de Student.* ***Resultados:*** *El consumo de alimentos fue menor en el grupo experimental. El peso corporal fue en controles 179±1 g y 128±11 g en el grupo experimental. La cetonemia fue significativamente mayor en el grupo experimental (0,29 ± 0,042 vs. 1,89 ± 0,7 mmol/l). La longitud del proceso condilar y la convexidad del proceso angular fueron significativamente menores en el grupo experimental.* ***Conclusiones:*** *Estos resultados sugieren que la dieta cetogénica altera el desarrollo del proceso condilar y del ángulo mandibular en ratas, promoviendo un patrón de crecimiento dolicofacial.*

Palabras clave: *cuerpos cetónicos - desarrollo óseo - mandíbula - desarrollo cráneo facial.*

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INTRODUCTION

In humans[1] and rodents[2], it is well established that the anterior portion of Meckel's cartilage initially serves as a template for intramembranous ossification of the mandible, and is then completely resorbed. However, the posterior region of the condylar processes is made of accessory cartilage and in humans, begins endochondral ossification around the 20th week of development. This condylar cartilage is the main mandibular center of growth by endochondral ossification until approximately twenty years of age, and plays a key role in maxillofacial development[1].

Craniofacial development is a highly complex process in which the movement of the mandibular symphysis in relation to the rest of the face is of fundamental importance. It is well documented that in normal conditions, the gnation moves downward and forward along the Ricketts facial axis[3]. This type of growth axis is the result of vertical and sagittal displacement of the mandible[3], whose normal development is assumed to be a condition for orthognathic-type facial development.

Bones may be vulnerable to genetic and environmental factors. While genetic factors determine the shape and size of the head, face and dental arches[4], craniofacial development is also affected by external factors during growth. Studies have reported that certain habits, trauma and premature extraction of deciduous teeth alter the normal growth direction and reduce arch length, causing crowding[5].

Additionally, nutritional alterations that may occur during growth can affect bone development, causing marked variations in bone shape and size[6,7]. "Atkins-style" diets, characterized by low-carbohydrate and high-fat content (LC–HF), are usually promoted for weight loss. These diets encourage the intake of fat and protein, while limiting carbohydrates[8]. Ketogenic diet results from further restricting the protein content of LC–HF diets, and is an established treatment for epilepsy in children[9,10]. Although its efficacy in seizure control has been widely reported in the international literature, ketogenic diets have been reported to have several unfavorable metabolic effects, such as increased adiposity, glucose intolerance, higher serum leptin concentrations, and concurrent lean body mass loss in human and animal studies[11–15]. Additionally, diets with an altered relative abundance of fat, protein and carbohydrates affect bone metabolism, altering the skeletal system. These alterations could include disturbances in endochondral ossification[8], as well as loss of bone mineral density, bone volume, trabecular number, and biomechanical properties in long bones[16].However, the effects of ketogenic diet on growth, shape and displacement of the mandible during maxillofacial development have not yet been evaluated.

When well-defined ketogenic low-carbohydrate, high-fat (LC–HF) diets are used to treat epilepsy in growing children[9,10], it is evident that the mandible develops under the negative influence of this nutritional alteration. Considering the effects of the ketogenic diet on long bones, we hypothesize that this diet could impact mandibular growth and maxillofacial development.

Given that clinical trials are restricted, rats are widely used as experimental models, despite their many morphological and functional differences with humans. Rats can perform free lateral and antero-posterior movements of the mandible due to their diet. Consequently, their mandibular body develops in an anteroposterior direction and has a flat glenoid cavity that differs from the human glenoid cavity, which is deeply shaped by opening and closing movements. These mandibular dynamics result in vertical facial development[17].

Based on the above, the aim of the present experimental work is to assess the influence of a ketogenic diet on condylar process length and angular process convexity through radiographic evaluation.

MATERIALS AND METHOD

Animals

Twenty male Wistar rats, weaned at 21 days, were housed individually with water available ad libitum. Room temperature was maintained at 25ºC with a 12:12 h light-dark cycle.

The experimental procedures were carried out in accordance with The National Institutes of Health Guidelines for the Care and Use of Laboratory Animals (NHI publication 85-23 Rev. 1985). The ethics committee of the School of Dentistry of the National University of Tucuman approved the protocol according to RES - ODO - DCHCD - 13765 / 2025

Experimental design

The animals were assigned to one of the following groups: control group (n=10), fed a regular hard diet ad libitum, and experimental group (n=10), fed a ketogenic hard diet ad libitum (Table 1)[18]. Animal body weights and food consumed were recorded throughout the experimental period. Animals were euthanized after 30 days of consuming the diet, following international protocols. Once sedated with an intraperitoneal injection of 0.25 ml of a 5% ketamine solution (Holliday-Scott SA) at a dose of 40 mg/kg body weight, and 0.5 ml of a 1% acepromazine solution (Acedan, Holliday-Scott SA) at a dose of 160 mg/kg body weight, euthanasia was performed with an IP injection of 0.20 ml of 4% sodium pentobarbital and 0.5% sodium diphenylhydantoin (Euthanyle, Brouwer SA) at a dose of 160 mg/kg body weight. The mandibles were then carefully dissected and fixed in 10% formalin, after which the soft tissue was removed. In order to perform the radiographic study, the mandibles were hemisected at the symphysis. Metallic landmarks consisting of L-shaped 0.2mm steel ligature wires[19] were placed in the mandibular foramen of one hemimandible of each rat (Fig. 1). The marked hemimandibles were positioned laterally on the film next to a 10mm long wire and radiographed using standard X-ray equipment at 70Kv and 8mA, 0.3 sec exposure time, with focus to film distance 40 cm.

Table 1. Comparison between standard diet and ketogenic diet

Projected (per 100 g)	Standard diet	Ketogenic diet
Energy	1338 kJ	1748 kJ
Protein	14.5 g	6.1 g
Fat	4 g	37.1 g
Carbohydrates	55.5 g	3.1 g
Dietary Fiber	4.5 g	27.5 g
Natrium	130 mg	144 mg
Calcium	720 mg	117 mg
Phosphorus	600 mg	81 mg
Vitamin D	2.5 µg	0.03 µg

Displayed nutrients of ketogenic diet is less than 100g due to the loss in manufacture[18]

Biochemical determinations

The state of ketosis was verified at the end of the experiment, by visualizing ketonemia determined by colorimetric methods in blood samples obtained by cutting tail veins.

Radiographic determinations

Reference points were marked on paper tracings of the projected image of the radiographs (X7 magnification) in order to perform the radiographic measurements (Fig. 1). Radiographic reference points identifications, tracings and measurements were conducted by the same author. Linear mandibular dimensions measured on the lateral roentgenograph of the mandible are shown in Table 2[19]. The projected image of the wire served as a scale and the measurements were calibrated according to the image of the standard length of wire and expressed in millimeters.

Statistical Analysis

Data were analyzed using SPSS software for Windows (version 22.0, IBM Corp, Armonk, NY, USA). The normal distribution of continuous variables was assessed using the Shapiro-Wilk test. Student's "*t*" test was used to compare linear mandibular dimensions between control and experimental groups. Differences were considered significant with $p<0.05$.

RESULTS

Body weight and food consumed

Ketogenic diet led to a statistically significant weight reduction at the end of the experimental period (128±11gr) compared to controls (179±1 g) $p<0.05$.

Total food consumed per rat was: Control group 745 g/rat vs. Experimental group 335g/rat.

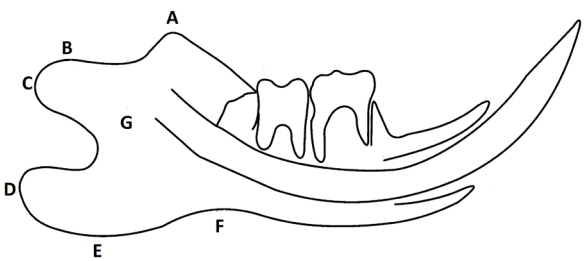

Fig. 1: Reference points defined on the lateral roentgenograph of the mandible, according to Kiliaridis S[19]. A: The most superior point of coronoid process. B: Fovea pterigoidea. C: The most posterior point of the condylar process. D: The most posterior point of the angular process of the mandible. E: The most inferior point of the lower border of the angular process F: The deepest point of the inferior border of the mandible. G: Position of markers in mandibular foramen.

Table 2. Variables measured on the lateral roentgenograph of the mandible.

Code	Description
A-G	Length of the coronoid process.
B-C	Length of the condylar head.
C-G	Length of the condylar process.
D-F	Length of the angular process.
E-DF	Convexity of the angular process. The shortest distance between point E and line DF.

The variables were defined between two points or between a point and a line. (According to Kiliaridis)[19].

Biochemical determinations

Ketonemia was significantly higher in the experimental group. Control group:0.29±0.042 mmol/l vs. Experimental: 1.89±0.7 mmol/l $p<0.05$.

Radiographic determinations

The cephalometric study showed that the condylar process and the convexity of the angular process were significantly lower in the experimental group (Table 3).

DISCUSSION

This study shows that ketogenic diet alters condylar process length (C-G) and angular process convexity (E-DF) in mandibles of growing rats. This is the first study to report mandibular disorders with this diet.

Ketogenic diet negatively influences longitudinal long bone growth in patients with intractable epilepsy[6,20,21]. In this context, it should be noted that histomorphometric studies have reported that this type of diet alters endochondral ossification in children[8,14,16]. Against this background, the growth of the mandibular condylar process that develops through endochondral ossification would also be affected, which would also have a negative impact on normal maxillofacial development.

In rats, the consistency of the diet affects mandibular growth[22], so it is important to emphasize that in the present study, both the control and the ketogenic diets were administered in the form of hard pellets. Although there is a publication[23] reporting that the ketogenic diet in mice does not induce any difference in muscle contraction strength; it should be noted that it was not performed on masticatory muscles. Thus, it is unlikely that the alterations observed in the present study are due to mechanical stimuli, to which the mandible is very sensitive.

Table 3. Radiographic determinations

	Control: Standard Diet	Experimental: Ketogenic Diet	p value
A-G	4.5 ±0.7	4.8 ±0.7	> 0.05
B-C	1.8±0.1	1.6±0.6	> 0.05
C-G	5.2±0.1	4.6±0.4	< 0.05*
D-F	8.8±1	7.5±0.6	> 0.05
E-DF	6.9±0.02	5.6±0.3	< 0.05*

X±SD. Measurements in millimeters. *Student's t test

It has been clearly demonstrated that, in an orthognathic growth pattern, the descent of the glenoid fossae and growth of the condylar process counterbalance the total vertical downward movement of the upper jaw and upward movement of the lower alveolar process[3, 24]. Clearly, if the vertical development of the maxilla and inferior alveolar processes is less than that of the glenoid fossae and condyles, the mandibular symphysis will predominantly move forward[3, 24]. However, if the vertical development of the maxilla and alveolar processes exceeds the contribution of the condyles, the symphysis will move exclusively downwards, thereby opening up the Rickets facial axis[3, 24].

According to the results obtained in this study, the lack of development of the condylar process in animals fed on a ketogenic diet could alter the normal pattern of facial growth. A significant decrease in the size of the condylar process could lead to a loss of vertical control. This would alter the spatial position of the mandible in relation to the base of the skull, creating ideal conditions for a growth pattern characterized by a downward and backward displacement of the mandibular symphysis and a posterior rotation of the mandible. This could result in dolichofacial growth. Such an alteration to the growth pattern caused by a ketogenic diet could also result in an increased angle to the base of the skull and a downward inclination of the occlusal plane. Under these conditions, it would be plausible to posit that the lower incisors would drift upwards and forwards in order to compensate for the posterior rotation of the mandible, thereby determining a pronounced occlusal curve. On the other hand, the lack of condylar growth likely resulted in premature contact in the molar area. Therefore, an anterior open bite may be a consequence of these nutritional alterations. Furthermore, the significant differences in convexity of the angular process (E-DF) described herein reveal a lack of angular

process development. It is well known that a lack of angular process development clinically results in an increased gonial angle. These conditions lead to the occlusal plane inclining logically towards posterior displacement of the mandibular symphysis. This pattern of mandibular development is characteristic of individuals with dolichofacial growth[3]. However, extrapolation of these results to humans should be done with caution.

The results of this study suggest that the ketogenic diet can alter mandibular and angular processes, affecting maxillofacial development, a condition that could be exacerbated if it coexists with oral habits or parafunctions. As the ketogenic diet is effective for controlling seizures in children with epilepsy, it cannot be discontinued. In this context, it is recommended that patients consult an orthodontist with the aim of using interceptive orthodontics to prevent or minimize the negative effects of the ketogenic diet on facial development.

CONFLICT OF INTEREST

The authors declare no potential conflicts of interest regarding the research, authorship, and/or publication of this article.

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Effects of resveratrol-enriched endodontic sealants on pulp stem cell viability and collagen production

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ABSTRACT

Root canal sealers should provide effective sealing while remaining biocompatible with periapical tissues. Strategies to further improve their biological performance are desirable. Resveratrol, a bioactive polyphenol with antioxidant and regenerative properties, may enhance cellular responses and tissue repair, representing a promising adjunct for endodontic materials. ***Aim:*** *The aim of this study was to evaluate cell viability and type I collagen expression in undifferentiated mesenchymal stem cell cultures exposed to EndoSequence BC bioceramic or AH Plus resin-based endodontic cements, with or without the addition of resveratrol.* ***Materials and Method:*** *Cement specimens were prepared in standardized molds (5 mm × 2 mm), allowed to set for 48 hours, sterilized, and conditioned in culture medium at a concentration of 0.2 g/mL at 37 °C. Resveratrol was added to the culture media at concentrations of 0.25 µM and 0.5 µM. The mesenchymal cell cultures were exposed to the different experimental conditions for 24, 48 and 72 hours, after which viability was assessed by MTT assay, and type I collagen secretion was quantified using the enzyme-linked immunosorbent assay (ELISA).* ***Results:*** *EndoSequence BC demonstrated significantly greater cell viability than AH Plus ($p<0.05$). At 48 hours, AH Plus combined with 0.5 µM resveratrol showed significantly greater cell viability than the cement alone and the 0.25 µM resveratrol group ($p<0.05$). In addition, supplementation with 0.5 µM resveratrol significantly increased type I collagen secretion for both cements compared to the corresponding groups without resveratrol ($p<0.05$), indicating a positive effect on extracellular matrix production.* ***Conclusions:*** *These findings suggest that resveratrol is a promising additive for improving the bioactivity of endodontic cements, enhancing collagen production and potentially contributing to tissue regeneration and bone repair.*

Keywords: *bioceramic cement - resveratrol - mesenchymal stem cells - collagen*

Efeitos de selantes endodônticos enriquecidos com resveratrol sobre a viabilidade de células-tronco pulpares e a produção de colágeno

RESUMO

Os cimentos endodônticos devem proporcionar um selamento eficaz, mantendo a biocompatibilidade com os tecidos periapicais. Estratégias para melhorar ainda mais seu desempenho biológico são desejáveis. O resveratrol, um polifenol bioativo com propriedades antioxidantes e regenerativas, pode potencializar as respostas celulares e o reparo tecidual, representando um adjuvante promissor para materiais endodônticos. ***Objetivo:*** *Este estudo teve como objetivo avaliar a viabilidade celular e a expressão de colágeno tipo I em culturas de células-tronco mesenquimais indiferenciadas expostas aos cimentos endodônticos biocerâmico EndoSequence BC e resinoso AH Plus, com ou sem a adição de resveratrol.* ***Materiais e Método:*** *Os cimentos foram preparados em moldes padronizados (5 mm × 2 mm), mantidos para presa por 48 horas, esterilizados e condicionados em meio de cultura na concentração de 0,2 g/mL a 37°C. O resveratrol foi adicionado ao meio de cultura nas concentrações de 0,25 µM e 0,5 µM. A viabilidade celular foi avaliada pelo ensaio de MTT após a exposição às diferentes condições experimentais, e a secreção de colágeno tipo I foi quantificada por meio do ensaio imunoenzimático (ELISA).* ***Resultados:*** *O EndoSequence BC apresentou viabilidade celular significativamente maior que o AH Plus ($p<0,05$). Em 48 horas, o AH Plus combinado com 0,5 µM de resveratrol apresentou viabilidade celular significativamente maior do que o cimento isolado e do que o grupo com 0,25 µM de resveratrol ($p<0,05$). Além disso, a suplementação com 0,5 µM de resveratrol aumentou significativamente a secreção de colágeno tipo I para ambos os cimentos em comparação com os respectivos grupos sem resveratrol ($p<0,05$), indicando um efeito positivo na produção da matriz extracelular.* ***Conclusões:*** *Esses achados sugerem que o resveratrol é um aditivo promissor para melhorar a bioatividade dos cimentos endodônticos, aumentando a produção de colágeno e potencialmente contribuindo para a regeneração tecidual e o reparo ósseo.*

Palavras-chave: *cimento biocerâmico - resveratrol - células tronco mesenquimais - colágeno*

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INTRODUCTION

During endodontic treatment, the root canal system is disinfected chemically and mechanically, and then filled to ensure adequate seal[1]. Properly performed endodontic treatment effectively reduces the bacterial load, thereby minimizing the risk of periapical lesions[2]. Periapical lesions of endodontic origin arise from an inflammatory response to microorganisms located around the root and within the root canal[3].

The most frequently used materials for root canal obturation are gutta-percha and root canal cements[4,5]. These materials must have dimensional stability, biocompatibility, and favorable handling properties[6]. An ideal root canal filling material should promote periradicular tissue regeneration, including cementogenesis on its surface, ensure an adequate seal, and be well tolerated by periapical tissues[7,8].

During conventional restorative techniques, vertical compression may cause extrusion of small amounts of restorative cement through the apical foramen and/or secondary canals into the surrounding tissues[9], harming periapical tissues and inducing inflammation[10]. Although extrusion generally does not compromise the success of endodontic treatment, it may delay the repair process[9]. Moreover, even in the absence of extrusion, these materials may release soluble substances that can be toxic to periapical tissues, affecting local bone metabolism and the healing process. It is therefore important that these cements should be highly biocompatible and not cause adverse or contact reactions in biological tissues[11].

A wide range of endodontic cement compositions is available for root canal obturation, including zinc oxide-eugenol, epoxy resin, and calcium silicate-based sealants[12-14]. Resin-based or resin-containing sealants and cements are often cytotoxic to various cell subpopulations[15]. In contrast, calcium silicate-based cements are characterized by adequate biocompatibility and bioactive properties, demonstrating non-cytotoxic behavior when in contact with periradicular tissues[16]. These cements promote the formation of a hydroxyapatite layer on their surface[17], which facilitates the creation of a mineral bond with dentin tissue[18].

AH Plus (De Trey-Dentsply, Konstanz, Germany) is an epoxy resin-based cement, widely recognized as the gold standard among endodontic cements due to its excellent physicochemical properties, despite lacking bioactive potential[16]. Endosequence BC Sealer (BCS) cement (Brasseler USA, Savannah, GA, USA) is a ready-to-use, premixed, injectable calcium silicate-based material designed for root canal filling and sealing[19], which has good physicochemical properties and cures in moist environments such as dentin tubules[20]. Resveratrol (trans-3,4,5-trihydroxystilbene) is a dietary antioxidant polyphenol found in various plants, including grapes, red wine, fruits, and peanuts. Over recent decades, it has gained significant attention for its potential to promote health and combat disease[21,22]. Resveratrol prevents ofatherosclerosis[22], and has cardioprotective and neuroprotective effects, as well as anticarcinogenic, antioxidant and photoprotective properties[22–24]. It also enhances collagen production which, in combination with its antioxidant effects, protects cells from oxidative damage caused by free radicals. The aim of this study was to evaluate cell viability and type 1 collagen secretion by adult mesenchymal stem cells exposed to an epoxy resin-based restorative cement and a bioceramic cement, both with and without the addition of resveratrol at different concentrations, at different time points.

MATERIALS AND METHOD

Undifferentiated mesenchymal cell culture

Immortalized stem cells derived from dental pulp were obtained from Lonza (USA, catalog #PT-5025). The cells were thawed and cultured in a growth medium composed of α-MEM supplemented with 10% fetal bovine serum (Gibco, USA), 50 µg/mL gentamicin, and 0.3 µg/mL fungizone. Cultures were maintained at 37 °C in a humidified atmosphere with 5% CO_2 and 95% atmospheric air. The medium was replaced three times per week. Upon reaching subconfluence, the culture medium was removed, and a solution containing 0.25% trypsin (Gibco, USA), 1.3 mg/mL type II collagenase (Gibco, USA), and 1 mM EDTA (Gibco, USA) was added to obtain a cell suspension for the subsequent experiments.

Experimental groups

The materials tested in this study were: EndoSequence BC Sealer (Brasseler, Savannah, Georgia, USA), AH Plus (De trey- Dentsply, Konstanz, Germany) and Resveratrol ≥ 99% (Sigma-Aldrich, St. Louis, MO, USA).

Cement specimens were prepared under aseptic conditions according to the manufacturer's instructions in compliance with ISO 10993-12 standards in inert silicone molds measuring 5 mm in diameter and 2 mm in height. They were placed in an oven for 48 hours to set, sterilized with ultraviolet light for 15 minutes[15], and stored in the culture medium to obtain eluates for the subsequent experiments.

Eluates were prepared following the ISO 10993-12 (International Organization for Standardization) guidelines, using a proportion of 0.2 g/mL by placing each cement specimen in a polypropylene tube (Eppendorf, Hauppauge, NY, USA) containing 1 mL of Dulbecco's Modified Eagle's Medium (DMEM, Nutricell Nutrientes Celulares, Campinas, SP, Brazil), and inclubating at 37°C for 24 hours in a humidified atmosphere with 5% CO_2 and 95% atmospheric air. After incubation, the culture medium was collected, filtered through a 0.22 µm filter, aliquoted, and stored at 4 °C. All experiments were performed in three independent biological replicates, with each condition analyzed in technical triplicate.

Resveratrol was dissolved in 1% dimethyl sulfoxide (DMSO) and added to the eluate at concentrations of 0.25 µM and 0.5 µM. The experimental groups consisted of eluates obtained from:

- EndoSequence BC Sealer (ES),
- EndoSequence BC Sealer supplemented with 0.25 µM resveratrol (ES+R0.25 µM),
- EndoSequence BC Sealer supplemented with 0.5 µM resveratrol (ES+R0.50 µM),
- AH Plus (AH),
- AH Plus supplemented with 0.25 µM resveratrol (AH+R0.25 µM), and
- AH Plus supplemented with 0.5 µM resveratrol (AH+R0.50 µM).

All groups were compared to a negative control group, which consisted of culture medium without the addition of resveratrol or cement eluates.

Cell viability assay

Cell cultures were seeded in 96-well polystyrene plates at a density of 110 cells/mm² and exposed to the prepared eluates. An MTT assay was conducted at 24, 48 and 72 hours after cell plating. Briefly, 10 µL of MTT solution (5 mg/mL; Sigma, USA) diluted in serum-free DMEM culture medium was added to the treated cultures, and incubated at 37 ºC for 3 hours. Following incubation, 100 µL of a 10% dimethyl sulfoxide (DMSO; LGC, São Paulo, Brazil) solution was added to solubilize the formazan crystals. Quantification was performed using an ELX800 microplate reader (Epoch Biotek Instruments, Inc.) at a wavelength of 590 nm to determine the optical density (OD) values.

Enzymatic immunoassay for type I collagen quantification (ELISA)

Cell cultures were seeded in 96-well polystyrene plates at a density of 110 cells/mm² and exposed to the prepared eluates. At 24, 48, and 72 hours, the culture supernatant was aspirated and centrifuged at 5000 × g for 15 minutes at 4 °C. Aliquots of each supernatant were analyzed according to the manufacturer's protocol (R&D Systems, USA) to determine type I collagen secretion by cells cultured under different conditions.

The reaction was finalized by adding 50 µL of 2N sulfuric acid (H_2SO_4) to the substrate solution in each well, and the resulting color intensity was measured using a spectrophotometer (Epoch, Biotek, Winooski, VT, USA) at a wavelength of 450 nm. Protein concentrations were expressed in picograms per milliliter (pg/mL).

Statistical analysis

Descriptive and exploratory analyses were initially conducted on the dataset. Variables were summarized using means, standard deviations, medians, and minimum and maximum values. Since the data did not satisfy the assumptions required for classical analysis of variance (ANOVA), generalized linear mixed models were employed to account for repeated measures over time. All statistical analyses were performed using R software, with significance level set at 5%.

RESULTS

The results of cell viability after exposure to the different eluates are presented in Table 1. When comparing the cements alone, EndoSequence cement displayed significantly higher cell viability than AH Plus at all evaluated time points (p<0.05). For EndoSequence cement, no significant increase in cell viability was observed when associated with resveratrol at either concentration compared to the cement alone (p<0.05).

In contrast, for AH Plus cement, no significant

Table 1. Quantification of cell viability (MTT) at different conditions. Data presented as mean (standard deviation) and median (minimum and maximum value) expressed in Arbitrary Units (AU).

Group	Time (hours)					
	24		48		72	
	Mean (standard deviation)	Median (minimum and maximum value)	Mean (standard deviation)	Median (minimum and maximum value)	Mean (standard deviation)	Median (minimum and maximum value)
Control	0.633 (0.052) Ba	0.608 (0.599; 0.693)	0.629 (0.070) Ba	0.662 (0.549; 0.677)	0.846 (0.042) Aa	0.836 (0.810; 0.892)
ES	0.600 (0.087) Aa	0.572 (0.530; 0.698)	0.528 (0.100) Aa	0.567 (0.414; 0.602)	0.569 (0.077) Ab	0.596 (0.483; 0.629)
ES+R0.25μM	0.457 (0.033) Bb	0.474 (0.419; 0,477)	0.588 (0.054) Aa	0.599 (0.529; 0.635)	0.628 (0.057) Ab	0.656 (0.562; 0.665)
ES+R0.5μM	0.434 (0.029) Bbc	0.425 (0.41; 0.466)	0.599 (0.012) Aa	0.594 (0.590; 0.612)	0.523 (0.015) ABb	0.517 (0.513; 0.540)
AH	0.365 (0.035) Ac	0.377 (0.325; 0.392)	0.364 (0.070) Ab	0.334 (0.314; 0.444)	0.395 (0.066) Ac	0.365 (0.350; 0.471)
AH+R0.25μM	0.434 (0.082) Abc	0.480 (0.340; 0.483)	0.345 (0.019) Bb	0.347 (0.326; 0.363)	0.281 (0.022) Cd	0.272 (0.266; 0.306)
AH+R0.5μM	0.611 (0.063) Aa	0.586 (0.564; 0.683)	0.627 (0.067) Aa	0.643 (0.554; 0.685)	0.534 (0.082) Ab	0.537 (0.451; 0.615)

Caption: p(group) < 0.0001; p(time)= 0.7913; p(interaction) < 0.0001. Different letters (uppercase horizontally and lowercase vertically) indicate statistically significant differences ($p < 0.05$); ES: Endo Sequence; AH: AH Plus; R: Resveratrol; Control: culture medium without resveratrol or cement eluates.

increase in cell viability was observed at 24 hours when associated with resveratrol at either concentration compared to the cement alone. At 72 hours, the AH+R0.25 μM group showed lower cell viability than the cement-only group. At 48 hours, however, a significant increase in cell viability was observed when AH Plus was combined with resveratrol at a concentration of 0.5 μM, compared to either the cement alone or the lower concentration of resveratrol.

The results of collagen I secretion are presented in Table 2. Significantly higher levels of collagen I expression were observed when cells were cultured on basal medium without resveratrol or cement eluates (control) compared to all other groups (p<0.05).

For both cements, the incorporation of resveratrol at a concentration of 0.5 μM resulted in significantly higher levels of collagen I secretion compared to the absence of the polyphenol (p<0.05) across all evaluated time points.

Table 2. Quantification of type I collagen secreted by undifferentiated mesenchymal cell culture under different conditions. Data presented as mean (standard deviation) and median (minimum and maximum value) expressed in pg/ml.

Group	Time (hours)					
	24		48		72	
	Mean (standard deviation)	Median (minimum and maximum value)	Mean (standard deviation)	Median (minimum and maximum value)	Mean (standard deviation)	Median (minimum and maximum value)
Control	1532.085 (265.612) Aa	1537.323 (1263.893; 1795.039)	1849.156 (434.385) Aa	2099.897 (1347.572; 2100.000)	1730.348 (276.842) Aa	1573.073 (1567.966; 2050.004)
ES	255.032 (30.296) Bc	271.532 (220.068; 273.496)	166.519 (83.566) Ccd	120.928 (115.664; 262.964)	453.272 (64.915) Ac	428.676 (404.249; 526.89)
ES+R0.25μM	335.306 (43.44) Ac	321.818 (300.211; 383.890)	194.27 (19.287) Bc	188.639 (178.425; 215.746)	415.413 (52.232) Ac	387.425 (383.139; 475.675)
ES+R0.5μM	495.331 (52.217) Ab	504.104 (439.283; 542.605)	509.343 (86.376) Ab	489.569 (434.568; 603.891)	600.617 (109.376) Ab	643.962 (476.212; 681.677)
AH	190.975 (15.517) Ad	182.332 (181.703; 208.889)	193.179 (28.348) Ac	209.086 (160.450; 210.000)	194.836 (10.881) Ad	198.164 (182.68; 203.664)
AH+R0.25μM	124.865 (11.211) Be	124.21 (113.996; 136.389)	143.722 (29.755) Bd	128.139 (124.996; 178.032)	239.27 (32.171) Ad	242.674 (205.532; 269.603)
AH+R0.5μM	443.485 (34.097) Bb	430.64 (417.676; 482.139)	415.413 (47.69) Bb	409.853 (370.746; 465.639)	594.449 (36.233) Ab	578.211 (569.175; 635.961)

Caption: p(group) < 0.0001; p(time) < 0.0001; p(interaction) < 0.0001; Different letters (uppercase horizontally and lowercase vertically) indicate statistically significant differences (p<0.05); ES: Endo Sequence; AH: AH Plus; R: Resveratrol; Control: culture medium without resveratrol or cement eluates.

DISCUSSION

This study investigated the viability and type I collagen expression of undifferentiated mesenchymal cells following exposure to EndoSequence BC bioceramic cement and AH Plus resin-based cement, with and without the incorporation of resveratrol at varying concentrations and exposure times.

The findings revealed that EndoSequence cement consistently promoted greater cell viability than AH Plus across all evaluated time points. For EndoSequence cement, the addition of resveratrol at different concentrations did not enhance cell viability relative to the cement alone. For AH Plus, the addition of resveratrol did not improve cell viability at 24 hours compared with the cement alone. At 72 hours, the lower concentration of resveratrol (0.25 μM) resulted in reduced cell viability, whereas the higher concentration (0.5 μM) improved cell viability. Notably, at 48 hours, the association of AH Plus with 0.5 μM resveratrol resulted in significantly higher cell viability compared to the cement alone or to the lower resveratrol concentration.

The potential toxicity of restorative materials has been a key concern in endodontic research since they were first used in 1962[8]. *In vitro* studies have demonstrated that materials exhibiting high toxicity in cell cultures are likely to exhibit similar toxicity when in contact with living tissues[25]. This makes *in vitro* testing a crucial step in assessing biocompatibility and cytotoxicity, providing essential information before progressing to *in vivo* evaluations.

Endodontic cements used during the obturation phase have biological properties that can influence their cytotoxicity and potential for leakage through the apical and secondary foramina during root canal treatment. Such leakage can vary in amount and may harm periapical tissues, potentially causing inflammation[10,13]. Therefore, these cements must have adequate biocompatibility, i.e., they should not elicit adverse reactions or undesirable responses from the surrounding biological tissues[14].

In the present study, the comparison between the isolated cements revealed consistently lower cell viability in cells exposed to AH Plus cement compared to EndoSequence BC Sealer at all evaluated time points. These findings are consistent with previous studies reporting greater biocompatibility and bioactivity of EndoSequence BC Sealer than AH Plus[15,26]. This behavior may be related to the calcium ion release and alkaline pH promoted by EndoSequence BC Sealer[20,27], which foster cell viability and mineralization potential[28]. In contrast, the lower cell viability observed for AH Plus may be associated with the release of residual cytotoxic components, including trace amounts of formaldehyde and unreacted resin compounds, even after setting[29].

For EndoSequence cement, different concentrations of resveratrol did not increase cell viability. Although previous studies have found EndoSequence BC Sealer to have lower cytotoxicity and genotoxicity than AH Plus, the present findings suggest that the addition of resveratrol did not further enhance the already favorable biological behavior of the bioceramic sealer under the evaluated conditions[30]. Moreover, other studies have shown that resin-based or resin-containing cements such as AH Plus often exert cytotoxic effects on various cell subpopulations[15]. In contrast, calcium silicate-based cements, including EndoSequence, are generally recognized for their adequate biocompatibility and bioactive properties, with no cytotoxicity when in contact with periradicular tissues. This discrepancy highlights the complex interactions between cement composition, experimental conditions, and cellular responses[16].

The results for AH Plus cement showed that the addition of resveratrol did not increase cell viability at 24 hours compared to the cement alone. At 72 hours, the lower concentration of resveratrol (0.25 μM) resulted in reduced cell viability, whereas the higher concentration (0.5 μM) improved cell viability. Notably, at 48 hours, the addition of 0.5 μM resveratrol significantly enhanced cell viability compared to the cement alone or to the lower resveratrol concentration. These findings suggest that the association of resveratrol with AH Plus may promote cell viability in a concentration-dependent manner, particularly at 0.5 μM.

The expression of type I collagen was also evaluated in undifferentiated mesenchymal cells after exposure to EndoSequence BC bioceramic filling cement and AH Plus resin-based cement. Type I collagen is a key structural protein in the extracellular matrix of bone tissue and serves as a chemotactic agent for critical repair cells. It plays a fundamental role in bone tissue formation and is a significant marker indicating the initiation of the mineralization phase[31,32]. The results showed significantly higher

levels of collagen I expression when cells were cultured on basal medium without resveratrol or cement eluates (control) compared to all other groups. For both cements, the incorporation of 0.5 μM resveratrol consistently resulted in increased collagen I secretion compared to the cements without the polyphenol at all evaluated time points. Resveratrol exhibits an affinity for estrogenic protein receptors found on fibroblasts and macrophages, which are key cells involved in the production of type I and type II collagen[33]. By interacting with these receptors, resveratrol may enhance their activity, thereby promoting the synthesis of these collagen types.

CONCLUSIONS

Based on the findings for cell viability and type I collagen secretion, it can be concluded that incorporating resveratrol into the tested cements enhanced type I collagen production without adversely affecting their cytotoxicity. However, as this is an *in vitro* study, certain limitations must be acknowledged, particularly regarding the extrapolation of these results to clinical practice. Despite being conducted on isolated cells, this study provides promising insights into improving type I collagen production, which may support bone repair in periapical lesions and potentially reduce the cytotoxicity of the evaluated endodontic cements.

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CONFLICT OF INTEREST

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Comparative Analysis of Graph Attention and Graph Convolutional Networks for Predicting Candidate SNP–miRNA–Drug Interactions in Integrated Regulatory Datasets

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ABSTRACT

Complex genetic and epigenetic factors, such as single-nucleotide polymorphisms (SNPs) that influence the function of microRNAs (miRNAs), are involved in periodontal inflammation and tissue regeneration. However, computational approaches for predicting potential SNP–miRNA interactions that may influence inflammatory signaling and drug responses remain limited in precision periodontology. ***Aim:*** *To implement and compare two graph neural network–based machine learning models—Graph Attention Network (GAT) and Graph Convolutional Network (GCN)— for predicting potential SNP–miRNA–drug interactions that may be relevant to inflammatory and regenerative pathways associated with periodontal disease.* ***Materials and Method:*** *SNPs found in miRNA precursor and target regions associated with immune and drug-response genes were included in three selected miRNA SNP v3.0 database datasets. After preprocessing, composite vectors were created by early fusion of heterogeneous features. Principal component analysis (PCA) was then used to normalize and reduce the composite vectors. Using matched hyperparameters, the GAT and GCN models were built in PyTorch Geometric and trained with cross-entropy loss. Regression metrics (RMSE, MAE, R2) and classification metrics (accuracy, precision, recall, F1-score, ROC-AUC, PR-AUC) were used to evaluate performance.* ***Results:*** *With a perfect recall (1.0), F1-score of 0.666, and PR-AUC of 0.750, GAT performed better in classification than GCN, demonstrating higher sensitivity for identifying candidate interactions within the integrated dataset. Although R^2 values were close to zero, indicating limited variance explanation, regression performance was comparable for both models (RMSE ≈ 0.524). Visual diagnostics showed that GAT maintained more prediction variability, while GCN predictions collapsed around mean values.* ***Conclusions:*** *Because of its attention-based architecture, GAT showed improved performance in classification metrics compared with GCN, suggesting potential advantages for prioritizing candidate SNP–miRNA–drug interactions within integrated regulatory datasets. However, the limited predictive performance observed indicates that these findings should be interpreted primarily as a comparative computational evaluation rather than as direct biological evidence. Future studies incorporating periodontal-specific molecular datasets and experimentally validated interactions will be necessary to improve biological interpretability and translational relevance.*

Key words: *periodontitis - polymorphism - single nucleotide - microRNAs - machine learning*

Análisis comparativo de redes de atención de grafos y redes convolucionales de grafos para predecir interacciones candidatas entre SNP, miRNA y fármacos en conjuntos de datos regulatorios integrados

RESUMEN

Factores genéticos y epigenéticos complejos, como los polimorfismos de nucleótido único (SNPs) que influyen en la función de los microARNs (miRNAs), participan en la inflamación periodontal y la regeneración tisular. Sin embargo, en el marco de la periodoncia de precisión siguen siendo limitados los enfoques computacionales para predecir interacciones potenciales SNP–miRNA que podrían influir en la señalización inflamatoria y en las respuestas a fármacos. ***Objetivo:*** *Implementar y comparar dos modelos de aprendizaje automático basados en redes neuronales de grafos—Graph Attention Network (GAT) y Graph Convolutional Network (GCN)— para predecir posibles interacciones SNP–miRNA–fármaco que podrían ser relevantes para las vías inflamatorias y regenerativas asociadas con la enfermedad periodontal.* ***Materiales y Método****: Se incluyeron SNPs ubicados en regiones precursoras de miRNA y en regiones diana, asociados con genes vinculados a respuesta inmune y respuesta a fármacos, a partir de tres conjuntos de datos seleccionados de la base miRNA SNP v3.0. Tras el preprocesamiento, se construyeron vectores compuestos mediante fusión temprana de características heterogéneas. Posteriormente, se aplicó análisis de componentes principales (PCA) para normalizar y reducir la dimensionalidad. Los modelos GAT y GCN se implementaron en PyTorch Geometric, se entrenaron con pérdida de entropía cruzada y se evaluaron usando métricas de regresión (RMSE, MAE, R^2) y de clasificación (exactitud, precisión, sensibilidad, F1-score, ROC-AUC, PR-AUC).* ***Resultados****: Con una sensibilidad perfecta (1,0), una puntuación F1 de 0,666 y un área bajo la curva precisión–recobrado (PR-AUC) de 0,750, el modelo GAT superó al modelo GCN en el desempeño de clasificación, mostrando una mayor sensibilidad para la identificación de interacciones candidatas dentro del conjunto de datos regulatorio integrado. Aunque los valores de R^2 fueron cercanos a cero—lo que sugiere una explicación limitada de la varianza—el rendimiento en regresión fue comparable entre ambos modelos (RMSE ≈ 0,524). Los diagnósticos visuales evidenciaron que GAT conservó mayor variabilidad en las predicciones, mientras que las predicciones de GCN tendieron a concentrarse alrededor de valores medios.* ***Conclusiones:*** *Debido a su arquitectura basada en mecanismos de atención, el modelo GAT mostró un mejor desempeño en métricas de clasificación en comparación con GCN, lo que sugiere posibles ventajas para priorizar interacciones candidatas SNP–miRNA–fármacos dentro de conjuntos de datos regulatorios integrados. No obstante, los valores de R^2 cercanos a cero indican una capacidad limitada para modelar la fuerza continua de interacción, por lo que los resultados deben interpretarse principalmente como una evaluación computacional comparativa. Estos hallazgos destacan el potencial de los enfoques de aprendizaje automático basados en grafos para explorar interacciones regulatorias candidatas y sugieren la necesidad de futuros estudios que incorporen conjuntos de datos moleculares específicos de enfermedad, modelos más interpretables y arquitecturas híbridas para mejorar la relevancia biológica y la aplicabilidad translacional.*

Palabras claves: *periodontitis - polimorfismo de nucleótido único - microARNs - aprendizaje automático*

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INTRODUCTION

Chronic inflammatory diseases known as periodontal diseases majorly impact oral and systemic health because they cause tooth-supporting tissues to deteriorate gradually. Although regenerative therapies that aim to repair periodontal tissues, including bone, cementum, and connective tissue, have gained clinical traction, patient response variability remains a significant obstacle[1–3]. Small noncoding RNA molecules known as microRNAs (miRNAs) are essential for post-transcriptional regulation. They can alter gene regulation by binding to the 3′ untranslated region of mRNAs. Gene regulation may be modulated by genetic variations in miRNAs or target binding sites that impact miRNA biogenesis or target binding. Research has indicated that human diseases such as autoimmune disorders and breast cancer are linked to an increase in miRNA-related SNPs[4,5]. Understanding miRNA regulation and choosing functional variants depend on identifying and analyzing these miRNA-related SNPs. Although many resources have been created to find and examine miRNA-related SNPs, most mainly concentrate on humans. SNPs have become much more numerous, especially for animals. It is now crucial to have a thorough database that includes miRNA-related SNPs and their functional profiles across several species[3-5].

According to recent developments in precision periodontology, interindividual genetic variations, specifically single-nucleotide polymorphisms (SNPs) that impact regulatory noncoding RNAs like miRNAs, may be partially responsible for this variability. Important post-transcriptional modulators of gene expression are miRNAs. SNPs in miRNA sequences or their target locations can alter miRNA biogenesis, binding affinity, and ultimately, downstream gene expression linked to tissue remodeling and inflammation. The importance of these SNP–miRNA interactions in modifying drug response and periodontal tissue regeneration is becoming more widely acknowledged. The intricate interactions among genomic variations, miRNA regulation, and pharmacological response are still poorly understood in periodontal disease[6,7].

Graph Neural Networks (GNNs)[8,9] since the advent of graph-based deep learning, which encodes relational structures between entities like genes, miRNAs, SNPs, and medications, has demonstrated promise in modeling such complex biological systems. Graph Attention Networks (GATs) and Graph Convolutional Networks (GCNs), in particular, provide strong frameworks for learning informative node representations and capturing topological dependencies in biological networks. While GCNs aggregate neighborhood information based on graph Laplacians, GATs employ attention mechanisms to learn adaptive weights for neighboring nodes—an advantage in heterogeneous networks where regulatory influence varies across connections[10,11].

A systematic comparison of GCN and GAT architectures for predicting SNP–miRNA–drug interactions in periodontal inflammation and regeneration is still largely unexplored despite the growing use of GNNs in systems biology. Furthermore, not many integrated models capture the complexity of regulatory SNPs influencing miRNA-mediated gene control within pharmacogenomic landscapes pertinent to periodontal health. Instead, most studies concentrate either on genomic features or on drug-target prediction separately. GCNs and GATs[9,12] have been effectively used in earlier research to model intricate biological networks, such as multi-view attention-based regulatory networks, drug–target interactions, and miRNA–disease associations. However, their use in SNP-modulated post-transcriptional regulation in regenerative periodontology has not yet been investigated. This study addresses this gap by constructing a fused multi-modal dataset integrating SNPs in pre-miRNA and miRNA target regions with immune- and drug-response-related genes. We evaluate the performance of GAT and GCN models in both regression and classification settings to predict functionally relevant SNP–miRNA–drug interactions. Our goal is to identify critical regulatory variants that may influence individual responses to regenerative therapies and inflammatory modulation, offering a step toward more personalized and predictive periodontal care.

Therefore, this study aims to implement and compare two graph-based deep learning models—GAT and GCN—for predicting SNP–miRNA–drug interactions relevant to periodontal inflammation and tissue regeneration. By constructing a biologically curated fused dataset that integrates SNPs in pre-miRNA and target site regions with drug-responsive and inflammation-related genes,

we leverage the power of graph representation learning to model complex regulatory relationships. Specifically, we evaluate the models in regression and classification settings to assess their ability to prioritize SNPs that may modulate miRNA function and therapeutic response. Through this comparative framework, we seek to uncover novel regulatory insights and provide a foundation for precision-guided therapeutic strategies in periodontology.

MATERIALS AND METHOD

Data Source and Retrieval

The datasets used in this study came from the publicly available miRNA SNP v3.0 database[4], which provides comprehensive annotations of SNPs that affect miRNA function, such as interactions between miRNA target sites and miRNA biogenesis. The "utr_immune" dataset contains predicted SNPs within miRNA target sites located in the 3′ untranslated regions (3′UTRs) of immune-related genes; the "utr_drug_response" dataset, which includes SNPs within miRNA target sites of genes associated with pharmacogenomic responses; and the "DRV_premiRNA" dataset, which contains information on SNPs located within precursor miRNA (pre-miRNA) sequences that may have an impact on miRNA maturation, stability, or expression. It is important to note that the filtering strategy was designed to enrich the dataset with genes associated with inflammatory and regenerative processes, which are relevant to periodontal biology but not exclusive to periodontal disease. Therefore, the dataset should be interpreted as a general regulatory interaction resource rather than a periodontal-specific dataset. All three datasets were specifically retrieved in tab-separated values (TSV) format.

An illustration of the comprehensive workflow of this study, from data acquisition to final evaluation, is shown in Figure 1. The analysis began with the extraction of SNPs located in miRNA precursor and target regions from the miRNA SNP v3.0 database, specifically focusing on genes associated with immune response and drug metabolism. Following data collection, composite feature vectors were created through early fusion of heterogeneous features, which were then normalized using principal component analysis (PCA) and divided into training and validation datasets. For model development, we implemented both GAT and GCN architectures in PyTorch Geometric, ensuring consistent hyperparameters across both models. The evaluation phase employed rigorous metrics, including regression analysis (RMSE, R^2) and classification performance indicators (F1-score, ROC-AUC), ultimately revealing GAT's superior sensitivity in detecting biologically significant SNP-miRNA-drug interactions.

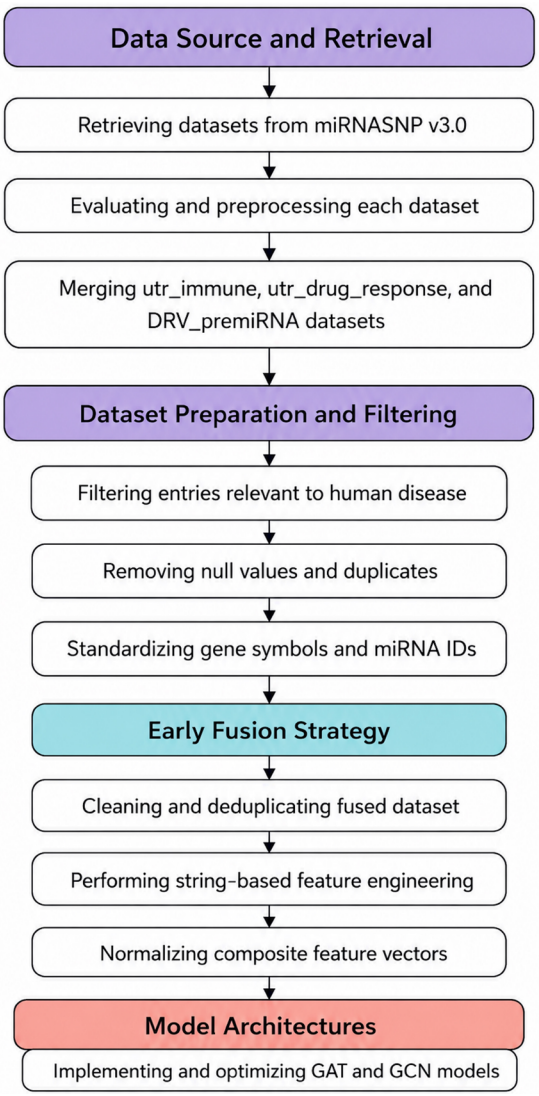

Fig. 1: Workflow of graph-based machine learning analysis of SNP–miRNA–drug interaction candidates using an integrated miRNA-SNP dataset.

Data Preprocessing and Quality Control

Before data preprocessing using Python (Pandas Library) standardized protocol, each dataset was independently assessed for formatting consistency, null values, and duplication. Column headers, including miRNA_ID, Gene_Symbol, SNP_ID,

and Chromosome_Position, were aligned across datasets to enable precise merging and downstream analysis. The analysis did not include records that lacked critical characteristics such as chromosomal coordinates, gene symbols, miRNA names, or SNP identifiers. To guarantee consistency across datasets, chromosome identifiers were standardized by eliminating the "chr" prefixes, and SNP positions were converted to integer formats.

Dataset Merging and Fusion Strategy

We first used key-based merging to combine the utr_immune and utr_drug_response datasets through an outer join using shared keys (miRNA_ID, Gene_Symbol, SNP_ID) to preserve unique features. This allowed us to integrate the SNP information about immune response, drug response, and miRNA biogenesis. The alignment of SNPs that affect both miRNA processing and target site binding was then made possible by integrating the DRV_premiRNA dataset using miRNA_ID as the linking field. Then, using distinct tuples of SNP_ID, miRNA_ID, and Gene_Symbol, we concentrated on deduplication and redundancy removal, locating and removing duplicate entries produced from shared SNP-miRNA mappings. To improve biological interpretation, we finally consolidated functional annotation by combining metadata from various datasets, such as predicted ΔΔG binding energy changes, seed-region mismatches, and drug/immune pathway associations, into enriched annotation fields.

Dataset Preparation and Filtering

The initial combined dataset consisted of 52,129 entries, which were then filtered using biologically significant terms like inflammation, cancer, regeneration, tumor, oncology, and healing to determine their relevance to human disease and tissue remodeling. These keywords were compared to miRNA associations, functional descriptors, and gene annotations. Several quality control procedures were used to guarantee data integrity: duplicate rows, particularly from overlapping annotations or redundant gene-SNP relationships, were eliminated to preserve uniqueness; entries with null values in essential fields were systematically removed; and final feature standardization was completed by harmonizing gene symbols to HGNC-approved nomenclature, normalizing miRNA IDs, and unifying column data types. The final curated and fused dataset is a thorough, highly reliable resource designed for AI-based computational modeling. It facilitates multi-domain analyses of how SNP variations affect the maturation of miRNAs and their downstream regulatory functions in pharmacogenomics, immune modulation, and disease-related regenerative pathways.

Early Fusion Strategy

Data Cleaning, Feature Engineering, and Dimensionality Reduction

The fused dataset underwent systematic preprocessing using the Pandas and Scikit-learn Python libraries to guarantee logical consistency and high-quality input for further analysis. After importing the original fused dataset from a fused_early_dataset.csv CSV file, several procedures were carried out to guarantee consistency and remove duplication. To start, a clean and legitimate subset was created by deleting entries in the early_fusion column that contained null or empty values. To enable consistent string comparisons, the leading and trailing whitespace in each early_fusion entry was removed using the.str.strip function. Additionally, redundant entries in the early_fusion column were found and eliminated using drop_duplicates to ensure a non-redundant data representation. To confirm the integrity of the transformation, a preview was printed after the cleaned dataset was saved as cleaned_fused_dataset.csv.

Feature Engineering via Early Fusion

String-based features from several columns were concatenated into a single composite column called early_fusion to incorporate heterogeneous features into a single vector representation. This method guarantees a linearized representation appropriate for numerical transformation while maintaining semantic dependencies among various modalities. A delimiter-based string merge was used to apply concatenation across the chosen feature columns.

Feature Normalization and Dimensionality Reduction

A high-dimensional feature matrix was then produced by vectorizing the early_fusion strings using tokenization and numerical encoding methods (such as TF-IDF or embedding-based models). The resultant feature vectors were normalized to unit length using L2 normalization before dimensionality

reduction to ensure scale invariance across features and prevent dominance by higher magnitude components. The normalized vectors were then subjected to Principal Component Analysis (PCA) to minimize dimensionality while maintaining maximum variance. Using cumulative variance thresholds (>90%), the principal components were chosen to preserve important signal properties while increasing computational efficiency and reducing noise. The input for ensuing machine learning tasks was this converted low-dimensional feature space.

The fused dataset was randomly partitioned into training and validation subsets using a standard data-splitting procedure implemented in the Scikit-learn Python library. The split preserved the distribution of positive and negative interaction labels to avoid class imbalance between training and validation sets.

Model Architectures

Graph Neural Network Model Architectures

We implemented and optimized two different GNN models, GAT and the GCN, to investigate graph-based feature representation learning on the fused dataset. Both models had similar architectural depths and hyperparameters to guarantee a controlled experimental evaluation. Model performance was evaluated using multiple classification metrics including accuracy, precision, recall, F1-score, ROC-AUC, and PR-AUC.

Configurations of the Model

The GAT model improves conventional graph learning by adding attention mechanisms that give neighbor contributions learnable weights. On the other hand, the GCN model uses a normalized graph Laplacian-based transformation to aggregate neighbor features. Both architectures used dropout for regularization and two hidden layers. PyTorch Geometric was used to implement all models, and the Adam optimizer was used for training. Table 1 provides a summary of the hyperparameters used for both models.

This standardized setup enables a robust comparison of attention-based versus convolution-based neighborhood aggregation mechanisms in graph learning tasks applied to SNP–miRNA–gene networks. Both models were trained for 200 epochs with early stopping based on validation loss.

Table 1. Architecture and Hyperparameters of GAT and GCN Models

Hyperparameter	GAT	GCN
Input Dimension	256	256
Hidden Layers	[128, 64]	[128, 64]
Attention Heads	4	—
Activation Function	LeakyReLU (α = 0.2)	ReLU
Dropout Rate	0.2	0.2
Optimizer	Adam	Adam
Learning Rate	0.001	0.001
Loss Function	Cross-Entropy	Cross-Entropy
Framework	PyTorch Geometric	PyTorch Geometric

RESULTS

Regression analysis aimed to estimate a continuous interaction score representing the predicted strength of SNP-modulated miRNA effects on drug-response pathways. When the GAT and GCN models are compared, it can be seen that both have moderate prediction error margins, roughly 36–38%. While GAT performs marginally better than GCN in mean absolute error, it performs worse in terms of MAPE. The root mean square error (RMSE), which is approximately 0.524, is almost the same for both models, indicating a steady distribution of residuals. Nevertheless, the R^2 values of each model are close to zero, indicating a poor fit for continuous outcome prediction, and demonstrating the model's incapacity to capture variance. In particular, GCN's marginally better R^2 (0.0002) suggests that it performs similarly to a mean predictor, whereas GAT's negative R^2 raises concerns about overfitting or inadequate generalization. More sophisticated modeling approaches, such as hybrid graph-transformers or knowledge-guided GNN models, are required, as these high error rates and nearly zero R^2 values highlight the complexity and nonlinear character of SNP-driven effects on miRNA function and drug interactions in periodontal regeneration.

A comparison of the training dynamics and predictive performance of GAT and Graph GCN models in analyzing SNP-miRNA-drug interactions for periodontal applications is shown in Figure 2. The left panel reveals GAT's (blue) superior optimization trajectory, starting with a lower initial loss (~2.3 vs. GCN's ~6.5) and stabilizing faster with minimal oscillations after just three epochs, while the right panel shows both models converging

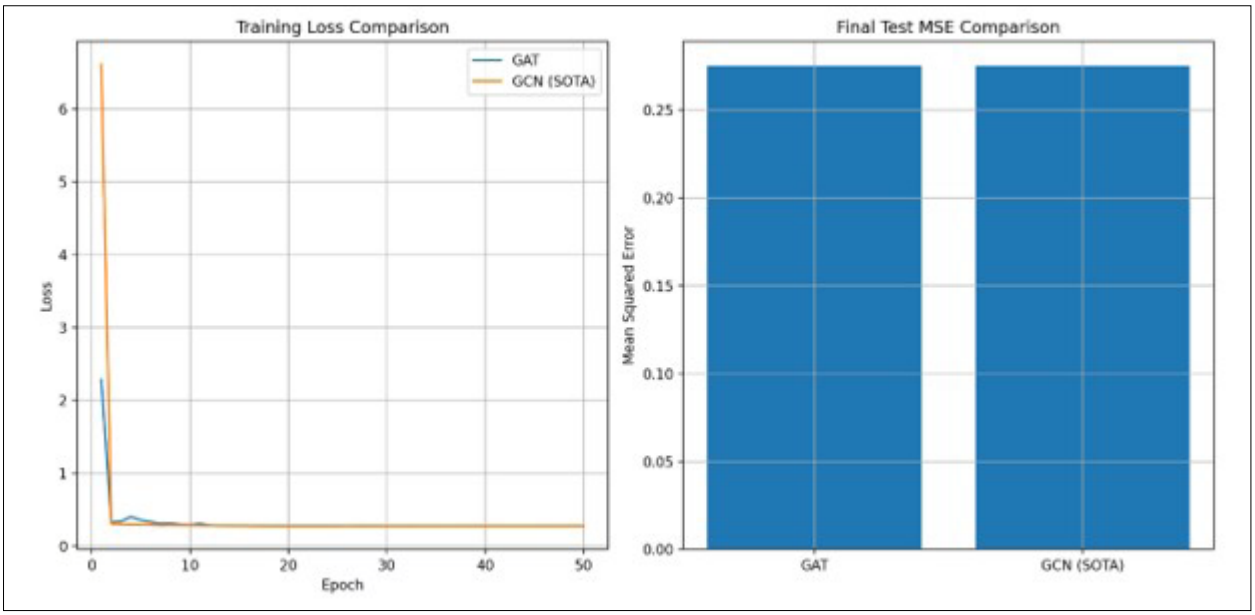

Fig. 2: Training dynamics and regression error comparison between GAT and GCN models on the fused SNP–miRNA dataset. Left panel: training loss trajectories for GAT and GCN across epochs. Right panel: test mean squared error (MSE) comparison after convergence. Although both models reached similar final regression error values, this analysis is intended to describe optimization behavior and comparative model performance on this dataset.

to similar test MSE values (~0.275). However, this apparent parity in regression accuracy masks critical differences: GAT maintains distinct advantages in biological interpretability and prediction variability despite matching GCN's (orange) final error metrics. The ability of the attention mechanism to capture nuanced feature relationships proves particularly valuable in this data-sparse biomedical context, where GCN's constrained expressivity limits its capacity to reveal subtle regulatory patterns. These findings suggest that the GAT architecture may provide advantages for identifying candidate SNP–miRNA–drug interactions in classification tasks. However, the limited regression performance observed in both models indicates that predicting continuous interaction strength remains challenging in this dataset.

The comparative prediction performance and error distributions between GAT and GCN models in analyzing SNP-miRNA-drug interactions relevant to periodontal regeneration are depicted in Figure 3. The top row features a residual plot highlighting GAT's superior performance, with well-dispersed predictions and smaller residuals, while GCN exhibits systematic underfitting, evidenced by large, uniform residuals due to its tendency toward flat predictions. The accompanying kernel density (KDE) plot of errors reveals a subtle right skew in both models, masking GCN's symmetric but biologically implausible error profile.

The middle row's scatter plot demonstrates GAT's ability to capture a broader range of target values with adaptive variability, contrasting sharply with GCN's tightly clustered predictions that confirm its prediction collapse. Supporting boxplots in the bottom row further underscore GCN's instability through higher variance and outlier susceptibility compared to GAT's more consistent predictions. This performance gap stems from GAT's attention mechanism, which dynamically weights node features to capture biologically meaningful signal variance – a capability fundamentally limited in GCN by its rigid aggregation strategy.

Together, these visualizations provide compelling evidence for GAT's superiority in modeling complex regulatory networks, establishing it as the preferred framework for precision applications in periodontal biology.

The 50-epoch training trajectories of GAT and GCN models are illustrated in Figure 4, revealing critical differences in their learning capabilities. The GCN model struggles initially with a significantly higher loss (≈6.5 vs. GAT's ≈2.2), reflecting its inherent challenges in processing heterogeneous fused graph

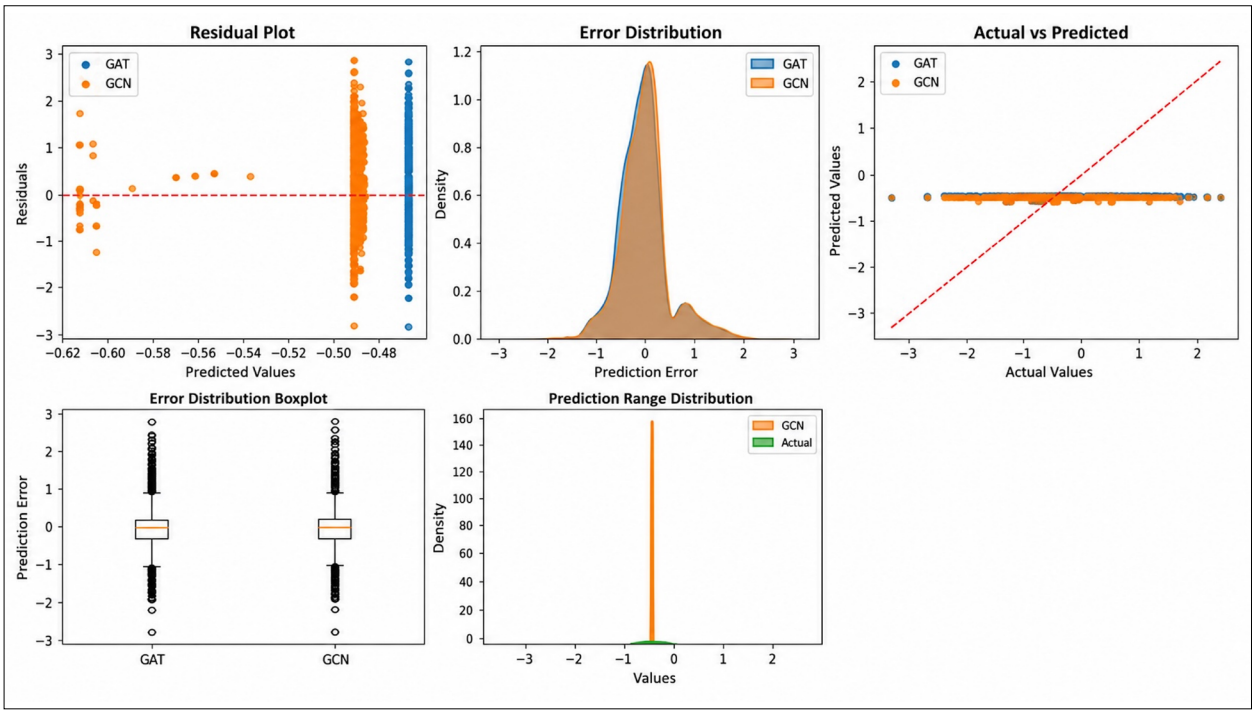

Fig 3: Prediction behavior and error distribution of GAT and GCN in regression analysis.
Panels show residual plots, error density distributions, predicted vs. observed scatter plots, and summary boxplots of errors for both models. The visual diagnostics indicate that GCN predictions tend to collapse toward central values (underfitting behavior), whereas GAT produces a wider spread of predictions. These plots complement the quantitative regression metrics, including near-zero R^2 values, and should be interpreted as model-behavior diagnostics on this dataset rather than as evidence of biological mechanism.

features. While both architectures demonstrate rapid loss reduction during early epochs - emphasizing the importance of initial training phases - their subsequent behaviors diverge markedly. GAT maintains a consistently lower loss trajectory, indicating superior feature adaptation through its attention mechanism, while GCN's flat convergence suggests architectural limitations in capturing complex biological relationships. Notably, neither model shows overfitting beyond epoch 5, but GAT's stable, lower loss plateau demonstrates its enhanced capacity for modeling intricate SNP–miRNA–drug interactions. These dynamics underscore GAT's advantages for periodontal regeneration research: its attention-based weighting excels at parsing high-dimensional biological graphs where GCN's rigid architecture falters, particularly when predicting context-dependent therapeutic targets in inflammatory and regenerative pathways. The figure ultimately positions GAT as the more robust framework for precision periodontology applications requiring nuanced biological inference.

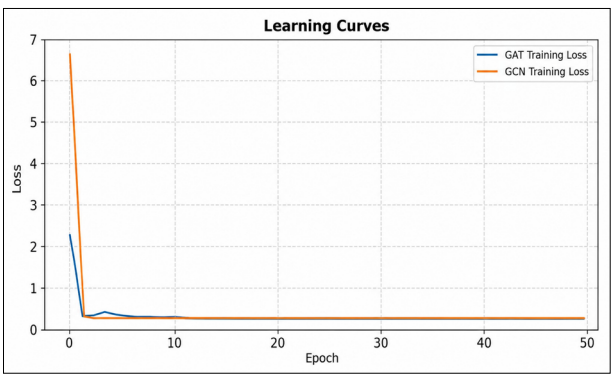

Fig. 4: Comparative training loss trajectories of GAT and GCN across 50 epochs.
The figure illustrates differences in optimization dynamics, with GAT showing a lower loss curve than GCN under matched hyperparameters and training conditions. The plot is presented to summarize training stability and convergence patterns in this comparative evaluation.

The predictive capabilities of GAT and GCN models in forecasting SNP–miRNA–drug interaction scores relevant to periodontal inflammation and tissue regeneration are compared in Figure 5. The left panel reveals GAT's strength through its unimodal, right-skewed distribution of predicted

values, which effectively captures the biological variability inherent in these interactions. In stark contrast, the middle panel exposes GCN's critical limitation - its predictions collapse around a single point, demonstrating severe underfitting that aligns with previous regression metrics. This fundamental shortcoming becomes even more apparent in the right panel's scatter plot, where GAT maintains a broad, biologically plausible range of predictions while GCN's outputs cluster narrowly, reflecting its inability to learn complex patterns. The comparative analysis highlights how GAT's attention mechanism successfully deciphers subtle regulatory effects on key pathways (e.g., Wnt and NF-κB) through adaptive weighting of node features, whereas GCN struggles with the heterogeneity of molecular interactions. While GAT emerges as clearly superior for this application, the figure also suggests that both models could benefit from further refinement to enhance their regression fidelity and biological interpretability for precision periodontology applications.

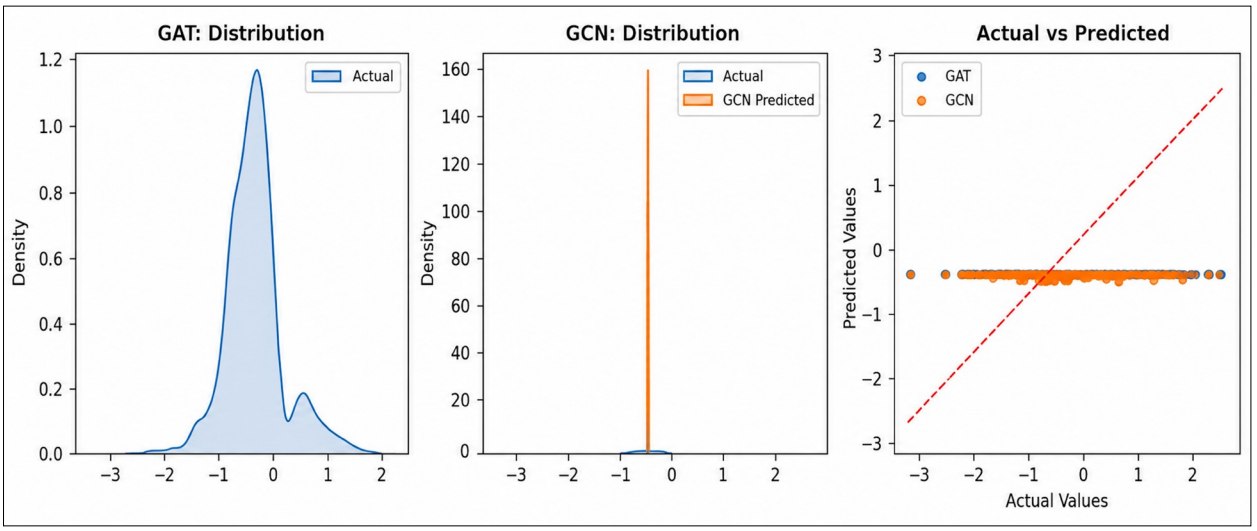

Fig. 5: Distribution of predicted interaction scores and prediction range comparison between GAT and GCN. Left and middle panels show the distributions of predicted scores for GAT and GCN, respectively. Right panel shows predicted vs. observed values, highlighting that GAT outputs span a broader range while GCN outputs are more clustered. These visualizations provide qualitative support for underfitting patterns in GCN and should be interpreted alongside the reported regression metrics (including near-zero R^2) as evidence of limited regression fidelity on this dataset.

Classification Metrics Analysis

Binary classification was used to assess the functional relevance of SNP–miRNA–disease interactions as either neutral or disease-modulating in periodontal disease. While the GAT model had low accuracy and precision (0.499), leading to many false positives, it demonstrated perfect recall (1.0) and an F1-score of 0.666, indicating effective detection of positive cases. Nevertheless, a PR_AUC of 0.750 indicates reasonable performance in ranking relevant interactions. In contrast, the GCN model showed a low PR_AUC (0.461), a very low F1-score (0.118), and poor recall (0.070), underscoring its conservative nature and propensity to overlook important interactions. These results suggest that while GCN's fixed graph propagation rules restrict its ability to capture important biological associations, GAT, with its attention mechanism, better identifies SNP-miRNA–drug relationships relevant to inflammation and regenerative pathways, even in the face of more false positives. The error distributions in both models showed mild right skewness and sporadic high-magnitude underestimations, but their mean error was nearly zero, indicating no significant systematic bias. Accurately predicting miRNA-SNP interactions is crucial for individualized regenerative treatment because SNPs in pre-miRNA and mature miRNA regions can also significantly impact miRNA stability, drug sensitivity, and successful treatment pathways in periodontology. Even though both models showed shortcomings in regression performance, GAT's classification capabilities make it more suitable for giving clinically actionable SNP-miRNA-drug interactions priority in the

complex regulatory environment of periodontal inflammation and regeneration. This suggests that more advanced models and biologically informed priors are required to improve predictive accuracy in precision periodontology.

DISCUSSION

Different strengths and limitations are revealed by comparing the GAT and GCN models. GAT performs better than GCN in the classification domain, showing perfect recall (1.0) and a significantly higher F1-score (0.666 vs. 0.118), albeit at the expense of lower precision (0.499) (Figs 2-5). Because of its attention mechanism, which dynamically weighs node neighbors and captures subtle variations in graph topology—a crucial advantage in sparse biological networks—these findings imply that the GAT model is highly sensitive to detecting SNP–miRNA–drug associations. On the other hand, GCN, which is limited by fixed aggregation rules, exhibits a low F1-score and poor recall (0.070), suggesting an excessively cautious approach that overlooks biologically significant positive interactions. The present results suggest that GAT may offer advantages over GCN in classification-based prioritization of candidate SNP–miRNA–drug interactions. However, the limited regression performance observed in both models indicates that current architectures still face substantial challenges in accurately modeling continuous interaction strength. Furthermore, recent explainable GNN initiatives reflect the need for improved interpretability in SNP–miRNA–-drug modeling. Despite differences in classification performance, both models show similar regression metrics, with mean absolute errors (MAE) and root mean square error (RMSE) values circling 0.36 and 0.524, respectively. However, when predicting continuous interaction strength or influence scores, the R^2 values near zero (or slightly negative) indicate limited explanatory power. This limitation might result from the linearity assumptions of the regression loss function and the high biological heterogeneity inherent in SNP-miRNA-target interactions. These assumptions may fail to capture the intricate nonlinear dependencies involved in pharmacodynamic response and post-transcriptional regulation[9,12].

The error distribution statistics, such as skewness (~0.61) and kurtosis (~2.9), also reveal a slightly right-skewed and leptokurtic pattern, indicating the existence of sporadic large-magnitude prediction errors. Underrepresented SNPs or low-frequency miRNA variants that have disproportionate regulatory effects on inflammation and tissue remodeling, as observed in regenerative periodontology, may be the source of these errors. To enhance predictive performance in overcoming constraints, several future directions are proposed: integrating molecular fingerprints to provide contextual understanding of SNP-miRNA-drug interactions; employing multi-task learning to optimize classification and regression objectives[13–15]. simultaneously, utilizing deeper or hybrid architectures to capture complex dependencies in regulatory landscapes, applying transfer learning to improve model generalizability by fine-tuning on specific datasets, and implementing techniques for model interpretability, including attention visualizations and importance attribution, to derive biological insights about regulatory SNPs and miRNAs in periodontal disease and healing.

An important consideration is that the miRNA SNP v3.0 database is not specific to periodontal disease but represents a broad collection of SNP–miRNA regulatory interactions across multiple biological contexts. Consequently, although our filtering strategy focused on genes associated with inflammation, immune response, and tissue repair, these biological processes are not exclusive to periodontal pathology. The present study should therefore be interpreted as a computational framework for prioritizing candidate interactions that may be relevant to periodontal inflammation and regeneration, rather than as an analysis based on a periodontal-specific molecular dataset.

Notwithstanding the knowledge acquired, this study has several drawbacks[16,17].

First, even after being selected and screened for biological significance, the dataset size is still small enough to train deep learning models, which may restrict generalizability. Second, model performance may be impacted by possible problems with data quality, such as inconsistent annotations, redundant SNP-miRNA mappings, and uneven class distributions. Third, hyperparameter optimization and architecture experimentation were limited due to the models' limited computational resources during training[13,18,19]. Fourth, despite the structural advantages of GNNs, their interpretability is still limited, making it difficult to convert predictions

into molecular biology mechanistic hypotheses. Finally, the continuum of biological interactions, where SNP effects can differ in magnitude, context-dependence, and downstream phenotypic impact—is oversimplified by using binary classification[20–22].

Even though the GAT model shows promise in detecting SNP–miRNA–drug interactions associated with periodontal inflammation and regeneration, substantial improvements in data diversity, model complexity, and interpretability are required to fully utilize graph learning in precision periodontology.

Another limitation is that the dataset was derived from a general miRNA SNP database rather than a periodontal-specific molecular dataset. Although filtering strategies were applied to enrich inflammation- and regeneration-related genes, these biological processes may also be involved in other inflammatory diseases. Future studies should incorporate periodontal-specific molecular datasets, including markers such as MMP-8, MMP-9, and pathways associated with key periodontal pathogens such as *Porphyromonas gingivalis*.

The dataset fusion strategy represents an important methodological contribution of this study, as it integrates heterogeneous SNP–miRNA regulatory information and drug-response annotations into a unified analytical framework. However, the results obtained should be interpreted primarily as a computational proof-of-concept. Because the dataset is derived from a general regulatory database rather than a periodontal-specific molecular resource, the findings cannot be directly extrapolated to all periodontal biological contexts. Future studies incorporating periodontal-specific molecular markers and experimentally validated regulatory interactions will be necessary to strengthen biological interpretability.

Another limitation of this study is the absence of classical machine learning baseline models such as logistic regression or random classifiers. The present work focused primarily on comparing two graph neural network architectures applied to the same fused dataset. Future work should incorporate additional baseline models to better contextualize the predictive performance of graph-based approaches.

CONCLUSIONS

This study compares GAT and GCN to model SNP-modulated miRNA-drug response interactions potentially relevant to inflammatory and regenerative pathways associated with periodontal disease. Our results indicate that the GAT architecture achieved higher sensitivity in classification tasks compared with GCN when identifying candidate SNP–miRNA–drug interactions. However, the low R^2 values observed in regression analyses highlight the difficulty of accurately modeling continuous interaction strength in complex post-transcriptional regulatory systems. Even though both models performed similarly on regression tasks, their low predictive ability (shown by nearly zero R^2 values) highlights the difficulty involved in modeling post-transcriptional regulatory dynamics with existing graph-based architectures. Error statistics and performance metrics analysis provide valuable insights into model behavior provide preliminary evidence that graph neural network architectures can identify patterns within integrated SNP–miRNA–drug datasets despite biological heterogeneity and sparse data. However, the study also points out important drawbacks that limit the translational applicability of existing models, such as dataset scale, interpretability, and architectural depth.

Predictive performance may be greatly improved by incorporating domain-specific features like molecular fingerprints, implementing multi-task learning frameworks, and embracing more expressive and interpretable architectures. These findings highlight the potential of graph-based machine learning approaches for prioritizing candidate regulatory interactions within integrated SNP–miRNA datasets. However, given the general nature of the dataset and the limited predictive performance observed, the results should be interpreted primarily as a computational proof-of-concept. Future studies incorporating periodontal-specific molecular datasets and experimentally validated regulatory interactions will be required to strengthen biological interpretation and translational applicability in periodontology.

CONFLICT OF INTEREST

The authors declare no potential conflicts of interest regarding the research, authorship, and/or publication of this article.

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Compressive strength of endodontically treated mandibular premolars rehabilitated with three techniques: core buildup material, intraradicular fiber posts and polyethylene fibers

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ABSTRACT

The biomechanical rehabilitation of endodontically treated premolars with extensive mesio-occluso-distal (MOD) cavities is a clinical challenge due to their increased susceptibility to fracture. The aim of this in vitro study was to compare the compressive strength of endodontically treated mandibular premolars restored using different post-endodontic reconstruction protocols, using intact teeth as a reference for maximum mechanical performance. ***Materials and Method:*** *Forty single-rooted premolars were distributed into four groups: one control group with no interventions, and three experimental groups with standardized mesio-occluso-distal (MOD) preparations. Experimental groups were restored respectively with core buildup, glass fiber posts, and polyethylene fiber reinforcement. After final composite resin restoration, specimens were tested for compressive strength using an INSTRON 1011 universal testing machine. Data were analyzed using the Kruskal–Wallis test (p<0.05).* ***Results:*** *When examining median (95%CI) compressive strength in Newtons (N), the highest value was found in the control group with 557.4 N (100.8-574.8), followed by the polyethylene fiber group with 471.4 N (194.2 to 565.5), the glass fiber posts group with 427.5 N (146.3 to 495.4) and the core buildup group with 345.1 N (216.8 to 574.9), (p = 0.84).* ***Conclusion:*** *Under the experimental conditions of the present study, the compressive strength of endodontically treated mandibular premolars did not differ significantly among the evaluated reconstruction protocols. Although polyethylene fiber reinforcement showed a tendency toward higher resistance values, the results do not enable the establishment of conclusive mechanical advantages among the restorative systems assessed.*

Keywords: *bicuspid - root canal therapy - compressive strength - composite resins*

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Resistencia a la compresión de premolares endodónticamente tratados y rehabilitados con tres tipos de técnicas: sustituto dentinario, anclaje intrarradicular y refuerzo con fibras

RESUMEN

La rehabilitación biomecánica de premolares tratados endodónticamente con cavidades mesio ocluso distales (MOD) extensas continúa siendo un desafío clínico debido a su mayor susceptibilidad a la fractura. El objetivo de este estudio in vitro fue comparar la resistencia a la compresión de premolares mandibulares tratados endodónticamente y restaurados mediante diferentes protocolos de reconstrucción postendodóntica, utilizando dientes íntegros como referencia de máximo desempeño mecánico. ***Materiales y Método:*** *Se utilizaron 40 premolares unirradiculares divididos en cuatro grupos: un grupo control sin intervenciones y tres grupos experimentales con preparaciones estandarizadas de cavidades mesio ocluso distales (MOD). Los grupos experimentales incluyeron restauraciones con sustituto dentinario, postes de fibra de vidrio y refuerzo con fibras de polietileno. Tras la reconstrucción final con resina compuesta, las muestras se sometieron a pruebas de resistencia a la compresión en una máquina de ensayo universal INSTRON modelo 1011. Los datos se analizaron mediante la prueba de Kruskal-Wallis (p<0,05, significativo).* ***Resultados:*** *Al analizar la mediana (IC95%) de la resistencia a la compresión en Newtons (N), el valor más alto se observó en el grupo control 557,4 N (100,8-574,8), seguido del grupo de fibras de refuerzo 471,4 N (194,2 a 565,5), postes de fibra de vidrio 427,5 N (146,3 a 495,4) y grupo con sustituto dentinario 345,1 N (216,8 a 574,9), respectivamente (p = 0,84).* ***Conclusión:*** *Bajo las condiciones experimentales del presente estudio, la resistencia a la compresión de premolares inferiores tratados endodónticamente no difirió significativamente entre los distintos protocolos de reconstrucción evaluados. Aunque el refuerzo con fibras de polietileno mostró una tendencia a mayores valores de resistencia, los resultados no permiten establecer ventajas mecánicas concluyentes entre los sistemas restauradores analizados.*

Palabras clave: *bicúspide - tratamiento de conducto radicular - resistencia a la compresión - resinas compuestas*

INTRODUCTION

The choice of the ideal restoration for endodontically treated teeth (ETT) has been widely discussed in the dental literature[1]. The outcome of root canal treatment is known to be positively correlated with the technical quality of the root filling, while high failure rates are mainly associated with a lack of adequate coronal sealing. Post-endodontic restoration therefore plays a key role. Not only should it provide function, aesthetics and marginal sealing, but it must also protect the remaining tooth structure[2,3].

One of the issues for ETT is the significant loss of tooth structure due to carious/non-carious lesions, previous restorations or fractures, and access cavities performed during the endodontic procedure[4]. Thus, endodontically treated teeth have a higher risk of biomechanical failure than do vital teeth[5].

The endodontic and restorative procedure outcome on the stiffness of teeth under occlusal stresses has been widely discussed. It has been shown that endodontic access reduced stiffness by about 5%, while proximal-occlusal cavity or the MOD preparations reduced resistance by 46% and 63%, respectively. The removal of one or both proximal marginal ridges is the major factor contributing to tooth strength loss[6].

This reduced structural strength is relevant in premolars, since, unlike the molars, they are more frequently exposed to destructive lateral forces, making them more prone to cuspal fractures[7]. Likewise, when cervical dentin is lost due to endodontic access or cavity preparation, the placement of a fiber post may be indicated. This may help to compensate for the remaining structure loss by avoiding stress concentration and enabling better distribution of stress to other areas of the tooth[8]. These posts are made of a resin matrix with reinforcing glass, carbon or quartz fiber[9], and because their elastic modulus is similar to that of dentin, they are fatigue-resistant and have tensile strength.

Another option for restoring ETT is the use of high molecular-weight polyethylene fibers. Ribbond fibers (Ribbond Inc., Seattle, WA, USA) are among the most commonly used. Their surface is plasma-treated to improve wettability and enhance adhesion to resin-based restorative materials. In addition, they exhibit an elastic modulus of 117 GPa and a tensile strength of 3 GPa. These mechanical properties, together with their flexibility, facilitate adaptation to the dental cavity[10].

Composite resins show good compressive strength but are weak against tensile forces; therefore, fiber reinforcement is proposed. It has been demonstrated that Ribbond fibers can increase the flexural strength and fracture toughness of composite resin restorations. The fiber design can increase the flexural strength through its pattern based on a dense network of locked nodal intersections, which are able to act as a crack stopping mechanism by changing direction that ultimately dissipates the strain. Furthermore, incorporating Ribbond fibers into composite resin restorations may reduce marginal microleakage in high C-factor cavities by decreasing the amount of resin matrix required for the final restoration[11].

In the area of coronal reconstruction, another widely used material is dual-curing resin, which behaves as a dentinal substitute in extensive cavities, and enables both direct and indirect teeth restorations[12]. This material is also recommended for intracanal cementation of fiber posts and core build-up due to its good mechanical properties and curing ability in areas where the light wave does not achieve adequate polymerization[13]. Its bonding protocols are specific, according to the manufacturer's instructions, as is the case of the ParaCore system (Coltene/Whaledent AG, Altstätten, Switzerland).

The aim of this *in vitro* study was to compare the compressive strength of endodontically treated mandibular premolars with MOD preparations restored using different post-endodontic reconstruction protocols (fiber posts, polyethylene fibers, and core buildup material), using intact teeth as a reference for maximum mechanical performance. The null hypothesis was that no significant differences in compressive strength would be observed among the evaluated groups.

MATERIALS AND METHOD

Sample selection

We used 40 single-rooted mandibular premolars which had been extracted for orthodontic reasons. Organic debris and periodontal tissue were removed with a G1 scaler tip (Woodpecker Medical Instrument Co., LTD, Guilin, China). The teeth were decontaminated in 2.5% sodium hypochlorite

solution and stored in a solution of equal parts of alcohol and glycerin at room temperature for two weeks. Teeth with open apices, carious or non-carious lesions, preexistent restorations, root resorption, fractures, excessively wide root canals, or severely calcified canals were excluded. The sample dimensions of the coronal region were taken into account as selection criteria: mesiodistal (MD) 6 mm +/- 0.5 mm and buccolingual (BL) 7.5 mm +/- 0.7 mm. Digital preoperative radiographs were taken in MD and BL direction with radio-visiograph (RVG) Carestream 5200 (Carestream Dental LLC, Atlanta, GA, USA) to confirm the presence of a single root canal (type I) according to Vertucci et al. classification[14].

Endodontic procedure

Endodontic access was performed with a diamond round bur, round tungsten carbide bur #06 (Microdont, São Paulo, SP, Brazil) followed by Endo-Z bur (Coltene/Whaledent AG, Altstätten, Switzerland) to finish and refine the access cavity. The first exploration was done with a #10 25 mm K-file (MicroMega SA, Besançon, France) to ensure the patency of the teeth, and the cervical and middle thirds of the root canal were prepared with One Flare File N25 9% L17 (MicroMega SA, Besançon, France). The working length was established by inserting a size #15 25 mm K-file (MicroMega SA, Besançon, France) into the canal until it was visible at the apical foramen, then subtracting 1 mm.

Endodontic treatments were performed by the same operator through the crown-apical technique using the MicroMega One RECI #25.04 reciprocating single-file system (MicroMega SA, Besançon, France) and X-Smart Plus motor (Dentsply Maillefer, Ballaigues, Switzerland), according to the manufacturer's instructions.

The canals were irrigated with 2.5% sodium hypochlorite throughout the preparation process using a 10-mL Luer syringe and a 25-gauge needle. The irrigant was activated with the EndoActivator system (Dentsply, Tulsa, OK, USA). The penultimate irrigation was done with 17% EDTA (Farmadental, Ultra D SRL, CABA, Argentina), followed by a final irrigation with 2.5% sodium hypochlorite.

Once the chemical-mechanical preparation had been completed, the coronal area was dried with cotton pellets, and the root canal with paper points (Meta Biomed Co. LTD., Chungcheongbuk-do, Korea). The gutta-percha point selected as the master point was #20.04 (Meta Biomed Co. LTD., Chungcheongbuk-do, Korea), accurately gauged with the endodontic gauge (Maillefer, Dentsply Sirona, Switzerland) consistently with the preset working length, and the obturation was performed according to the hybrid Tagger technique using accessory gutta-percha points (Meta Biomed Co. LTD., Chungcheongbuk-do, Korea) and AH26 sealer (Dentsply De Trey GmbH, Konstanz, Germany). A radiograph of each tooth was made to evaluate the quality of the endodontic treatment. Finally, the samples were stored at 37 °C and 100% humidity for one week.

In all the teeth except those of the control group, 6 mm deep standardized MOD cavities were prepared with the diamond instrument FG 846 KRF-018 (JOTA AG, Rüthi, Switzerland), which was changed after every six uses to ensure its grinding power (Fig. 1). The widths of the lingual and buccal walls were standardized at 2.5 +/- 0.3 mm with an approximate angulation of 6° given by the same instrument, and all the angles were rounded. All the preparations were performed by one operator.

The teeth were divided into 4 groups:

1. Control GROUP (n= 10): Intact teeth, with neither endodontic treatment nor restoration, included as a reference group to represent the maximum compressive resistance of sound dental structure.

2. Core buildup material GROUP (n= 10): Endodontically treated teeth (ETT), MOD preparation, ParaCore buildup (Coltene/Whaledent AG, Altstätten, Switzerland) and direct final restoration with microhybrid composite Brilliant Everglow Body 4g shade A2/B2 (Coltene/Whaledent AG, Altstätten, Switzerland).

3. Intraradicular fiber post GROUP (n= 10): ETT, MOD preparation, placement of glass fiber posts #1 ParaPost Taper Lux (Coltene/Whaledent AG, Altstätten, Switzerland) and direct final restoration with microhybrid composite Brilliant Everglow Body 4g shade A2/B2 (Coltene/Whaledent AG, Altstätten, Switzerland).

4. Polyethylene fibers GROUP (n= 10): ETT, MOD preparation, placement of fiber continuous strip (Ribbond Inc., Seattle, USA) and direct final restoration with microhybrid composite Brilliant Everglow Body 4g color A2/B2 (Coltene/Whaledent AG, Altstätten, Switzerland).

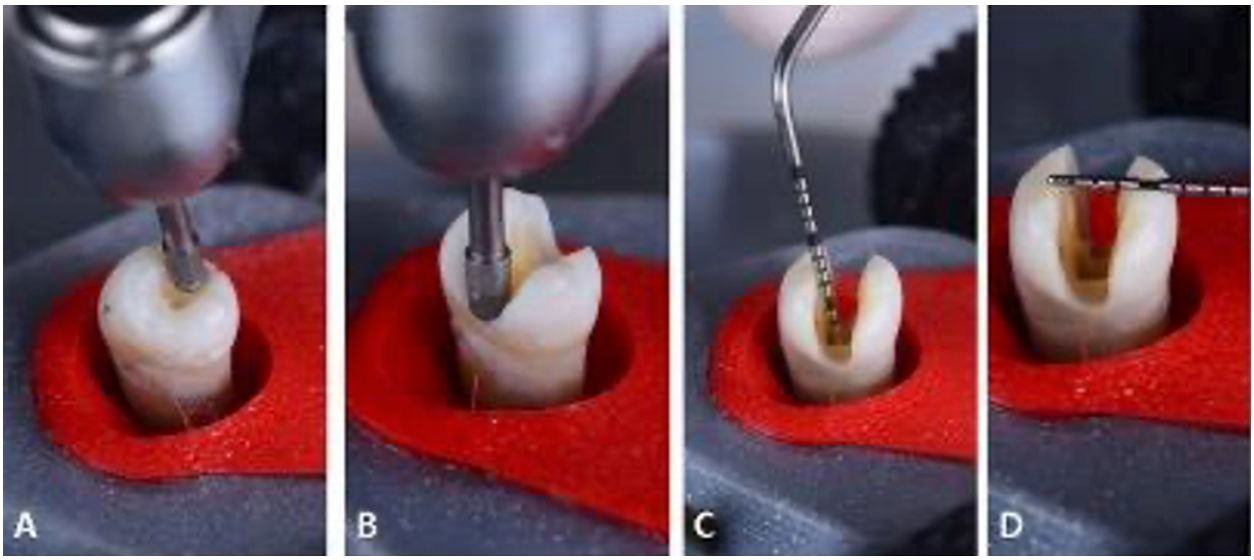

Fig. 1: MOD cavity preparation. **A:** *Extension of the cavity to the distal ridge.* **B:** *Extension to the mesial ridge* **C:** *Checking the axial wall depth* **D:** *Checking the isthmus width.*

Restoration procedure

Core buildup material GROUP: a 1-2 mm desobturation was performed on the teeth with Gates-Glidden burs (Dentsply, Sirona, Switzerland). Teeth were selectively etched with 35% phosphoric acid (Etchant Gel S, Coltene/Whaledent AG, Altstätten, Switzerland) on the dental enamel for 15 seconds, and rinsed for 30 seconds with water followed by water spray. Each specimen was air dried and the root canal entrance sealed with composite Brilliant Flow (Coltene/Whaledent AG, Altstätten, Switzerland). Dentinal substrate was prepared according to the manufacturer's instructions. The dual-cured composite resin system ParaCore (Coltene/Whaledent AG, Altstätten, Switzerland) was dispensed up to the dentinoenamel junction (DEJ) through the automix tip of the kit. After the initial setting time, polymerization was performed on each tooth surface for 20 seconds, until the curing process was completed (Fig. 2).

Intraradicular fiber post GROUP: Desobturation was performed with a Peeso drill #1 (Deltajet, Guangdong, China) 9 mm apically from the cementoenamel junction (CEJ). A #1 ParaPost drill

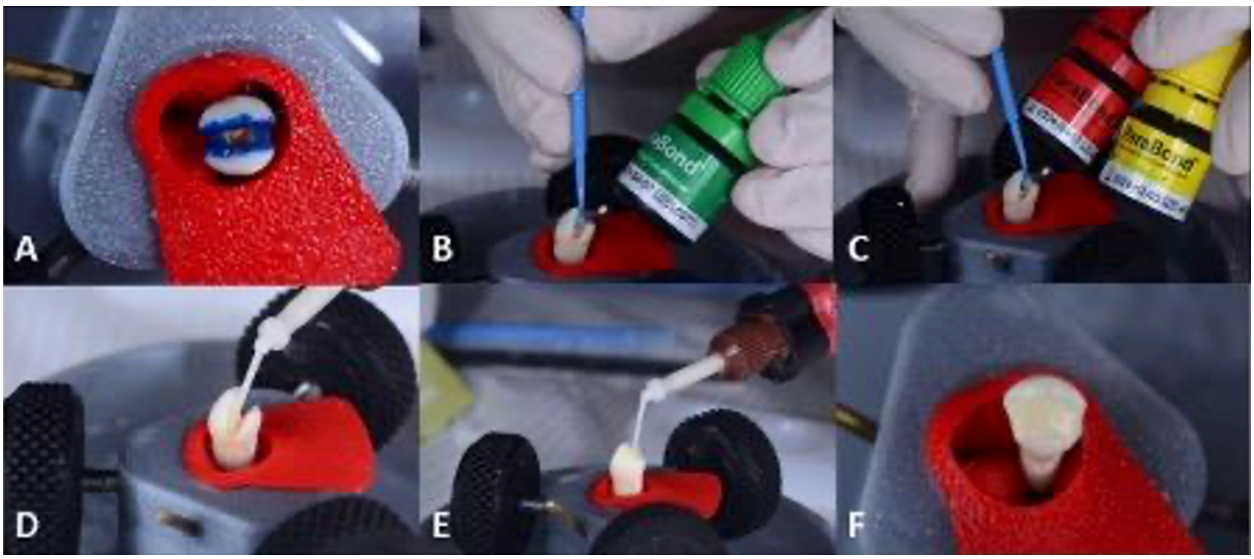

Fig. 2: Restoration protocol with core build-up material. **A:** *Selective etch of the enamel* **B:** *Primer application.* **C:** *Applying the adhesive.* **D** *and* **E:** *Applying ParaCore automix syringe tip* **F:** *After polymerization.*

(Coltene/Whaledent AG, Altstätten, Switzerland) was used to shape the root space, and control radiographs were taken. Each tooth was etched selectively with 35% phosphoric acid (Etchant Gel S, Coltene/Whaledent AG, Altstätten, Switzerland), following the same protocol as described above. Post cementation and core reconstruction were done with the resin system ParaCore (Coltene/Whaledent AG, Altstätten, Switzerland), according to the manufacturer's instructions (Fig. 3).

Polyethylene fibers GROUP: 1-2 mm root canal filling was removed from the entrance of the canal with Gates-Glidden burs (Dentsply, Sirona, Switzerland). Enamel was etched selectively with 35% phosphoric acid (Etchant Gel S, Coltene/Whaledent AG, Altstätten, Switzerland), as in the other two groups. Then, the adhesive system ONE COAT 7 Universal (Coltene/Whaledent GmbH Co., Langenau, Germany) was applied for 20 seconds over the whole preparation. The adhesive solvent was evaporated with air, and polymerization was conducted for 20 seconds with a Led D curing light lamp (Woodpecker Medical Instrument Co., LTD, Guilin, China). The root canal entrance was sealed with composite Brilliant Flow (Coltene/Whaledent AG, Altstätten, Switzerland). A 2-mm-wide polyethylene fiber strip (Ribbond Inc., Seattle, WA, USA) was cut to a length sufficient to span the MOD preparation in a buccolingual direction, using the scissors provided in the kit. The fibers were coated in the pure waterproof adhesive ClearFil SE Bond (Kuraray Medical Inc., Okayama, Japan) to enhance their handling and adaptation. Once inside the cavity, they were fixed with composite Brilliant Flow (Coltene/Whaledent AG, Altstätten, Switzerland) and light cured (Fig. 4).

The final restoration was done with microhybrid composite Brilliant Everglow A2/B2 (Coltene/Whaledent AG, Altstätten, Switzerland) in the core buildup material, intraradicular fiber post and polyethylene fiber groups, with the aim of performing the anatomic restoration. To hold the restorative material inside the proximal aspects of the tooth, a metal matrix assembled in a Tofflemire matrix retainer was used.

Roots were coated with light-body silicone (President Coltene/Whaledent AG, Altstätten, Switzerland) 1 mm below the CEJ to simulate the 0.3 mm periodontal membrane space, and inserted into a block of transparent self-curing acrylic resin (Subiton, Laboratorio SL, Prov. de Bs. As., Argentina). When specimen preparation was complete, they were stored at 100% humidity and at 37 °C for 15 days.

Specimens were tested for compressive strength in a universal INSTRON type testing machine, model 1011 (Instron Mechanical Testing Systems, Norwood, MA, USA). A round metallic tip 5 mm in diameter was placed parallel to the long axis of the tooth against the occlusal surface restoration, connecting the buccal and lingual cusps simultaneously. Each tooth was submitted

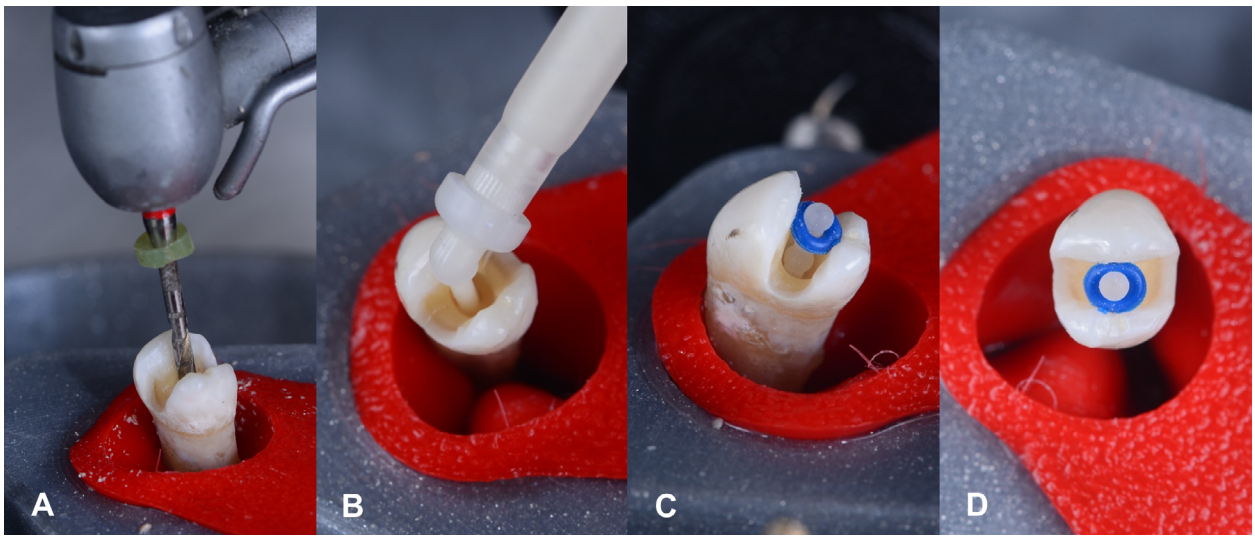

Fig. 3: Intraradicular fiber post cementation protocol: **A:** *Preparation of the post cavity.* **B:** *Cementation with ParaCore.* **C:** *Insertion of the post, lateral view.* **D:** *Insertion of the post, occlusal view.*

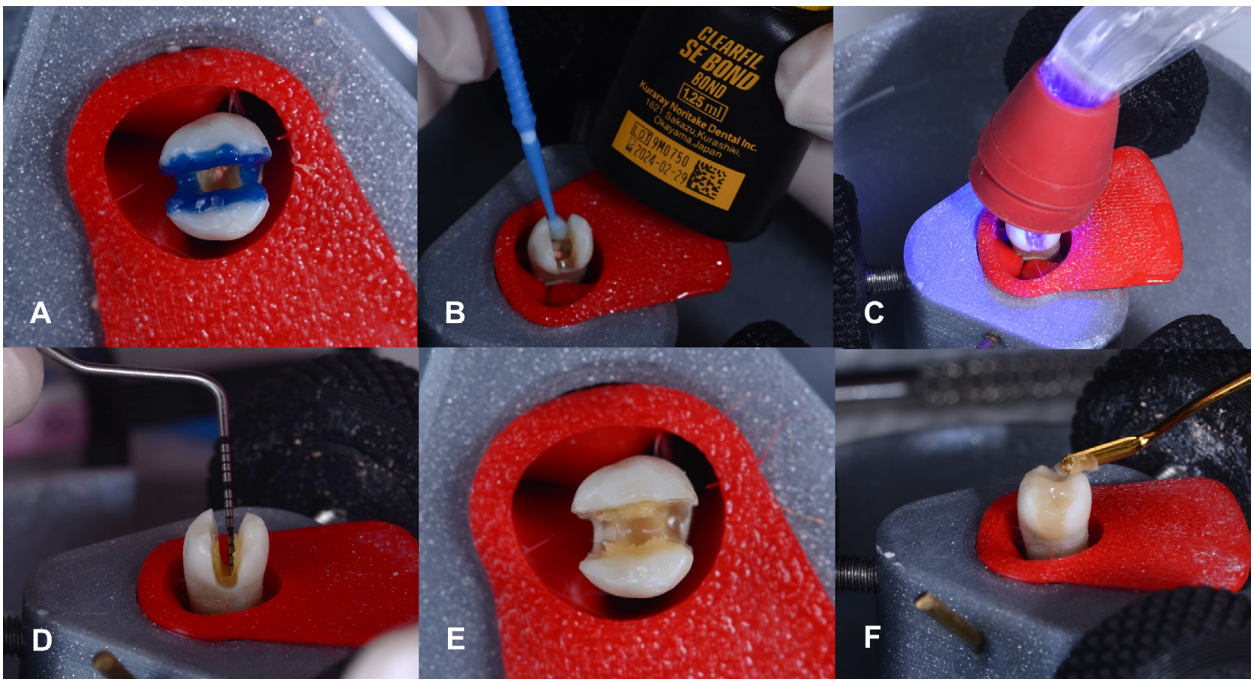

Fig. 4: Polyethylene fibers protocol. **A:** *Selective etch of the enamel.* **B:** *Application of the adhesive system.* **C:** *Light activation.* **D:** *Depth control with periodontal probe.* **E***: Following the fiber polymerization inside the cavity.* **F:** *Insertion of direct composite resin.*

to axial compressive stresses at 1 mm/min and the maximal load reached at the fracture was recorded in Newtons, as suggested by different authors[15,16].

Data were distributed normally, and analysis of variance (ANOVA) was applied in statistical software. Compressive strength (N) was described by the following measurements: median with 95% confidence interval (CI95) estimated by exact method, mean standard deviation (SD), first quartile (Q_1), third quartile (Q_3), minimum (Min) and maximum (Max). Resistance was compared among the four protocols using non-parametric Kruskal-Wallis test. One-way parametric ANOVA test was not used because it does not meet the assumptions of normality, although it does meet the homoscedasticity. The assumptions of normality and homoscedasticity were assessed by the Shapiro Wilk test with approximations of Royston and Levene, respectively. A p-value lower than 0.05 was considered significant. The program R v. 4.3.1 (R Core Team, 2023)[17] was used: database package, *DescTools*[18], *ggplot2*[19] and *lawstat*[20].

RESULTS

The compressive strength values (N) showed numerical differences among the evaluated groups; however, no statistically significant difference was observed (Kruskal–Wallis: $H = 0.85$; $df = 3$; $p = 0.84$). The median (95% CI) compressive strength values were 557.4 N (100.8–574.8) for the control group, 345.1 N (216.8–574.9) for the core buildup material group, 427.5 N (146.3–495.4) for the intraradicular fiber post group, and 471.4 N (194.2–565.5) for the polyethylene fiber group (Fig. 5, Table 1).

DISCUSSION

This *in vitro* study investigated the compressive strength of endodontically treated premolars with MOD cavities restored with three different restorative protocols. No significant difference was found among the 4 groups (p=0.84).

Intact teeth were included as a control group to provide a reference value for the maximum compressive resistance of non-restored dental structures. Although the primary aim of the study was to compare different post-endodontic reconstruction protocols, the inclusion of intact teeth enabled biomechanical comparison with the original condition of the dental substrate, and facilitated interpretation of the restorative behavior relative to intact teeth.

Premolars are more prone than molars to being submitted to lateral forces during mastication. The remaining dental structure and the functional

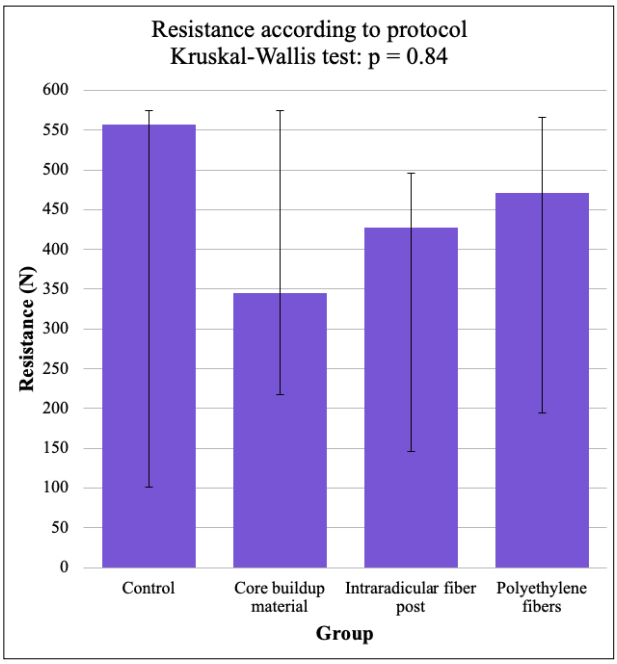

Fig. 5: Compressive strength compared in the four protocols (Median, CI95).

demands are decisive factors, as they must ensure compressive strength and long-term clinical performance. Premolars were used in the current study because they exhibit characteristics that make them more prone to being subjected to those stresses[21].

Although fiber posts are generally recommended in teeth with extensive loss of coronal structure, they were included in the present study to evaluate their reinforcing effect under standardized experimental conditions and compare their mechanical behavior with alternative restorative strategies. The use of fiber posts in ETT has been associated with improved stress distribution because their elastic modulus is similar to that of dentin. However, their use should be considered carefully, according to the amount of remaining dental structure[8,9,21].

Cavity preparations which cause a tooth further weakening due to their depth or extension must be restored by means of adhesive restorations with cuspal coverage[22]. This approach contributes to preventing cuspal flexion and reduces marginal leakage, which are crucial to ensure long-term durability of the restoration[23].

This study compared the axial stress loads of the restorative materials in anatomical restorations of endodontically treated teeth. Currently, restorative dentistry is based on biomimetic adhesive techniques, and consists of preserving the greatest possible amount of dental tissue. The restoration type (direct vs. indirect, and composite vs. ceramics) should be selected according to the extent of the cavity[24]. The restorative procedure and the filling material must provide sufficient mechanical resistance to a tooth with an MOD cavity[25]. In teeth with missing walls and high C factor cavities, the restorative technique is the key to achieving the adhesion of restorative materials[26].

It should also be considered that direct composite restorations in premolars with MOD cavities may present a greater risk of structural failure under functional loading due to the loss of both marginal ridges. This structural compromise may negatively affect the distribution of occlusal forces and increase susceptibility to cuspal or radicular fractures[22,26]. Therefore, in clinical situations with extensive loss of tooth structure, indirect restorations with cuspal coverage could provide a more predictable long-term restorative alternative[22,24].

Daher et al.[27] evaluated the fracture resistance and failure mode of non-invasively reinforced endodontically treated mandibular molars, and reported no significant difference in fracture resistance among the evaluated restorative approaches. However, the use of glass fiber reinforcement was associated with a more favorable failure pattern, suggesting a potential biomechanical benefit despite the absence of differences in maximum fracture resistance values.

Table 1. Comparison of the compressive strength in the four protocols

Group	Resistance (N)				
	Median (CI95)	Mean (SD)	Q_1 / Q_3	Min/Max	N
Control	557.4 (100.8 to 574.8)	419.5 (207.4)	293.5 / 572.1	63.6 / 575.5	10
Core buildup material	345.1 (216.8 to 574.9)	361.2 (158.8)	233.6 / 501.4	132.3 / 576.4	10
Intraradicular fiber post	427.5 (146.3 to 495.4)	386.6 (152.6)	389.2 / 457.3	86.4 / 576.1	10
Polyethylene fibers	471.4 (194.2 to 565.5)	408.4 (171.8)	243.3 / 560.7	153.0 / 574.5	10

Kruskal-Wallis test: H = 0.85; DF = 3; p = 0.84

Another study assessed the fiber positioning and the orientation of fracture resistance in mandibular premolars with MOD cavities. The results showed that horizontally positioned fibers, oriented in a buccolingual direction, increased fracture resistance[15]. This orientation was also used in the present study. Previous investigations showed that the reinforcement with fibers, regardless of position or orientation, resulted in an increase in resistance to fracture of teeth[28-32]. These results are consistent with the findings of the present work.

Different studies have reported that fiber placement is an effective, practical treatment option to reinforce the material in endodontically treated teeth, with no posts that distribute the stresses along the interface tooth-restoration. The restoration transfers the matrix stresses to the fibers, preventing cracks from spreading through the filling materials[8,9]. The present study supports the research of Soto-Cadena et al.[7] on the reinforcement of endodontically treated MOD cavities with Ribbond fibers and a resin subsequently used as build-up material increased the resistance to fracture, suggesting that it may be adequate for the direct coronal restoration of large posterior cavities in stress-bearing areas.

Göktürk et al.[16] investigated fracture resistance in endodontically treated maxillary premolars, with Class II cavities with different types of restorations. They analyzed the following groups: intact teeth; unrestored endodontically treated teeth; direct resins; direct resin buccolingually reinforced with glass fiber, and inlay ceramic restorations. All restorative techniques increased fracture resistance compared with the unrestored group; however, the values remained lower than those of intact teeth. No significant differences were observed among the restored groups, which is consistent with the findings of the present study.

According to Sengun et al.[33], fiber-reinforced composite restoration seemed to be a more reliable restorative technique than traditional composite restorations for endodontically treated teeth with MOD cavities, since was associated with a more favorable failure pattern under occlusal loading.

The present study has limitations inherent to *in vitro* investigations. The compressive load was applied under static axial conditions, which do not completely reproduce the multidirectional and cyclic forces present in the oral environment. In addition, thermal cycling, fatigue loading, aging of restorative materials, and indirect restorations with cuspal coverage were not evaluated. Another limitation is that fracture mode was not analyzed. Future studies should include these variables to better simulate clinical conditions and assess the long-term biomechanical behavior of restored endodontically treated teeth.

CONCLUSION

Under the experimental conditions of the present study, the evaluated post-endodontic reconstruction protocols showed similar mechanical behavior regarding compressive strength in endodontically treated mandibular premolars. Although polyethylene fiber reinforcement demonstrated a tendency toward higher resistance values, the results do not enable the establishment of any conclusive mechanical advantages of the restorative systems assessed.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interests.

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MRSA carriage rate among dental healthcare personnel: a multicentric cross-sectional study

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ABSTRACT

Methicillin-resistant Staphylococcus aureus (MRSA) represents a significant global healthcare challenge, yet its prevalence among dental personnel remains poorly understood. ***Objective:*** *The aim of this study was to determine the carriage rate and related risk factors of MRSA among Colombian oral healthcare providers.* ***Materials and Method:*** *A multicenter descriptive cross-sectional study was conducted. Nasal swabs were obtained from 457 randomly selected healthcare professionals from dental services across eight cities in Colombia, using proportional stratified sampling and snowball sampling in 29% of the cases. Inclusion criteria were being a full-time worker in a dentistry setting, and being healthy with no upper respiratory tract infection, fever, or use of antimicrobial therapy in the previous three months. Gram staining, catalase and coagulase tests were used to identify S. aureus colonies. The methicillin-resistant phenotype was detected using the Kirby-Bauer disc method. The virulence factor encoding genes mecA and Panton Valentine Leukocidine (PVL) were detected by polymerase chain reaction (PCR).* ***Results*****:** *Overall prevalence of MRSA was 4.4% [95% confidence interval (CI): 2.5% – 6.2%], determined using mecA gene detection. Women carried 75% of the MRSA strains, and dentists accounted for 60%. Carrier rates were 4.03% [95% CI: 1.8% - 6.2%] for dentists, and 5.03% [95% CI: 1.6% - 8.4%] for other personnel. These rates did not differ significantly from each other (Fisher's exact test, p=0.67). A personal history of hospitalization over the previous year was identified as a predisposing factor for the host (p=0.033).* ***Conclusion****: This multicenter study reveals a 4.4% MRSA carriage rate among Colombian dental healthcare providers. MRSA-carrying dental healthcare workers did not appear to have risk factors associated with professional activity.*

Keywords: *methicillin resistant staphylococcus aureus – prevalence – dentists - dental health services - carrier state*

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Tasa de portación de SAMR entre el personal de atención odontológica: un estudio transversal multicéntrico

RESUMEN

El Staphylococcus aureus resistente a la meticilina (SARM) representa un desafío global significativo para la salud, aunque su prevalencia entre el personal dental sigue siendo poco comprendida. ***Objetivo:*** *La colonización por Staphylococcus aureus resistente a la meticilina (SARM) entre profesionales odontológicos es incierta. Este estudio tuvo como objetivo determinar la tasa de portación y los factores de riesgo relacionados con portación de SARM entre profesionales de la salud bucodental colombianos. Diseño del estudio: Estudio transversal multicéntrico.* ***Materiales y Método:*** *Se obtuvieron aleatoriamente hisopados nasales en 457 profesionales de la salud en servicios odontológicos de ocho ciudades de Colombia. La tinción de Gram, las pruebas de catalasa y coagulasa indicaron colonias de S. aureus. El fenotipo de resistencia a la meticilina se detectó mediante el método del disco de Kirby-Bauer. Los genes que codifican el factor de virulencia mecA y la leucocidina de Panton Valentine (PVL) se detectaron mediante reacción en cadena de la polimerasa (PCR).* ***Resultados:*** *La prevalencia general de SARM fue del 4,4 % [intervalo de confianza (IC) del 95 %: 2,5 % – 6,2 %], determinada mediante la detección del gen mecA. Por sexo se observó que las mujeres portaban el 75% de las cepas de SARM, y por ocupación los profesionales portaban el 60%. Los odontólogos presentaron una tasa de portación del 4,03% [IC del 95%: 1,8% - 6,2%], mientras que el resto del personal tuvo un 5,03% [IC del 95%: 1,6% - 8,4%]. Entre las estimaciones no se observó diferencias estadísticamente significativas (prueba exacta de Fisher, p = 0,67). El antecedente personal de hospitalización durante el año previo se identificó como un factor predisponente para el huésped (p = 0,033).* ***Conclusión:*** *La prevalencia de SARM entre el personal odontológico es moderada. Los trabajadores de la salud dental portadores de SARM no parecieron tener factores de riesgo asociados con su actividad profesional.*

Palabras clave: *staphylococcus aureus resistente a la meticilina – prevalencia – odontólogos - servicios de salud dental - portador sano*

INTRODUCTION

Staphylococcus aureus is a common commensal bacterium found in normal human microbiota, primarily on skin, gastrointestinal tract, and nasal mucosa. Nasal colonization by *S. aureus* begins within the first month of life, with carriage rates of approximately 40 to 50% during the first 8 weeks, decreasing to 21% by six months of age. The mother is the usual source[1,2]. In healthy adults, review studies have reported frequencies of approximately 20 and 30% for permanent and intermittent carriers, respectively[3,4]. Methicillin-resistant *Staphylococcus aureus* (MRSA) was first reported in 1961, after it acquired the staphylococcal cassette chromosome *mec* element through gene transfer of the *mecA* gene encoding PBP-2α. It has become a significant global healthcare challenge and a major cause of a wide range of hospital-acquired infections, posing substantial threat to patient safety[5].

While MRSA infections are more common in healthcare settings, they can also affect healthy individuals and veterinary settings, compromising public health because of the high risk of cellulitis, abscesses and invasive infections such as pneumonia and bacteremia[6]. MRSA spreads through direct physical contact with healthy carriers and contaminated personal items, particularly in group settings such as sports teams, schools, households, and nursing homes. MRSA prevalence has been reported as around 14.69 to 22% in non-outbreak scenarios, with the nares being the most frequently colonized body site[7–10].

The emergence and transmission of MRSA among healthcare workers (HCWs) are important aspects of MRSA epidemiology. The prevalence of MRSA carriers among healthcare workers is an indicator of its spread within population groups, and of the increased risk of colonization by multidrug-resistant bacteria. Among HCWs, prospective and cross-sectional studies, mostly carried out in Europe, USA and Asia, have shown a pooled prevalence of MRSA carriage of up to 9.23%, with higher rates in nursing and medical staff involved in patient care[11–13]. However, few studies have examined MRSA nasal colonization prevalence among dental healthcare providers. In Latin America, only two studies, both in Brazil, have investigated the issue in dental personnel[14,15], and they were limited by convenience sampling, non-representative samples, small study populations, and unreported non-response rates. No such study has been conducted in Colombia. We hypothesized that dental healthcare personnel would exhibit a higher prevalence of MRSA carriage than the general population due to frequent occupational exposure to the oral and respiratory tracts of patients. Therefore, the aim of this study was to investigate MRSA carriage prevalence among dental healthcare workers and identify associated risk factors. Understanding MRSA epidemiology in dental personnel can inform targeted infection prevention and control strategies to minimize transmission and protect patients and providers.

MATERIALS AND METHOD

Study design and setting

This descriptive cross-sectional, multi-center study was conducted from January 2023 to April 2024. The study population consisted of active dental healthcare workers in eight cities in Colombia (Armenia, Bogotá D.C., Bucaramanga, Cúcuta, Neiva, Palmira, Popayán and Villavicencio).

Selection of participants

A total 457 participants were included in this study via a probabilistic, stratified sampling process. Initially, dental service institutions were identified from the data reported in the "Special Registry of Health Service Providers" on the website of Ministerio de Salud y Protección Social de Colombia (Colombian Ministry of Health and Social Protection)[16]. In the second phase, institutions were divided based on the city location, after which 400 institutions were selected from this list using random numerical allocation. To ensure geographical representativeness, the number of participants per city was adjusted proportionally to the density of dentists per inhabitant in each city. The samples were drawn from each city using simple random sampling methods. Where access to institutions was restricted or they were not located at the address indicated in the database, recruitment was supplemented by snowball sampling from the initial location. This situation occurred in 29% of cases. At least one participant was randomly selected from each institution. All participants were contacted and informed of the study purpose. Verbal and written consent was obtained from all participants. The study protocol was approved by the Ethics Committee of University of Antonio Nariño/Santo

Tomás (Approval number 2022220/May 2022).
As this is the first study of its kind in Colombia, a review of MRSA prevalence studies in other countries was conducted to calculate the sample size. The calculation considered 2.9% minimum expected prevalence[17], 95% confidence level, and 5% estimated margin of error. The sample size calculation for a proportion was based on the formula provided by García-García et al.[18]. The total number of samples was 385, increased by 18% to account for potential sampling issues and to achieve greater statistical power for detecting lower prevalence data.
Inclusion criteria were: (i) being a full-time worker in a dentistry setting and (ii) being healthy, with no upper respiratory tract infection, fever, or use of antimicrobial therapy in the last three months. Participants who refused to take part were excluded. The study achieved 100% response rate, with no data loss or refusals by participants during the nasal swab collection process. A questionnaire was used to record demographic and epidemiological data concerning the determinants of human carriage (e.g. family cohabitation, morbidity, history of hospitalization and risk factors such as drug exposure) and use of personal protective equipment.

Methods of measurement

Microbiological methods. Nasal swabs were obtained from all study participants. Nasal samples were collected by standardized students at the School of Dentistry. A sterile single-headed polystyrene swab was inserted into both the left and right nostrils of each study subject, touching the nasal vestibule and rotating for 5-10 seconds in each. The swabs were transferred in Amies transport medium CM0425-OXOID to the microbiology laboratory within 2 hours, and processed immediately upon arrival.
In the laboratory, the swabs were streaked onto Mannitol Salt Agar to enable selective growth of *S. aureus*. The bacterial strains were transferred to Blood Agar and incubated for 24 h at 36 °C. Suspected colonies were subjected to Gram staining, β-hemolytic phenotype analysis, and coagulase test (Bactident® coagulase EDTA-rabbit plasma). The susceptibility pattern for several antibiotics was determined using the disk diffusion method according to the Clinical & Laboratory Standards Institute (CLSI) guidelines. Oxacillin (1μg), rifampicin (5 μg), vancomycin (30 μg), gentamicin (10 μg), clindamycin (2 μg) and cefoxitin (10 μg) were tested, and the strains classified as susceptible or resistant based on the *S. aureus* epidemiological cut-off values issued by CLSI[19].

Polymerase chain reaction (PCR). Cefoxitin-resistant isolates were analyzed for the presence of *mecA* and Panton-Valentine Leukocidin (PVL) genes. The primers used to amplify the *mecA* gene were forward: 5'-AAAATCGATGGTAAAGGTTGGC- 3'and reverse: 5'-AGTTCTGCAGTACCGGATTTGC-3', as previously described by Sanchez M. et al.[20]. The expected amplicon size was 533 bp (GenBank: KC243783.1). For the *Luk-PV* gene, the forward primer was 5'-GTAAAATGTCTGGACATGATCCA- 3'and the reverse was 5'-TCTGCCATATGGTCCCCAAC-3.' The expected amplicon size was 969 bp (GenBank: MK902786.1). We amplified only one region, consisting of the last section of *lukS*-PV and the beginning of l*ukF*-PV, as previously described by Johnsson et al.[21]. Primer specificity was assessed using BLAST. DNA was extracted using the QIAamp DNA kit according to the manufacturer's guidelines. The concentration, purity and integrity of the DNA were checked using a NanoDrop 2000c spectrophotometer (Thermo Scientific). Conventional PCR was performed in a TC32 Mini Thermal cycler (Benchmark), in a final volume of 25 μL using GoTaq® Green Master Mix (Promega). Amplification was performed under the following conditions: initial denaturation 95 °C, 1 min, denaturation 94 °C, 1 min; annealing at 55 °C for 1 min; and extension at 72 °C for 1 min. PCR products were resolved at 100V on 1.5% agarose gel in 1X TAE buffer for 60 min using a 1000 bp DNA ladder, and visualized with SYBR™ Safe DNA stain gel (Invitrogen) under UV light in a myGel InstaView electrophoresis system (Accuris instruments). *Staphylococcus aureus subsp. aureus* derived from ATCC25923 (*mecA* -; PVL +) and ATCC33591 (*mecA* +; PVL -) were used as a quality positive control, and pure water was used as a negative control in every assay.

Data analysis

The proportion of *S. aureus* and MRSA carriage was measured by positive testing and calculating

95% confidence intervals (CIs). Chi-square test or Fisher's exact two-tailed test, as appropriate, were used to compare the prevalence proportions based on occupation and personal risk factors between the groups of *S. aureus* carriers and MRSA carriers, as well as between dentists and other dental worker groups, with P values set at <0.05 to indicate statistical significance. Statistical analyses were performed using GraphPad Prism version 10.

RESULTS

The characteristics of the samples studied are listed in Table 1. A total 457 nasal swabs were analyzed. The study sample was divided into two groups according to place of residence: Bogotá and other cities. Mean age and time since graduation differed significantly between dentistry professionals residing in Bogotá and those residing in other cities in Colombia.

Prevalence of MRSA carriers

Of the 457 dental services workers, 95 (20.8%; 95%CI: 17.1% - 24.5%) were nasal carriers of *S. aureus*. According to the cefoxitin test, 27 (5.91%; 95% CI: 3.7% – 8.1%) were MRSA carriers, and based on PCR *mecA* gene detection, 20 were confirmed as MRSA carriers (4.4%; 95% CI: 2.5% – 6.2%). Of the cefoxitin-resistant strains, 74.1% tested positive for *mecA*. 75% of the strains were found in women, and 60% in dental professionals. Regarding occupational distribution, phenotypic methods identified 17 dental professionals (dentists and specialists) as MRSA carriers, while genotypic methods identified 12.

Prevalence in each group of dental health workers is shown in Table 2. Among assistant personnel, students and hygienists (159 subjects; 34.8% of the

Table 1. Participant characteristics

Variable	Bogotá D.C. (n= 243)	Other cities (n=214)	Total (n=457)
Age (years) Mean ± SD (range)	**30.6 ± 9.7 (19 – 76)**	**36 ± 11 * (18 – 69)**	**33 ± 11 (19 – 76)**
Sex n (%)			
Female	168 (69.1%)	156 (72.9%)	324 (70.9%)
Male	75 (30.9%)	58 (27.1%)	133 (29.1%)
Occupation n (%)			
Dentist	107 (44.0%)	71 (33.2%)	178 (38.9%)
Dental specialist	47 (11.1%)	73 (34.1%)	120 (26.3%)
Assistant	50 (20.6%)	46 (21.5%)	96 (21.0%)
Oral hygienist	7 (2.9%)	19 (8.9%)	26 (5.7%)
Student	32 (13.2%)	5 (2.3%)	37 (8.1%)
Place of employment n (%)			
Student (University)			6 (1.3%)
Private Dental office			330 (72.2%)
Health provider entity			5 (1.1%)
Public Health service			44 (9.6%)
Private Health service			56 (12.3%)
Dental center			16 (3.5%)
Time since graduation (months) Median (CI 95%)			
Dentists	**60 (36 – 72)**	**120 (96 – 139) ***	**84 (72 - 96)**
Assistants, hygienists, students	**12 (9 – 23)**	**84 (60 – 104) ***	**30 (24 – 48) ***
Total	**24 (24 – 36)**	**102 (91 – 120) ***	**60 (48 – 72)**

Statistically significant difference, Mann-Whitney U-test $p < 0.001$

Table 2. Prevalence of *S. aureus* and MRSA in dental healthcare workers

Occupation	Total sample	% of S. *aureus* carriers	% (95% CI)	% MRSA carriers	% (95% CI)
Dentist	178	21.9%	22.5% (17.9% - 27.6%)	3.9%	4.03% (3.1%-8.3%)
Dental specialist	120	23.3%		4.2%	
Assistant	96	18.7%	17.6% (12.0% - 24.4%)	5.2%	5.03% (2.5%-10%)
Oral hygienist	26	15.4%		7.7%	
Student's test	37	16.2%		2.7%	
Fisher's test			*p*=0.059		*p*=0.670

sample), MRSA prevalence was identified as 6.3% [95% CI: 2.5%–10.1%] using phenotypic methods, and 5.03% [95% CI: 1.6% - 8.4%] using genotypic methods. There was no statistically significant difference between the dentists and other dental worker groups, according to Fisher's exact test (p=0.67). Prevalence of MRSA carriers was high among oral hygienists.

Most *S. aureus* and MRSA carriers resided in Bogotá D.C., accounting for 25%. In other cities, the prevalence of *S. aureus* carriers was 15.4%, and that of MRSA carriers was 2.3%. There was no statistically significant difference between the groups.

PCR amplification of the *mecA* gene (Fig. 1A) and *Luk-PV* gene were performed on all twenty-seven cefoxitin-resistant isolates. *Luk-PV* gene was detected in only 30% of the *mecA* gene carriers (Fig. 1B).

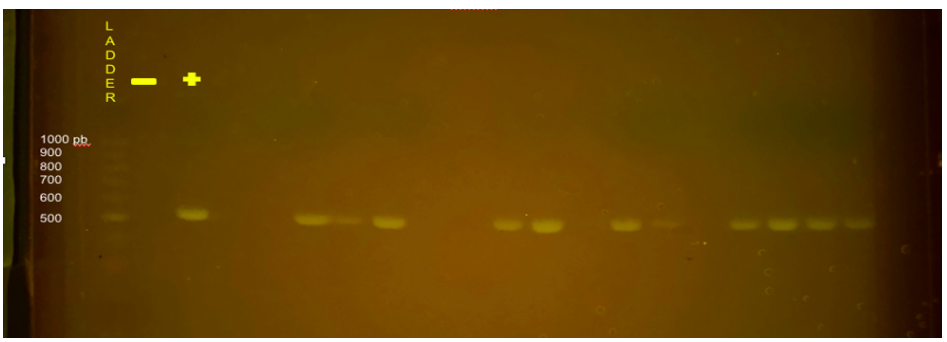


Fig. 1A: Gel electrophoresis - *mecA* gene: *Agarose gel electrophoresis for the detection of the mecA gene (533 bp) in Staphylococcus aureus strains by conventional PCR. Lane 1: molecular weight marker (1000 bp); lane 2: negative control (S. aureus ATCC 25923); lane 3: positive control (S. aureus ATCC 33591); lane 4: water; lanes 5–20: sample strains.*

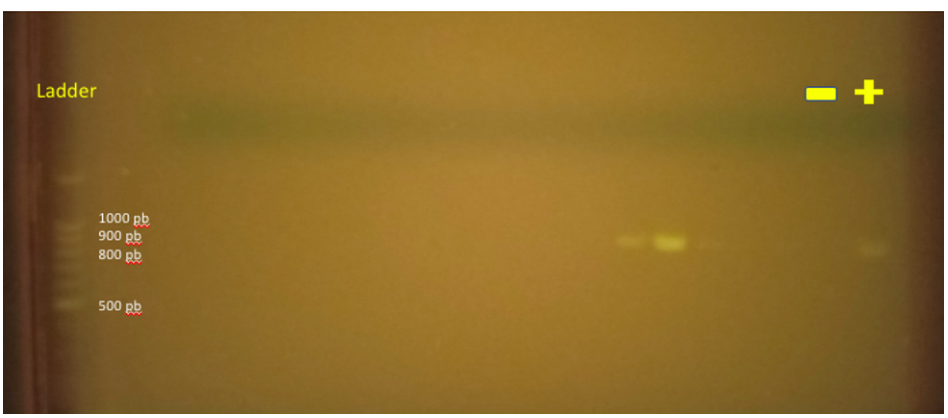


Fig. 1B: Gel electrophoresis - Luk-PV gene: *Agarose gel electrophoresis was used to detect the Luk-PV gene (969 bp) in Staphylococcus aureus strains by conventional PCR. Lane 1: molecular weight marker (1000 bp); lane 3: water; lanes 4–18: sample strains; lane 19: negative control (S. aureus ATCC 33591); lane 20: positive control (S. aureus ATCC 25923).*

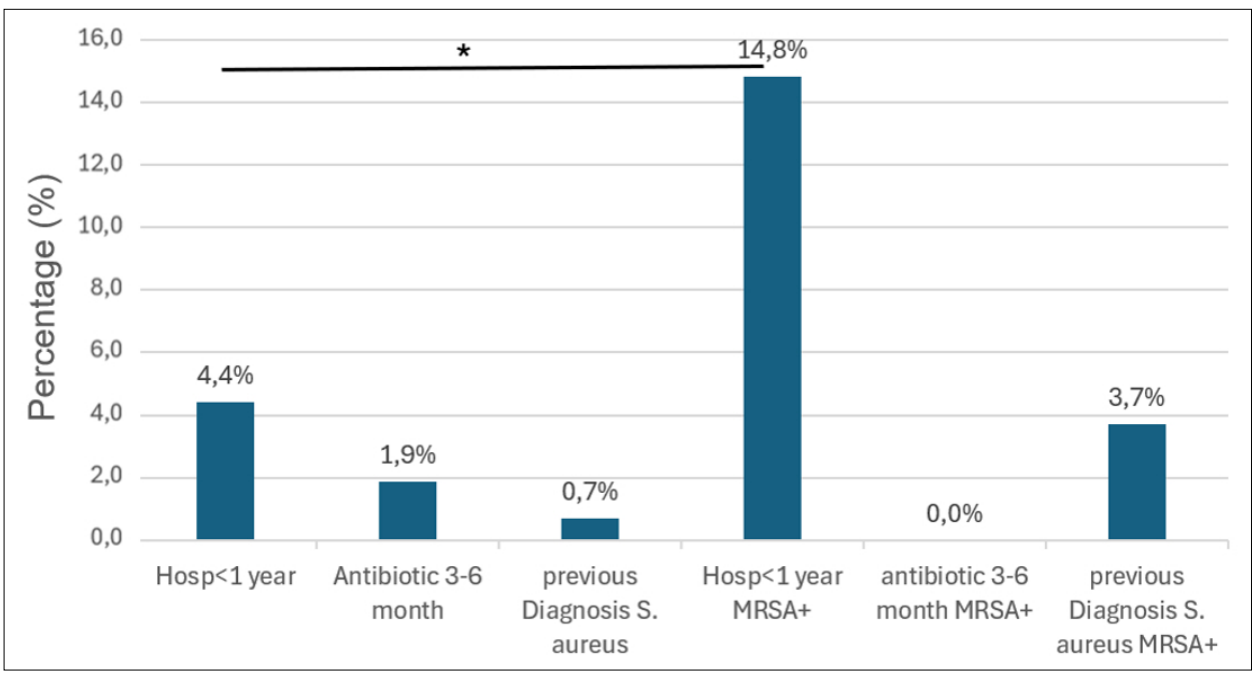
Factors associated with MRSA carriage

Host predisposing characteristics involving personal and drug exposure risk factors were analyzed and correlated with self-reported use of personal protective equipment. There was no statistically significant difference in the presence of risk factors between *S. aureus* and MRSA carriers (Table 3). Almost 52% of MRSA carriers did not live with other healthcare workers. However, among the dental staff living with HCWs, most MRSA carriers (61.5%, 8/13) lived with physicians and nurses. The history of hospitalization in the previous year was the only factor associated with MRSA carrier status (Fig. 2).

Table 3. Distribution of personal risk factors by carriage status

		Total sample	*S. aureus* carriers	MRSA carriers	*p value*
Family cohabitants	Any HCW	48.6%	48.4%	51.8%	*0.828*
	Physician/nurse	19.9%	21.0%	29.6%	*0.436*
	Dentist	19.3%	20.0%	18.5%	*1.000*
	Dental assistant	5.3%	5.3%	0.0%	*0.585*
	Other HCW	7.0%	5.3%	0.0%	*0.585*
	Total	457	95	27	
Morbidity	Any HCW	90.4%	89.7%	96.3%	*0.433*
	Obesity	3.5%	2.9%	0%	*1.000*
	Diabetes	0.9%	1.5%	3.7%	*0.489*
	Rheumatoid arthritis	0.2%	0.0%	0%	N/A
	Atopic dermatitis	4.8%	5.9%	0%	*0.575*
	Recurrent boils/chronic furunculosis	0.2%	0%	0%	N/A
	Total	457	68	27	

HCW= Healthcare worker



* Fisher exact test p <0.05

Fig. 2: Drug exposure risk factors

The analysis of use of personal protective equipment demonstrated exemplary compliance: over 98% for gloves and facemasks, 82.3% for face shields (masks and eye protection), and 74.2% for disposable gowns. The regular use of disposable gowns was lower among MRSA carriers than *S. aureus* carriers; however, this disparity was not statistically significant (p=0.621).

Antibiotic resistance patterns of *S. aureus* strains

All *S. aureus* strains isolated were tested for susceptibility to oxacillin, clindamycin, gentamicin and rifampicin. Approximately 90% of the isolates were susceptible to oxacillin and clindamycin, and 97% were susceptible to gentamicin and rifampicin. MRSA strains were 100% susceptible to gentamicin and 88.9% susceptible to rifampicin. The resistance rates to oxacillin and clindamycin were 55.6% and 14.8%, respectively. The antibiotic sensitivity patterns of *S. aureus* and MRSA strains are presented in Table 4.

Table 4. Antibiotic-resistance profile in *S. aureus* and MRSA strains

	Antibiotic	Susceptible %	Intermediate %	Resistant %
S. aureus	Oxacillin	89.7	5.1	5.1
	Gentamicin	96.2	2.6	1.3
	Clindamycin	88.5	9.0	1.3
	Rifampicin	98.7	1.3	0.0
MRSA	Oxacillin	44.4	0	55.6
	Gentamicin	100	0	0
	Clindamycin	70.4	14.8	14.8
	Rifampicin	88.9	11.1	0

DISCUSSION

The study found that *S. aureus* was consistently prevalent across all staff groups. MRSA carrier rates varied, with oral hygienists showing the highest rates, followed by assistants, dental professionals, and students. The analysis of risk factors indicated that a personal history of hospitalization in the past year was the only factor associated with being a carrier among dental healthcare staff. Adherence to protective equipment use was high.

To the best of our knowledge, this is the first multicenter observational study conducted in a Latin American population. A review of the literature revealed eight studies of dental healthcare professionals in Ukraine[22], Germany[23], Seul[17], United Arab Emirates[24], Egypt[25], India[26] and Brazil[14,15]. Only Lerche et al.[23] and Salmanov et al.[22] used large, randomly selected samples from different dental settings. The remaining studies used data from only one or two institutions with dental services. The largest Brazilian study included 31 participants and assessed colonization prevalence in salivary and nasopharyngeal secretions from professionals at two academic dental clinics[14]. Dental assistants provided data in half of these studies. The current study has the largest sample size and, unlike other studies, includes a randomly selected sample of dental graduates and specialists, assistants, and dentistry students.

Comparison of our results to those of other similar studies with randomly selected populations showed that the *S. aureus* carriage rate was very similar, with values between 22 and 30.9%, while the MRSA carriage rate was quite variable, with values of 0% in Mecklenburg Western Pomerania, Germany[23], 9.7% in Kyiv Ukraine[22], and 4.03% in Colombia, as reported herein. The prevalence of virulence factor *Luk*-PV genes was 55.5% in MRSA strains of dentistry staff from Kyiv, which was higher than the 30% of MRSA strains that were found in the current study. *Luk*-PV genes are mostly associated with community-acquired S. *aureus* strains and recognized as markers for virulent strains because they code for a cytotoxin that causes tissue necrosis and leukocyte destruction, which are related to furunculosis, cutaneous abscesses, severe necrotizing pneumonia, and sepsis. This finding indicates that one out of every ten MRSA strains is highly invasive, most probably community-acquired MRSA, and represents a serious health threat for carriers[27,28].

Previous studies on other Colombian populations have shown similar MRSA prevalence rates. For example, Rebollo-Perez J et al.[29] found a prevalence of 4.8% in preschool children. Their risk factors analysis showed no association with household healthcare workers but did find an association with antibiotic use in the previous 3 months. Unlike our findings, the authors identified that all MRSA strains were PVL-positive, suggesting community-associated MRSA (CA-MRSA) strains. Other researchers[30] have also suggested that outpatient treatment and antimicrobial therapy during hospitalization are important MRSA risk factors in Colombia.

Phenotypic resistance without detection of the *mecA* gene occurred in 25.9% of the participants, which may be due to alternative resistance caused by borderline strains with hyperproduction of beta-lactamase or even modified *S. aureus* (MODSA), which has mechanisms other than non-*mecA* and non-*mecC*-mediated resistance[31]. However, these mechanisms were not investigated in the current study.

The current study had some limitations. We did not sample hands, which may have either under or overestimated MRSA colonization frequency. Bacterial carriage varies across body sites[32], but few studies compare *S. aureus* or MRSA carriage data from two or more body sampling sites. The nasal vestibule is the most frequent carriage site, with highest colonization rates[25,33]. Furthermore, our results may not fully represent MRSA prevalence in Colombia or individual cities because we did not sample all regions, and the sample size was not calculated for each city's prevalence. Additionally, the non-probabilistic sampling in 29% of cases compromises statistical representativeness, introduces selection bias, and may limit the external validity of the findings. However, our study provides crucial epidemiologic data on MRSA prevalence in a wide range of healthcare workers, antibiotic resistance monitoring, and factors influencing the circulation of health-threatening microorganisms in healthcare settings. While Fisher's exact test was utilized appropriately for small subgroup analyses, the study may be statistically underpowered to detect significant associations for rare comorbidities or specific living arrangements.

CONCLUSIONS

This multicenter study reveals a 4.4% MRSA carriage rate among Colombian dental healthcare providers. Additionally, 30% of MRSA strains exhibit higher virulence due to the presence of the *luk*-PV gene. Continuous monitoring of MRSA carriers among healthcare workers is essential for understanding and controlling the spread of healthcare-associated infections in dental settings.

CONFLICT OF INTERESTS

All the authors declare that there is no conflict of interest regarding the publication of this manuscript.

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Effect of disinfection and storage media on surface microhardness of human enamel blocks

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ABSTRACT

Protocolized disinfection and storage are critical to ensuring the quality of dental specimens used in in vitro studies. However, few evaluations have addressed the effect of different combinations of disinfection and storage media on the surface microhardness of human enamel blocks. ***Aim:*** *To evaluate the effect of different disinfection and storage media on the microhardness of human enamel blocks over four time periods.* ***Materials and Method:*** *The study received ethical approval. The surface microhardness of human enamel blocks was measured at baseline (T0). Subsequently, three blocks were assigned to each of six different disinfection-storage media combinations: acetic acid (5%) – chloramine (1%) [*AA-CT*], hypochlorite (5.25%) – chloramine (1%) [*NaOCl-CT*], chloramine (0.5%) – chloramine (1%) [*CT-CT*], acetic acid (5%) – thymol (0.02%) [*AA-TH*], hypochlorite (5.25%) – thymol (0.02%) [*NaOCl-TH*], and chloramine (0.5%) – thymol (0.02%) [*CT-TH*]. After two weeks (T1), one month (T2), three months (T3) or six months (T4) of storage at 4°C, microhardness was reassessed. Percentages of microhardness variation (mineral gain/loss) from baseline were calculated for each time. Data were analyzed using one-way ANOVA and two-way ANOVA to identify differences among disinfection-storage medium combinations and storage times, respectively.* ***Results:*** *A total 72 enamel blocks were analyzed. The microhardness variation percentages showed that the CT-TH combination (T1: 0.00%; T2: 4.05%; T3: 2.78%; T4: -4.74%; p>0.005) had the least variation, and that the microhardness values in the six groups at T3 were the most stable and did not differ significantly (p= 0.630).* ***Conclusion****: Combinations of chloramine-thymol and chloramine-chloramine generated the fewest changes in microhardness in enamel blocks, and are suitable for preserving extracted teeth.*

Keywords: *tooth - enamel - disinfection - storage*

Efecto de los medios de desinfección y almacenamiento sobre la microdureza superficial en bloques de esmalte humano.

RESUMEN

La desinfección y el almacenamiento protocolizados son procesos fundamentales para garantizar la calidad de las muestras dentales necesarias en los estudios in vitro; sin embargo, son escasas las evaluaciones sobre el efecto que tienen las distintas combinaciones de medios de desinfección y de almacenamiento sobre la microdureza superficial de bloques de esmalte humano. ***Objetivo:*** *Evaluar el efecto de diferentes medios de desinfección y almacenamiento sobre la microdureza de bloques de esmalte humano durante cuatro periodos de tiempo.* ***Materiales y método:*** *Con la aprobación ética, se midió la microdureza superficial de bloques de esmalte humano en el momento inicial (T0). Posteriormente, se asignaron tres bloques a seis grupos de combinaciones de medios de desinfección y almacenamiento: ácido acético (5 %) - cloramina (1 %) [AA-CT], hipoclorito (5,25 %) - cloramina (1 %) [NaOCl-CT], cloramina (0,5 %) - cloramina (1 %) [CT-CT], ácido acético (5 %) - timol (0,02 %) [AA-TH], hipoclorito (5,25 %) - timol (0,02 %) [NaOCl-TH] y cloramina (0,5 %) - timol (0,02 %) [CT-TH]. Tras dos semanas (T1), un mes (T2), tres meses (T3) o seis meses (T4) de almacenamiento a 4 °C, se volvió a evaluar la microdureza. Se calcularon los porcentajes de variación de la microdureza (ganancia/pérdida mineral) con respecto al valor inicial para cada momento. Los datos se analizaron mediante las pruebas de ANOVA de una vía y un ANOVA de dos vías para identificar las diferencias entre los grupos de combinación de desinfección-medio de almacenamiento y los tiempos de almacenamiento, respectivamente.* ***Resultados:*** *Se analizaron un total de 54 bloques de esmalte. Los porcentajes de variación de la microdureza mostraron que la combinación CT-TH (T1: 0,00 %; T2: 4,05 %; T3: 2,78 %; T4: -4,74 %; p > 0,005) presentaba la menor variación, y que los valores de microdureza obtenidos en los seis grupos en T3 fueron los que se mantuvieron más estable y por tanto no diferían significativamente (p = 0,630).* ***Conclusión:*** *Las combinaciones de cloramina-timol y cloramina-cloramina generaron los menores cambios en la microdureza de los bloques de esmalte y son adecuadas para la conservación de piezas dentales.*

Palabras clave: *dientes - esmalte - desinfección - almacenamiento*

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INTRODUCTION

Dental research often uses natural teeth to investigate pathologies and conditions of hard tissues, as well as to evaluate dental materials. Although human teeth are the most suitable substrate, freshly extracted samples are not always readily available[1], making it necessary to collect and store them for variable periods of time until they are used.

Extracted teeth are disinfected and stored in different media which prevent microbial growth, dehydration, and mineral structure changes. It is essential to choose the right medium to ensure the quality of these tissues when the time comes to analyze and evaluate them[2]. Disinfection media include sodium hypochlorite[2–4], acetic acid[3,5], formalin[4], 0.5% chloramine T[6,7], and thymol T T[7], among others [8], while storage solutions include saline solutions, chloramine T, formalin and thymol[1,2,9,10], which have been used at different concentrations with varying degrees of success.

Sodium hypochlorite is a non-specific proteolytic agent that effectively removes organic components at room temperature[11]. However, it is not recommended as a storage medium because increases the permeability of dental enamel and decreases the adhesion strength of tissue[4,12,13].

Acetic acid (5%) has antimicrobial properties related to its ability to alter bacterial cell membranes, denature proteins and cause cell death[3,5], and has been proposed as a disinfectant for extracted human teeth[5].

Formalin (10%) has antimicrobial and protein-binding properties; however, it should be used with caution due to its carcinogenic properties[14].

Chloramine T (0.5%) is a disinfectant activity, and optimum capacity, like that of the oral environment, to preserve the properties the dental structure[15]. Despite its similarity to sodium hypochlorite, chloramine T does not degrade the collagen structure[10]. Its effect on adhesive resistance in teeth is comparable to that obtained with freshly extracted teeth[16].

Thymol (0.1%) is not considered the most effective disinfectant; however, it is a good storage medium that adequately preserves tooth color and adhesive strength. However, it may interfere with methacrylate polymerization[17].

Despite the availability of isolated information for each medium, few evaluations have addressed the combined effect of disinfection and storage media on the surface microhardness of teeth exposed for different periods of time. In this context, it is critical to define the influence of different combinations of disinfection and storage media on tooth specimens in order to manage the protocols applied for all stages, from tooth collection to experimental use, thereby ensuring the quality of the results. Thus, the aim of this study was to evaluate the effect of different disinfection and storage media combinations on the microhardness of human enamel blocks over four time periods.

MATERIALS AND METHOD

This exploratory experimental study was approved by the Institutional Research Ethics Committee (Approval number 012-2017). Teeth extracted for therapeutic reasons unrelated to this study were collected from the university's oral surgery clinics through voluntary donation after the donors signed an informed consent form. Sample size was established as 18 recently-extracted sound teeth. Any teeth with caries lesions, coronal/radicular restorations, enamel development defects, or endodontic treatments were excluded. Specimens were collected by dentists in high-density polyethylene containers, immersed in 0.5% chloramine T solution, and transferred to the laboratory. Teeth were cleaned and organic tissue remains removed with Gracey 11/12 curettes and endodontic files.

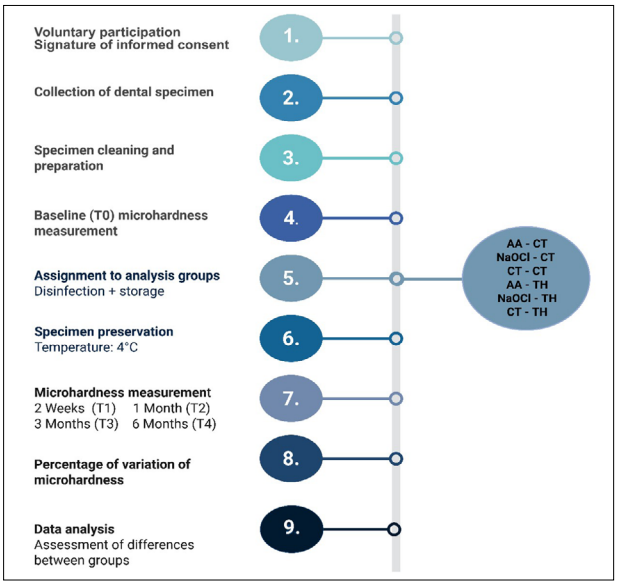

Fig. 1: Flowchart: The processing of enamel blocks exposed to different disinfection-storage media combinations.

A flowchart of the enamel block processing is shown in Fig. 1. Enamel blocks (4x4 mm) were

made from each surface of the tooth crowns (IsoMet 1000 - Buehler) and polished to obtain a flat surface (Bühler Metaserv 250). At baseline, transverse microhardness was assessed using microhardness tester (Wilson Hardness Model 1202) coupled with the FM-ARS analysis program. A Knoop-type diamond indenter with a load of 25 g for 5 seconds was used to make five indentations in each block. Values were expressed as Knoop Hardness Numbers (KHN) in kg/mm^2.

Then, three enamel blocks were randomly assigned to each of the following disinfection-storage media combination groups: acetic acid (5%) – chloramine (1%) [**AA-CT**], hypochlorite (5.25%) – chloramine (1%) [**NaOCl-CT**], chloramine (0.5%) – chloramine (1%) [**CT-CT**], acetic acid (5%) – thymol (0.02%) [**AA-TH**], hypochlorite (5.25%) – thymol (0.02%) [**NaOCl-TH**], and chloramine (0.5%) – thymol (0.02%) [**CT-TH**].

Each group of three blocks was immersed in the assigned disinfection medium for five minutes. Subsequently, the blocks were washed thoroughly with deionized water and transferred to the corresponding storage medium. Samples were stored at 4 °C and evaluated at four storage times: two weeks (T1), one month (T2), three months (T3), and six months (T4). At the end of each time, microhardness was reassessed. Change in microhardness was determined by calculating the difference between the baseline microhardness values and those obtained at each time point (T1–T4). The percentage of change was calculated through the MicroHardness Analysis (MHA) equation[18] and reported as a positive or negative value, indicating mineral loss or gain, respectively:

$$MHA = \frac{MHAbaseline - MHAfinal}{MHAbaseline} \times 100.$$

Statistical analysis

The percentage variations in the microhardness of the blocks exposed to different combinations of disinfection-storage media were recorded, organized in an Excel database, and analyzed using GraphPad Prism version 11.0.0 for Windows (GraphPad Software, Boston, Massachusetts USA). First, the normality of the data was assessed using the Shapiro-Wilk test. One-way ANOVA analysis of variance was used to evaluate the difference between groups, followed by two-way ANOVA with Tukey's post hoc test**,** to identify differences in the microhardness of enamel blocks exposed to disinfection-storage media combinations at four different times.

RESULTS

A total 72 enamel blocks were analyzed. Nineteen indentations were analyzed on the enamel blocks exposed to each disinfection-storage media combination, excluding any atypical microhardness measurements with values less than 100 kg/mm^2. Results of the analysis regarding the effect of the disinfection–storage medium combination on surface microhardness are shown in Fig. 2 as percentages of variation.

The Chloramine-Thymol (**CT-TH**) media combination (T1: 0.00%, T2: 4.05%, T3: 2.78%, T4: -4.74%, $p = 0.6301$) showed the most stable values of microhardness compared to the other combinations. Statistically significant differences ($p<0.05$) were observed in the microhardness variation of the blocks exposed to **NaOCl-CT** (T1: 0.00%, T2: 0.75%, T3: 21.93%, T4: 88.72%, $p<0.0001$) with respect to those exposed to **AA-TH** (T1: -2.77%, T2: 32.79%, T3: 17.11%, T4: 22.54%; $p = 0.0055$) and to **NaOCl-TH** (T1: 0.00%, T2: 3.31%, T3: 22.18%, T4: -7.55%; $p = 0.0055$) at the four analyzed times (Fig. 2). In addition, differences in microhardness were found, considering two factors – treatment and time, p<0.0001 (Table S1). Multiple comparisons test showed differences in microhardness measurements at 1 month (T2) and six months (T4) (Table S2).

The comparisons of the six disinfection-storage media combinations at each evaluated time point are shown in Fig. 3. No differences were found between the disinfection-storage media groups at T1 ($p=0.2227$), while significant differences were identified at T2 ($p=0.0060$). **AA-CT** (26.67%) and **NaOCl-CT** (0.75%) had statistically significant differences ($p=0.0099$), besides the microhardness observed for **AA-CT** (26.67%) and **CT-TH** (4.05%) ($p=0.0046$), and for **NaOCl-CT** (0.75%) and **AA-TH** (32.79%) ($p=0.0333$). The most stable values of dental samples microhardness were observed at T3 for all the combinations ($p = 0.2878$). At T4, the main differences were observed when comparing **AA-CT** (10.49%) and **NaOCl-CT** (88.72%) to the other analyzed combinations ($p<0.0001$) (Table 1).

Analysis based on disinfection-storage media combinations.

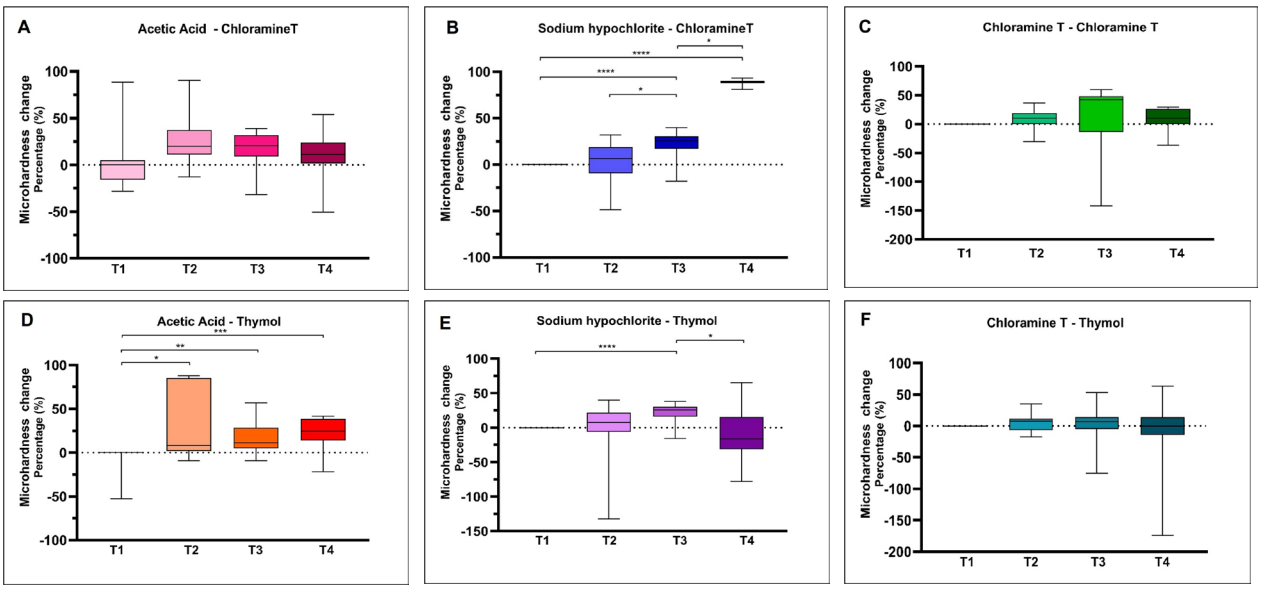

Fig. 2: Change in microhardness in enamel blocks exposed to disinfection-storage media. Colors represent the groups of analysis; color intensity increases over time. **A.** *Acetic Acid – Chloramine T (Pink),* **B.** *Sodium hypochlorite – Chloramine T (Blue),* **C.** *Chloramine T – Chloramine T (Green),* **D.** *Acetic Acid – Thymol (Orange),* **E.** *Sodium hypochlorite – Thymol (Purple),* **F.** *Chloramine T – Thymol (Bluish green). Differences between four times were evaluated for each treatment, p < 0.05 (*), p < 0.01 (**), p < 0.001 (***), p < 0.0001 (****).*

Analysis based on evaluation times.

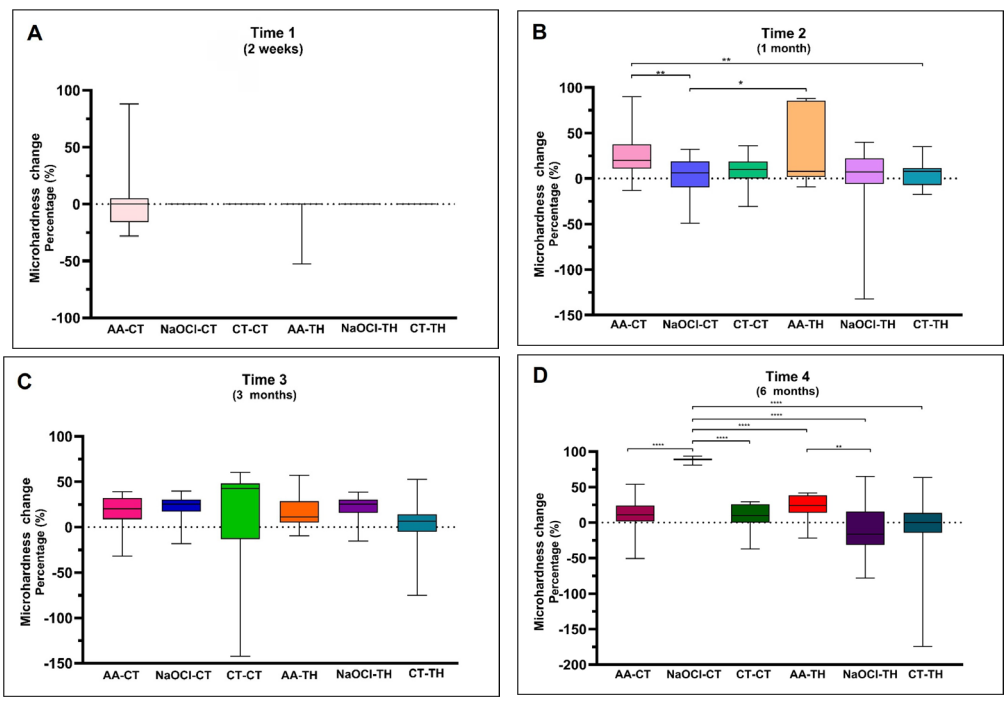

Fig. 3: Change in microhardness in enamel blocks exposed for four times (T1 to T4), to disinfection-storage media. Colors represent the groups of analysis; color intensity increases over time) Acetic Acid – Chloramine T (Pink); Sodium hypochlorite – Chloramine T (Blue); Chloramine T – Chloramine T (Green); Acetic Acid – Thymol (Orange); Sodium hypochlorite – Thymol (Purple); Chloramine T – Thymol (Bluish green). **A.** *Time 1 (2 weeks),* **B.** *Time 2 (1 Month),* **C.** *Time 3 (3 Months),* **D.** *Time 4 (6 Months). Multiple comparison tests were used to evaluate differences between combinations for each time. Statistical significances were presented p < 0.05 (*), p < 0.01 (**), p < 0.001 (***), p < 0.0001 (****).*

Table 1. Comparison of microhardness between treatments and times based on one-way ANOVA

Time	Disinfection-Storage Medium 1	%	Disinfection-Storage Medium 2	%	*Adjusted p value*
T1 2 weeks	NaOCl – CT	0.0	AA - CT	11.8	*0.8018*
	CT – CT	0.0	AA - CT	11.8	*0.8018*
	AA – TH	-2.77	AA - CT	11.8	*0.7604*
	CT – TH	0.0	AA - CT	11.8	*0.8018*
	NaOCl – TH	0.0	AA - CT	11.8	*0.8018*
	CT – CT	0.0	NaOCl - CT	0.0	--
	AA – TH	-2.77	NaOCl - CT	0.0	*0.9120*
	CT – TH	0.0	NaOCl - CT	0.0	--
	NaOCl – TH	0.0	NaOCl - CT	0.0	--
	AA – TH	-2.77	CT - CT	0.0	*0.9120*
	CT – TH	0.0	CT – TH	0.0	--
	NaOCl – TH	0.0	CT - CT	0.0	--
	CT – TH	0.0	AA - TH	-2.77	*0.9120*
	NaOCl – TH	0.0	AA - TH	-2.77	*0.9120*
	NaOCl – TH	0.0	CT - TH	0.0	--
T2 1 month	NaOCl – CT	0.75	AA - CT	26.67	***0.0099***
	CT – CT	8.36	AA - CT	26.67	*0.2218*
	AA – TH	32.79	AA - CT	26.67	*0.9947*
	CT – TH	4.05	AA - CT	26.67	***0.0046***
	NaOCl – TH	3.31	AA - CT	26.67	*0.2262*
	CT – CT	3.31	NaOCl - CT	0.75	*0.8610*
	AA – TH	32.79	NaOCl - CT	0.75	***0.0333***
	CT – TH	4.05	NaOCl - CT	0.75	*0.9938*
	NaOCl – TH	3.01	NaOCl - CT	0.75	*0.9999*
	AA – TH	32.79	CT - CT	8.36	*0.0834*
	CT – TH	4.05	CT - CT	8.36	*0.9694*
	NaOCl – TH	3.31	CT - CT	8.36	*0.9902*
	CT – TH	4.05	AA - TH	32.79	*0.1964*
	NaOCl – TH	3.31	AA - TH	32.79	*0.1503*
	NaOCl – TH	3.31	CT - TH	4.05	*>0.9999*
T3 3 months	NaOCl – CT	21.93	AA - CT	16.59	*0.8553*
	CT – CT	11.84	AA - CT	16.59	*0.9992*
	AA – TH	17.11	AA - CT	16.59	*>0.9999*
	CT – TH	2.78	AA - CT	16.59	*0.4226*
	NaOCl – TH	22.18	AA - CT	16.59	*0.8441*
	CT – CT	11.84	NaOCl - CT	21.93	*0.9704*
	AA – TH	17.11	NaOCl - CT	21.93	*0.9362*
	CT – TH	2.78	NaOCl - CT	21.93	*0.0584*
	NaOCl – TH	22.18	NaOCl - CT	21.93	*>0.9999*
	AA - TH	17.11	CT - CT	11.84	*0.9980*
	CT - TH	2.78	CT - CT	11.84	*0.9715*
	NaOCl - TH	22.18	CT - CT	11.84	*0.9775*
	CT - TH	2.78	AA - TH	17.11	*0.4514*
	NaOCl - TH	22.18	AA - TH	17.11	*0.9206*
	NaOCl - TH	22.18	CT - TH	2.78	*0.1395*

Table 1. continued

T4 6 months	NaOCl - CT	88.72	AA - CT	10.49	***<0.0001***
	CT - CT	8.45	AA - CT	10.49	*0.9990*
	AA - TH	22.54	AA - CT	10.49	*0.2937*
	CT - TH	-4.74	AA - CT	10.49	*0.7716*
	NaOCl - TH	-7.55	AA - CT	10.49	*0.4648*
	CT - CT	8.45	NaOCl - CT	88.72	***<0.0001***
	AA - TH	22.54	NaOCl - CT	88.72	***<0.0001***
	CT - TH	-4.74	NaOCl - CT	88.72	***<0.0001***
	NaOCl - TH	-7.55	NaOCl - CT	88.72	***<0.0001***
	AA - TH	22.54	CT - CT	8.45	*0.3274*
	CT - TH	-4.74	CT - CT	8.45	*0.8287*
	NaOCl - TH	-7.55	CT - CT	8.45	*0.6408*
	CT - TH	-4.74	AA - TH	22.54	*0.2873*
	NaOCl - TH	-7.55	AA - TH	22.54	***0.0073***
	NaOCl - TH	-7.55	CT - TH	-4.74	*>0.9999*

Differences between groups were assessed using one-way ANOVA. Multiple comparisons test was then performed, followed by Tukey's post-test. P-values less than 0.05 were regarded as significant, Not calculated --

DISCUSSION

This study evaluated the effects of disinfection and storage media on the microhardness of human enamel blocks evaluated after four different time periods. The combination of chloramine T and thymol showed the most stable surface microhardness values compared to the rest of the combinations at all evaluated times.

Disinfection and preservation processes need to be defined effectively in order to ensure the quality of dental samples to be used in research or academic processes. Two essential aspects are limiting cross-contamination and causing the least possible damage to the mineralized structure, since teeth that are not disinfected and are kept dry, even for short periods, have been found to be unsuitable for academic or research projects[10,18,19].

Extracted teeth should be immersed in a medium that (a) preserves their moisture, to reduce any structural changes, and (b) disinfects them, to reduce any potential health risks caused by blood-borne pathogens to people handling specimens during academic and research activities[20]. The latter is stated in the ISO guidelines for studies with dental materials (tooth adhesion tests)[21], and is the basis for the aim of this study.

This research helps highlight the importance of protocolized management of extracted teeth, which are used in different processes – mainly preclinical practices. The processes used by students to acquire extracted teeth are not currently regulated, exposing the dental school community to hazards that could easily be addressed through community awareness and protocol implementation[22].

The result of this study showed that the most stable microhardness values for the surface structure were observed with the chloramine T-thymol combination, in agreement with previous reports[7]. Thymol has been described as effective in preventing cross-contamination and sample dehydration, which supports its use as storage medium. However, using thymol to preserve extracted teeth should consider their intended use, as it has been reported to be suboptimal in adhesion studies[1,7,16,23]. Thymol has been described as an inhibitor of methacrylate polymerization[1], as well as causing some alterations in the calcium, potassium, sodium, and phosphorus content of enamel[24] and dentin[25].

The second option found in this study to be adequate for disinfection-storage was the combination of chloramine T at 0.5% and 1%, as it did not generate considerable structural changes. This agrees with previous reports on the use of chloramine as a disinfectant and storage medium for extracted teeth[7,26]. ISO guidelines and Colombian Technical Standard (NTC 4882) consider the use of chloramine T 0.5% as a storage medium for dental samples for a period not exceeding one week, considering that other chemical agents can be absorbed and generate alterations[21,27]. This alternative has been shown to have

no significant effects on the hardness, modulus of elasticity, or morphological characteristics of dental samples[26]. However, there are some contradictory reports regarding its effect on adhesion[7,9,26].

Not all combinations with chloramine T produced favorable results. The combination hypochlorite-chloramine T displayed the greatest variation in surface microhardness, especially at the longest exposure time. Although hypochlorite has been widely used in protocols for disinfecting and inactivating dental samples due to its antimicrobial properties, it affects dental hardness[28] and collagen content[29], making it an unsuitable storage medium for preserving dental samples to be used in research.

Although this study did not find optimal results with acetic acid, it has been described not only as having high efficacy as a disinfectant, but also for the preservation of the morphological properties of dental specimens[30]. Further studies are needed to determine the ideal disinfection and storage medium for different applications and the maximum storage time for human tooth banks.

CONCLUSION

This study found that the chloramine-thymol and chloramine-chloramine combinations of disinfection-storage media resulted in the most stable enamel surface microhardness values, suggesting that they are appropriate for maintaining tooth integrity during storage.

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CONFLICT OF INTERESTS

The authors declare no potential conflicts of interest regarding the research, authorship, and/or publication of this article.

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Table S1. Two-way ANOVA analysis

ANOVA table	SS	DF	MS	F (DFn, DFd)	*P value*
Time x T Disinfection-Storage Medium	106419	15	7095	F (15, 324) = 10.36	*p <0.0001*
Time	20566	3	6855	F (3, 324) = 10.01	*p <0.0001*
Disinfection-Storage Medium	38826	5	7765	F (5, 108) = 11.95	*p <0.0001*

Table S2. Comparison of microhardness between treatments and times based on two-way ANOVA

Time	Disinfection-Storage Medium 1	(%)	Disinfection-Storage Medium 2	(%)	*p value*
T2 (1 month)	NaOCl – CT	26.67	AA - CT	0.75	***0.0272***
	CT – CT	26.67	AA - CT	8.36	*0.2537*
	AA – TH	26.67	AA - CT	32.79	*0.9788*
	CT – TH	26.67	AA - CT	4.05	*0.081*
	NaOCl – TH	26.67	AA - CT	3.31	*0.0645*
	CT – CT	0.75	NaOCl - CT	8.36	*0.9459*
	AA – TH	0.75	NaOCl - CT	32.79	***0.0023***
	CT – TH	0.75	NaOCl - CT	4.05	*0.9988*
	NaOCl – TH	0.75	NaOCl - CT	3.31	*0.9997*
	AA – TH	8.36	CT - CT	32.79	***0.0456***
	CT – TH	8.36	CT - CT	4.05	*0.9957*
	NaOCl – TH	8.36	CT - CT	3.31	*0.9911*
	CT – TH	32.79	AA - TH	4.05	***0.0093***
	NaOCl – TH	32.79	AA - TH	3.31	***0.0069***
	NaOCl – TH	4.051	CT - TH	3.31	*>0.9999*
T4 (6 months)	NaOCl - CT	10.49	AA - CT	88.72	***<0.0001***
	CT - CT	10.49	AA - CT	8.453	*0.9999*
	AA - TH	10.49	AA - CT	22.54	*0.7098*
	CT - TH	10.49	AA - CT	-4.74	*0.4630*
	NaOCl - TH	10.49	AA - CT	-7.55	*0.2694*
	CT - CT	88.72	NaOCl - CT	8.45	***<0.0001***
	AA - TH	88.72	NaOCl - CT	22.54	***<0.0001***
	CT - TH	88.72	NaOCl - CT	-4.74	***<0.0001***
	NaOCl - TH	88.72	NaOCl - CT	-7.55	***<0.0001***
	AA - TH	8.45	CT - CT	22.54	*0.5522*
	CT - TH	8.453	CT - CT	-4.739	0.6229
	NaOCl - TH	8.453	CT - CT	-7.547	0.4056
	CT - TH	22.54	AA - TH	-4.739	**0.0165**
	NaOCl - TH	22.54	AA - TH	-7.547	**0.0053**
	NaOCl - TH	-4.739	CT - TH	-7.547	0.9995

Differences between groups were assessed using two-way ANOVA. A multiple comparisons test was then performed. followed by Tukey's post-test. P-values less than 0.05 were regarded as significant

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Trigeminal neuroplasticity after pulpitis and oral administration of paracetamol in rats

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ABSTRACT

Experimental stimulation of dental pulp nociceptors induces plastic changes in the trigeminal subnucleus caudalis (Vc) through increased intracellular calcium levels and discharge of nociceptive afferent fibers. Acetaminophen (paracetamol) is a potent and selective inhibitor of COX-2 within the central nervous system (CNS) and may act in association with the cannabinoid system and the descending serotonergic pathway. ***Aim:*** *In this context, the main objective of this study is to analyze the plastic changes induced by paracetamol in the nociceptive circuit of the Vc following unilateral dental pulp inflammation (pulpitis) in rats.* ***Material and Method:*** *Twenty-one female Wistar rats, 50 days old, were divided into three groups. In the experimental group 1 (EG1), a unilateral pulpitis was induced in the left mandibular first molar, and animals received no further treatment. In group 2 (EG2), the procedure was the same as in EG1, but 48 h later, animals received paracetamol in 3 daily doses (300 mg/kg each) every 4 h for 2 days. The control group (CG) received no treatment. After euthanasia, liver, hemimandibles and brainstem samples were analyzed. The results were analyzed using Student's t-test or ANOVA ($p<0.05$).* ***Results:*** *5-hydroxytryptamine transporter (5HT-T) analysis in the ipsilateral Vc demonstrated that paracetamol administration increased plexus length and varicosity density compared with EG1 and CG ($p<0.05$). Paracetamol also increased cannabinoid CB1 receptor (CB1r) expression ($p<0.05$), and decreased the number of calbindin D28K positive projection neurons ($p<0.05$).* ***Conclusion:*** *Following experimental pulpitis, oral administration of paracetamol induces plastic changes in the serotonergic and endocannabinoid systems within the Vc. This plasticity is reflected by a reduced number of fusiform calbindin D28K projection neurons in lamina I, associated with modulation of orofacial nociceptive processing.*

Keywords: *acetaminophen - receptor, cannabinoid - CB1- serotonin plasma membrane transport proteins - trigeminal caudal nucleus - nociception - pulpitis*

Neuroplasticidad trigeminal luego de una pulpitis y administración oral de paracetamol en ratas

RESUMEN

La estimulación experimental de los nociceptores de la pulpa dental induce cambios plásticos en el subnúcleo caudal del trigémino (Vc) generando un aumento de los niveles de calcio intracelular y la descarga de las fibras aferentes nociceptivas. El paracetamol es un potente inhibidor selectivo de la COX-2 en el sistema nervioso central (SNC) y puede actuar en asociación con el sistema cannabinoide y la vía serotoninérgica descendente. ***Objetivo:*** *el objetivo principal de este estudio es analizar los cambios plásticos inducidos por el paracetamol en el circuito nociceptivo del Vc tras una inflamación unilateral de la pulpa dental (pulpitis) en ratas.* ***Materiales y métodos:*** *21 ratas Wistar hembras, de 50 días de edad, se dividieron en tres grupos. En el grupo experimental 1 (GE1), se indujo una pulpitis unilateral en el primer molar mandibular izquierdo, y los animales no recibieron ningún tratamiento adicional. En el grupo 2 (GE2), el procedimiento fue el mismo que en GE1, pero 48 h después, los animales recibieron paracetamol en 3 dosis diarias (300 mg/kg cada una) cada 4 h durante 2 días. El grupo control (GC) no recibió ningún procedimiento. Tras la eutanasia, se analizaron muestras de hígado, hemimandíbulas y tronco encefálico. Los resultados se analizaron mediante la prueba t de Student o ANOVA ($p < 0,05$).* ***Resultados:*** *En el Vc ipsilateral del GE2 se demostró que la administración de paracetamol aumentó la longitud del plexo y la densidad de varicosidades del transportador de 5-hidroxitriptamina (5HT-T) en comparación con el EG1 y el CG ($p < 0,05$). También se demostró mayor expresión del receptor cannabinoide CB1 (CB1r) y menor número de neuronas de proyección positivas para calbindina D28K ($p < 0,05$).* ***Conclusión:*** *Tras una pulpitis experimental, la administración oral de paracetamol induce cambios plásticos en los sistemas serotoninérgico y endocannabinoide dentro del Vc. Esta neuroplasticidad se refleja en una reducción del número de neuronas de proyección fusiformes positivas para calbindina D28K en la lámina I, asociada a la modulación del procesamiento nociceptivo orofacial.*

Palabras clave: *paracetamol - receptor cannabinoide - CB1 - proteínas de transporte de serotonina en la membrana plasmática - núcleo caudal del trigémino - nocicepción - pulpitis*

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INTRODUCTION

Prostaglandins play an essential role in processing inflammatory pain, not only by sensitizing the peripheral terminals of primary afferent fibers, but also by increasing input of nociceptive information in the central nervous system (CNS)[1]. The sensory information of orofacial structures is relayed by the trigeminal system, which comprises principal, mesencephalic and spinal nuclei. Nociceptive information is processed in the spinal subnucleus oralis (Vo), interpolaris (Vi) and caudalis (Vc), depending on the orofacial region involved. Experimental stimulation of orofacial nociceptors has been shown to induce plastic changes (neuroplasticity) at various levels of nociceptive pathways[2,3]. Afferent projections from mandibular tooth pulp terminate in the dorsomedial region of the Vc, which has been identified as the first center involved in orofacial nociceptive modulation[4]. Glutamatergic trigeminal ganglion neurons carry the nociceptive information at the superficial laminae I and II and activate glutamatergic receptors. From laminae I (LI), those nociceptive messages are transmitted by fusiform projection neurons to other regions of the brainstem and to the thalamus[4,5]. The majority of fusiform neurons in LI project to supraspinal targets and more than half co-express glutamate and calbindin immunoreactivity. Calbindin may therefore serve as a marker of projection neurons and contribute to buffering intracellular calcium increases induced by discharge of nociceptive afferent fibers[5]. These transient changes in local Vc circuits are critical for maintaining balance between excitatory and inhibitory neurons in nociceptive pathways[6,7]. Neurons in substantia gelatinosa or lamina II (LII) are considered interneurons that process sensory information and do not project out of Vc. In addition to local modulation by the Vc, the descending system presents components functionally related with those of the opioid, endocannabinoid, noradrenergic and serotonergic systems, and play an important role in the orofacial nociceptive pathway[8,9].

Dental clinical evidence shows that patients with pulpitis frequently undergo analgesic therapy to mitigate treatment-associated pain. Acetaminophen (paracetamol) is an effective analgesic medication which is metabolized in the liver and can thus cause liver injury at high doses[10]. In terms of functional effects, paracetamol exerts weak inhibition of peripheral COX-1 and COX-2 linked to the inflammatory response following injury[11,12], but acts predominantly at the CNS. It also produces antinociceptive effects through interactions with serotonergic and endocannabinoid pathways[13-15]. Descending facilitatory and inhibitory serotoninergic fibers originated in the midbrain periaqueductal gray (PAG) and ventromedial medulla (VMM) descend and make contacts with primary afferents and interneurons at laminae of Vc[8,9]. The 5-hydroxytryptamine transporter (5HT-T) modulates neurotransmission by facilitating the removal of serotonin from synapses through neurotransmitter reuptake by presynaptic terminals[16]. In turn, the effects of endocannabinoids are mediated by receptors CB1 or CB2 and are responsible of physiological responses as analgesia and synaptic plasticity, among others[17]. Recent research has demonstrated that cannabinoid CB1 receptor (CB1r) is the most abundant in CNS and is expressed in glutamatergic, GABAergic, serotonergic, cholinergic, noradrenergic and dopaminergic neurons[18]. The activation of these receptors present in both the plasmatic membrane and on the outer mitochondrial membrane, decreases the release of glutamate and GABA[14,18,19]. Given the limited evidence available of the action of serotonin and CB1r on Vc cells, this study analyzed paracetamol-induced plastic changes in the trigeminal Vc nociceptive circuit after unilateral pulpitis (inflammation of the dental pulp) in rats.

We hypothesize that oral administration of paracetamol after pulpitis induces neuroplastic changes in the Vc through serotonergic and endocannabinoid mechanisms. These changes are reflected by alterations in 5HT-T expression, CB1 receptor distribution, and in the number of calbindin D28K-positive neurons. These mechanisms are likely interconnected rather than independent, as evidence suggests that paracetamol metabolites (e.g., AM404) simultaneously engage cannabinoid receptors and serotonergic pathways within central pain circuits[14, 15].

Current evidence indicates that paracetamol analgesia involves a multi-target central mechanism, including indirect activation of CB1 receptors and facilitation of descending serotonergic pathways, rather than isolated actions on COX enzymes.

MATERIALS AND METHOD

Animals

Animals were housed in galvanized steel cages, with three animals per cage, in a temperature-controlled room (21-24°C) under 12-hour light/dark cycle. Food (Standard diet rat-mouse chow – Cooperación) and water were provided ad libitum. Our protocol followed the internationally accepted standards of the Guidelines for the Care and Use of Laboratory Animals (NIH Publication 1985, and the National Academy of Science, US) and was approved by the Ethics Committee of the School of Dentistry at Universidad de Buenos Aires (Cicual-Odon/FOUBA 006/2023). Twenty-one female Wistar rats, 50 days old, weighing 120-140 g were randomly assigned to one of three groups of seven animals each (n=7), using a simple randomization method. In experimental group 1 (EG1), a unilateral dental pulp injury was induced in the left mandibular first molar, and animals received no further treatment. In experimental group 2 (EG2), the procedure was the same as in EG1, but 48 h later animals received paracetamol antinociceptive treatment, similar to the analgesia used when the nociceptive pathway is activated by inflammatory tissue injury in humans. The control group (CG) received no treatment.

Surgical procedure of molar access cavity

Animals were anesthetized by intraperitoneal injection of ketamine (50 mg/kg, Holliday Laboratory) and xylazine (10 mg/kg König Laboratory). In the pulpitis groups, i.e., EG1 and EG2, enamel and dentin were removed from the left mandibular first molar using a 1/4 round bur at low speed to expose the mesial horn of the pulp chamber (ipsilateral). The cavity was then sealed with phosphate cement (Harvard; Richter and Hoffmann, Berlin, Germany) to prevent bacterial contamination and stimulate pulp inflammation through direct contact with cement. After the procedure, animals were monitored for discomfort or pain, and body weight was followed up until euthanasia. Only in the first 24 h after surgery, EG1 and EG2 animals showed decreased activity and ate less than the previous day. In EG2, 900 mg/kg/day paracetamol (Termofren™ drops, Roemmers, Buenos Aires, Argentina) for 2d [10,20,21]. Paracetamol was administrated orally starting 48 h after pulp injury to simulate delayed clinical analgesic intervention. Animals received three doses (300 mg/kg each) per day (every 4 h starting at 8 am), resulting in a 12-hour drug-free interval. On day 4, EG2 and CG animals were anesthetized and 500 µl blood was collected from the rat tail vessels for analysis of serum transaminase levels (GPT: alanine transaminase and GOT: aspartate transaminase) and to evaluate the potential hepatotoxic effect of paracetamol. Serum transaminase levels in control animals were used as reference values.

Fixation and dissection

On day 4 after molar pulp injury, all animal groups were euthanized with anesthesia overdose and transcardially perfused through the left ventricle with cold saline solution (Ringer's sodium chloride - potassium chloride - calcium chloride) plus 50 IU heparin and 60 ml cold paraformaldehyde (Merck, Rahway, NJ, US) 4% (w/v) in 0.1 M phosphate buffer, pH 7.3-7.5. After euthanasia, animals were dissected and liver, hemimandibles and brainstem samples collected. Livers were removed and processed using routine histologic techniques. Paraplast (Sigma-Aldrich, Burlington, MA, US) sections of approximately 8-µm thickness were prepared and stained with hematoxylin and eosin (H&E). To detect glycogen deposition in hepatocytes some sections were processed through periodic acid-Schiff (PAS) histochemistry. The hemimandibles were decalcified in 10% EDTA/phosphate-buffered saline (PBS) 1X for 5 wk. and embedded in Paraplast. Mesiodistal longitudinal sections of approximately 8-µm thickness were obtained from the mandibular first molars, and sections were stained with H&E. After brainstem dissection, the right ventral side was marked with a blade to identify the contralateral side. Brainstems were cryoprotected in sucrose/PBS 30% (w/v), and frozen coronal sections (30 µm) from the C4 to -5.6 interaural level[22] were collected as floating sections into PBS/azide.

Immunofluorescence

Sections were incubated in blocking solution (3% normal sheep serum, Tris Buffer saline (TBS), 0.3% Triton X-100) for 2-4 hours at room temperature. At that time, sections were incubated for 48 hours at 4°C with combined primary antibodies: mouse monoclonal anti-5-hydroxytryptamine transporter (RRID Millipore Cat# MAB1564, RRID:AB_94220 1:1000), rabbit anti-calbindin D28K (1:5000; RRID Lot Nº 03, Swant Cat# CB38, RRID:AB_2721225) and rabbit anti-CB1r (1:3000; RRID Cayman Chemical Cat#

10006590, RRID:AB_10098690) in 1% normal sheep serum, 0.1% Triton X-100, TBS. Negative controls were performed by replacing primary antibodies with goat serum. After 40 min washing with TBS, rhodamine red (TRITC 1:2000 Jackson Immuno Research) or fluorescein isothiocyanate (FITC 1:2000 Jackson Immuno Research) conjugated secondary antibodies were used. Sections were incubated for 3 hours, and then washed with TBS for 30 min. Cell nuclei were labeled with the fluorescent DNA marker Hoechst 33342. Finally, sections were mounted on glass slides previously dipped in 1% gelatin, air dried and slides were coverslipped using TBS:glycerol (1:3) for UV microscopic observation.

Image analysis

Photomicrographs were acquired with a Zeiss Axiophot light microscope equipped with epifluorescence, alternating between FITC and rhodamine filter sets. Images were captured at 24-bit resolution (8 bpp × 3 channels) using an Olympus Q-color 5 camera. Histomorphometric analysis was performed on digital photomicrograph from histological sections using Image Pro Plus 6 software (Media Cybernetics, Silver Springs, MD, US). Image acquisition and analyses were performed by an observer blinded to group allocation. The following parameters were studied:

On livers: histological features on H&E stained and PAS histochemistry sections.

On left mandibular first molars: percentage of pulp inflammation volume per total cameral pulp volume (2D) (IV/PV_{2D}%) and histological features.

On dorsomedial area and two levels of ipsilateral Vc per animal:

I. 5HT-T plexus: determined as the total length of fibers (in μm) and quantification of 5HT-T varicosities (in number of varicosities) / area of 0.01 mm^2 in laminae I, II and III).
II. Calb+ 5HT-T: percentage of calbindin D28k positive neurons with 5HT-T varicosities around the soma / total calbindin D28k positive neurons per area of 0.01 mm^2 in laminae I, II and III, in %.
III. CB1r or Calbindin D28k positive cells: quantification of fusiform somas compatible with the morphology of projection neurons in LI and quantification of smaller somas compatible with modulatory interneurons in LII / area of 0.05 mm^2

Statistical analysis

Data are shown as means ± SEM. Data were statistically compared by Student t-test or two-way analyses of variance (ANOVA), followed by the post hoc Bonferroni test. Statistical differences were considered significant for p-values below 0.05. All statistical analyses were performed with Graph Pad Prism (version 5.03; GraphPad Software, Williston, VT, UD).

RESULTS

Analysis of hepatotoxicity

To evaluate hepatotoxicity associated with paracetamol, the livers of CG and EG2 were processed using routine histological techniques with H&E (Fig. 1A - C) and examined for glycogen deposits using PAS (Fig. 1D - F) staining. After paracetamol administration, the hepatic tissue and cell morphology showed normal histological appearance. Hepatocytes showed positive PAS staining for intracellular glycogen deposits. In the portal space, the morphology was preserved and exhibited no evidence of inflammatory infiltrate. Biochemical serum final transaminase levels (UI/L) exhibited no significant differences between EG2 and CG (final GPT EG2: 68.5 ± 12.0; CG: 78.5 ± 27.6; final GOT EG2: 292 ± 11.3; CG: 250 ± 19.8; $p>0.05$).

Analysis of left mandibular first molar in situ

In CG (Fig. 2 A), the molars showed normal histological features, with no signs of inflammatory process in the pulp or periodontal ligament. Sections of left mandibular first molar in EG1 and EG2 revealed an acute inflammatory infiltrate in the coronal pulp in direct contact with the sealing material 4 d post injury. Inflammatory reactions observed in both groups were similar in terms of the percentage of volume pulp inflammation in relation to the total volume cameral pulp (IV/PV_{2D}%) (EG1: 29% ± 2.92; EG2: 28% ± 2.41; $p>0.05$) and histological features (Fig. 2 B and C). This histopathological reaction may be compatible with a pulpitis diagnosis and the consequent nociceptive stimulation.

Analysis of 5HT-T expression in the Vc

Descending serotonergic fibers showed varicosities along their path and 5HT-T was expressed in these varicosities. 5HT-T analysis revealed that the descending fibers were distributed as a plexus

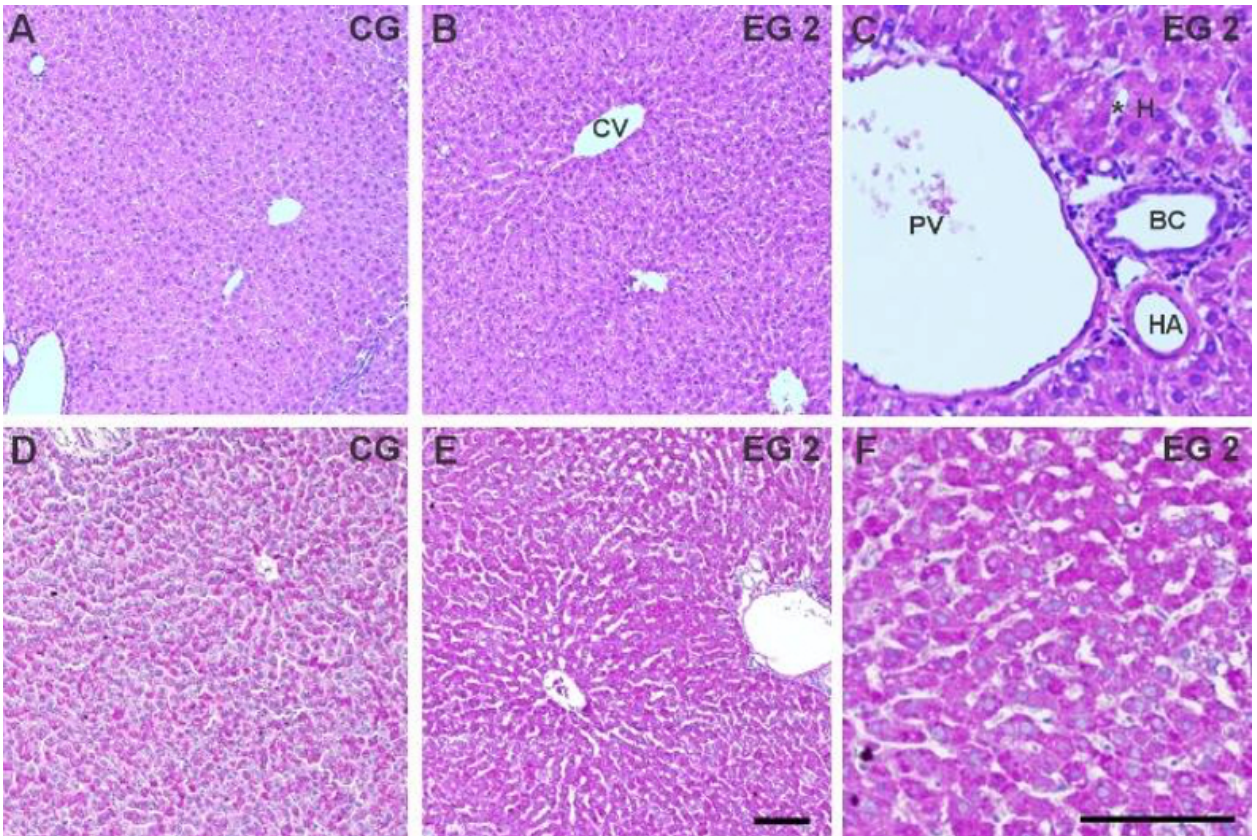

*Fig. 1: Microphotographs of the liver from representative animal specimens from CG (A and D) and EG2 (B, C, E and F) processed with H&E (A - C) and PAS (D - F). In both groups, the hepatic acinus and portal space display normal histological appearance (C) and hepatocytes retained intracellular glycogen deposits (F). HA: hepatic artery, PV: portal vein, BD: bile duct, CV: central vein, H: hepatocytes and *: sinusoidal capillaries and Kupffer cell. The scale bar represents 100 µm*

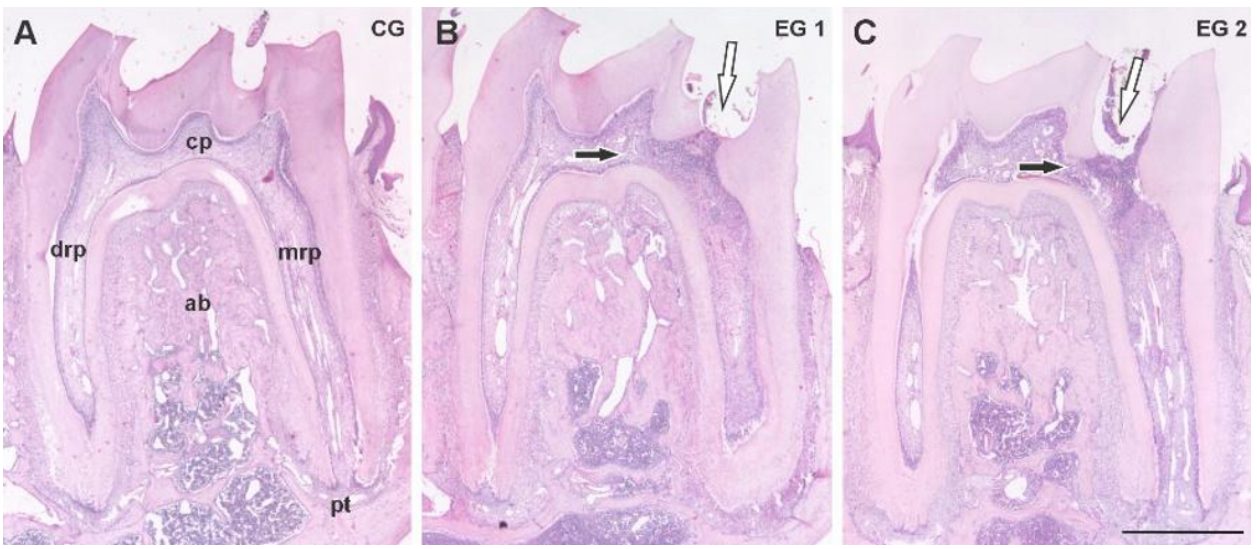

Fig. 2: Sections showing control (A) and experimental left mandibular first molar (B and C) in the alveolar bone (ab) stained with H&E. B and C show the mesial cavity (black arrow), and the extent of the inflammatory process (white arrow) in the cameral pulp (cp). The inflammatory reactions in EG1 (B) and EG2 (C) show numerous inflammatory cells, mainly lymphocytes and macrophages. Note that the mesial root pulp (mrp), distal root pulp (drp) and the periapical tissues (pt) appeared free of morphological signs of inflammation (A – C). The scale bar represents 1000 µm.

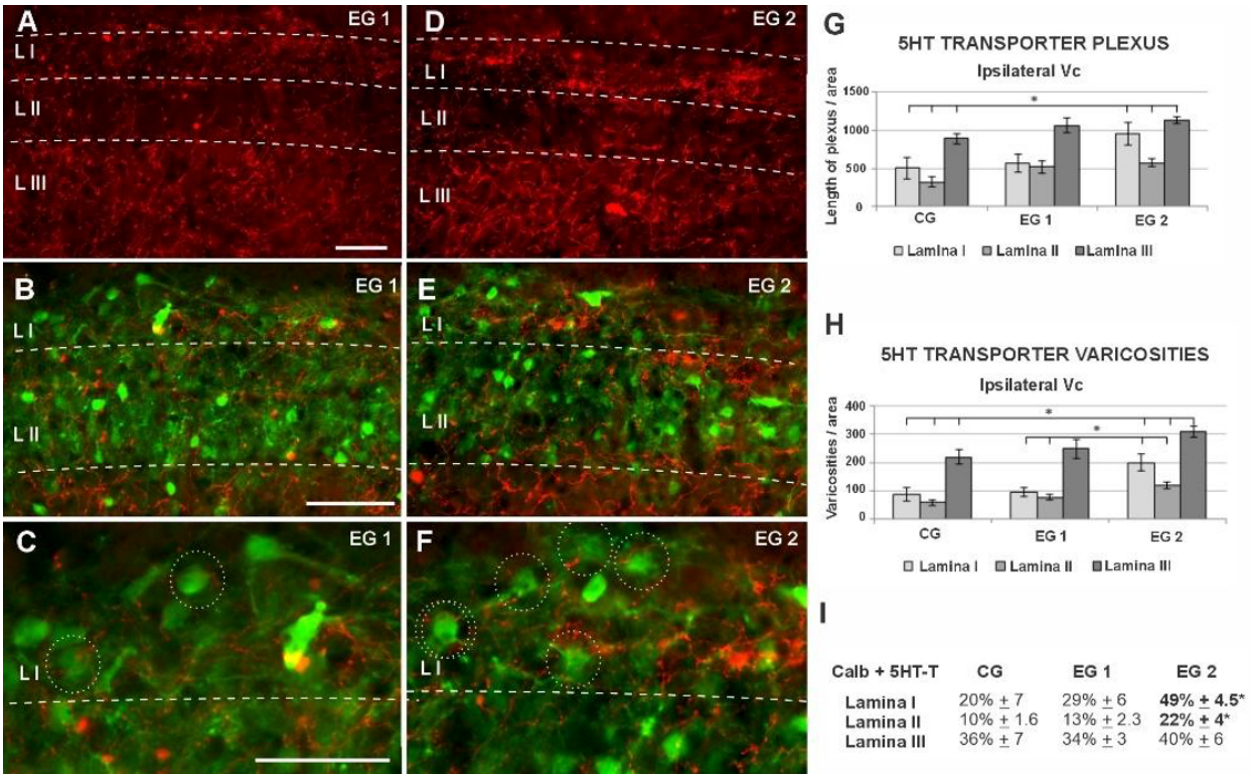

*Fig. 3: Analysis of the 5HT-T in the laminae of the ipsilateral Vc to molar pulpitis (A and D). The length of the plexus (G) and the quantification of its varicosities (H) in superficial laminae (LI and LII) and deeper lamina (LIII) are represented in the bar graphs for CG, EG1 and EG2. B-C and E-F show the co-expression of calbindin D28K (FITC) and 5HT-T varicosities (rhodamine). The table (I) presents the mean percentage + SEM of calbindin D28K neurons with 5HT-T varicosities around the soma for each lamina of the Vc in all groups. The scale bar represents 50 µm. * $p<0.05$*

in the Vc, primarily in LI and III, with a minimal presence in LII (Fig. 3 A and D). Comparisons of plexus length in each lamina of the ipsilateral Vc rendered significantly greater length in LI, II and III of EG2 compared to CG (Fig. 3 G). Paracetamol administration significantly increased the number of 5HT-T positive varicosities in LI (CG: 88.13 ± 24.40; EG1: 95.36 ± 14.47; EG2: 200.36 ± 28.99; $p<0.05$) and II (CG: 57.92 ± 10.78; EG1: 76.79 ± 9.43; EG2: 118.39 ± 12.48; $p<0.05$) (Fig. 3 A, D and H).

Furthermore, 5HT-T varicosities associated with calbindin D28K positive neurons were quantified. In LI, projection neurons showed a fusiform soma and two long primary extensions emerging from each end of the soma. LII showed smaller neurons compatible with the morphology of modulatory interneurons (Fig. 3 B-C, E-F). EG2 demonstrated a significant increase of varicosities associated with calbindin D28K positive somas in LI (CG: 20% ± 7; EG1: 29% ± 6; EG2: 49% ± 4.5; $p<0.05$) and LII (CG: 10% ± 1.6; EG1: 13% ± 2.3; EG2: 22% ± 4; $p<0.05$) (Fig. 3 I).

Analysis of CB1r expression in the Vc

CB1r expression was observed in the perinuclear cytoplasm and at the beginning of the neuronal processes in both projection neurons (P) and interneurons (M). Furthermore, the distribution of CB1r in nociceptive LI-II and V was studied (Fig. 4 A - C). Expression analysis of CB1r in the superficial laminae demonstrated that the number of projection neurons located in LI on the ipsilateral side was significantly greater in EG2 than in EG1 or CG (CG: 3.67 ± 0.33; EG1: 2.6 ± 0.24; EG2: 6.0 ± 0.58; $p< 0.05$). In LII, no significant changes were observed in CB1r expression (CG: 26 ± 3.06; EG1: 18 ± 2.10; EG2: 20 ± 2.16; $p > 0.05$) (Fig 4 D, E and H, I).

Analysis of calbindin D28K expression in the Vc

The number of calbindin D28K positive fusiform neurons per area of LI in the ipsilateral Vc was significantly larger in EG1 than in EG2 or CG at 4 d post-injury (CG: 3.3 ± 0.63; EG1: 4.29 ± 0.47; EG2:3.0 ± 0.19; $p<0.05$). In contrast, calbindin D28K expression in smaller modulatory interneurons of

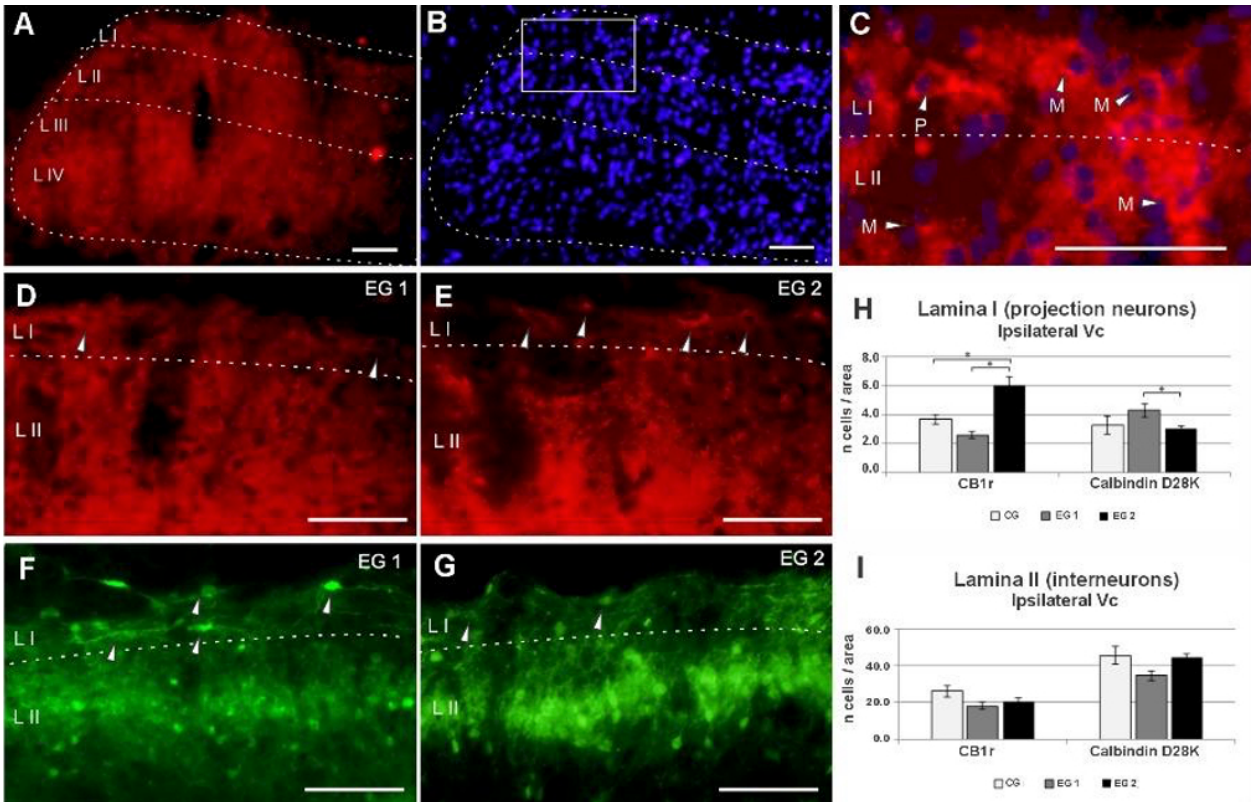

*Fig. 4: Coronal sections of the Vc using the immunofluorescence technique for CB1r (A) and fluorescent DNA marker Hoechst for cell nuclei labeling (B). Arrowhead in C indicate co-expression in projection (P) and modulatory (M) neurons LI and LII. Arrowheads indicate CB1r positive projection neurons (D and E) and calbindin D28K neurons (F and G) in the ipsilateral Vc of experimental groups. The bar graphs represent the number of positive projection neurons (G) and positive modulatory neurons (H) per area in LI or LII of each group. The scale bar represents 50 µm. * $p<0.05$*

LII showed non-significant differences (CG: 45.5 ± 6.19; EG1: 34.4 ± 10.34; EG2: 44.38 ± 7.85; $p > 0.05$) (Fig. 4 F - I).

DISCUSSION

Paracetamol is occasionally used to manage pain before and after dental procedures. In EG2, paracetamol was administrated orally starting 48 h after pulp injury to simulate delayed clinical analgesic intervention, once pulp inflammation (pulpitis) had developed. To maximize antinociceptive efficacy, the EG2 animals received three doses (300 mg/kg each) per day (every 4 h), resulting in a 12-hour drug-free interval. The dosing used in this study is higher than typical clinical doses in humans, however, such doses are commonly employed in experimental models[20,21] to ensure central pharmacological effects. Because 90–95% of paracetamol undergoes hepatic biotransformation and its toxicity increases with dose[12], we examined liver histology and serum transaminases in both control and paracetamol treated rats to check for possible liver toxicity. Importantly, no hepatotoxicity was detected, consistent with previous studies showing that toxicity depends on metabolic saturation rather than dose alone under controlled conditions[10,12,21]. Histological assessment showed preserved lobular architecture and portal tracts in paracetamol-treated animals. Hepatocytes contained cytoplasmic glycogen, and there were no signs of necrosis or inflammation. Serum transaminase levels in EG2 remained within CG ranges. These findings confirm that, under our dosing regimen, rats do not develop paracetamol-induced liver injury[10]. Nevertheless, caution is warranted when extrapolating these findings to clinical settings.

Different tooth injuries induce varying degrees of neuroplastic change at the trigeminal nucleus, depending on severity. In this study we used a type II intermediate molar tooth injury, which caused partial pulp loss, controlled infection with an inflammatory reaction, and occasionally necrosis of

the pulp, but did not spread to adjacent territories such as periapical tissues[23]. Pulp inflammation was induced in the left mandibular first molar by exposing the mesial pulp chamber, and sealing with restorative material was then performed to mimic what may happen in the dental clinic procedures. Histopathological examination of the exposed pulp confirmed the development of pulpitis. This inflammatory response generates nociceptive signals that are conveyed predominantly via Aδ and C primary afferent fibers originated from the trigeminal ganglion and carry nociceptive information to the Vc[4].

In recent years, we have studied neurons in LI, II, and V of the rat Vc and their modulatory role in the orofacial nociceptive pathway after pulpitis[7]. Our analysis focused on female rats, testing the hypothesis of sex-based differences in nociceptive thresholds. The literature indicates that these responses are highly genotype-dependent; for instance, while Sprague-Dawley and Long-Evans strains exhibit dimorphic sensitivity, Wistar-Kyoto rats often show no significant difference between sexes[24]. Consequently, the present study evaluates the potential antinociceptive effect of paracetamol in female Wistar rats. Within this framework, both EG1 and EG2 groups displayed a similar degree of pulp inflammation, indicating that paracetamol exerts a weak inhibitory effect on peripheral pulp inflammation—an observation consistent with its limited efficacy in other inflamed tissues[11,12]. In contrast, recent evidence indicates that the central analgesic effects of paracetamol are mediated by its metabolite AM404, which activates CB1 receptors and interacts with serotonergic pathways within central nociceptive circuits[14,15,25.] Nevertheless, how oral paracetamol modulates synaptic and cellular plasticity within the Vc in an odontogenic pulpitis model remains to be determined.

Paracetamol administration is associated with remodeling of the serotonergic pathways in the superficial dorsal laminae of the Vc. EG2 exhibited a significant increase in both the length of 5HT-T positive plexus and the density of 5HT-T–immunoreactive varicosities, indicative of enhanced serotonin reuptake capacity[16]. Co-localization analysis further revealed that these 5HT-T varicosities formed close appositions nearby calbindin-D28k–positive neurons. These findings suggest modulation of nociceptive processing rather than direct evidence of functional inhibition.

Moreover, the cannabinoid system modulates synaptic transmission within SNC[17]. Activation of CB1r in neurons in the trigeminal complex inhibits neurotransmitter release, leading to a decrease in nociceptive responses, or pain signaling[26]. Therefore, CB1r activation contributes to antinociception by blocking the progression of the pain pathway.

In line with Farquhar-Smith et al.[27], our immunohistochemical analysis revealed CB1r localization in the perinuclear cytoplasm of neurons within the superficial laminae of the Vc. EG2 showed a notable increase in CB1r immunoreactivity, specifically in ipsilateral LI projection neurons, that receive central projections from trigeminal primary afferent neurons innervating the mandibular division. Animal studies have demonstrated that calbindin D28K, a calcium-binding protein, is expressed by distinct neuronal populations in the Vc, suggesting different functional properties among these neurons. Small multipolar calbindin-positive neurons in LI and II modulate nociceptive input, whereas fusiform calbindin-positive neurons in LI constitute ascending projection cells targeting the medial thalamus and other pain-related subnuclei[5,28]. Multiple Ca^{2+} channels and Ca^{2+} influx are involved in the glutamatergic synaptic transmission[29]. Consistent with our preceding study[7], enhanced calbindin D28K expression in LI fusiform neurons, supports the activation of the ascending orofacial nociceptive pathway after pulpitis. In contrast, the present study demonstrates that oral paracetamol administration produces a significant reduction in the number of calbindin D28K–positive projection neurons in LI of the Vc. This decrease likely reflects enhanced presynaptic inhibition mediated by the concomitant increase of 5HT-T and CB1r expression in EG2, resulting in reduced calcium-dependent neurotransmitter release. In agreement with these findings, previous studies of excitatory synaptic transmission have indicated that presynaptic action of CB1r and 5HTr reduce directly or indirectly Ca^{2+} influx and glutamate release[29,30].

CONCLUSIONS

In conclusion, our findings indicate that after experimental activation of dental pulp nociceptors (pulpitis), oral administration of paracetamol produces plastic changes that can be observed at the level of the serotonergic and endocannabinoid

systems of the Vc. This plasticity is expressed as a lower number of fusiform calbindin D28K projection neurons in lamina I. The reduction in the number of calbindin D28K-positive neurons in lamina I may reflect adaptive neuroplasticity associated with the modulation of orofacial nociceptive processing.

From a clinical perspective, these findings support the concept that paracetamol may exert part of its analgesic effect through central modulation of trigeminal nociceptive circuits, which is particularly relevant in odontogenic pain conditions such as pulpitis.

A major limitation of this study is the absence of behavioral nociceptive assessment. Further research including behavioral and electrophysiological approaches, as well as co-expression analyses of fusiform neurons with glutamate, CB1r or 5HT receptor subtypes, are needed to validate the functional relevance of these findings. Elucidating these mechanisms may contribute to optimizing the central antinociceptive effects of paracetamol and analgesic strategies in dental practice.

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CONFLICT OF INTERESTS

The authors declare no potential conflicts of interest regarding the research, authorship and/or publication of this article.

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Chronology and sequence of permanent tooth eruption in children in Buenos Aires City

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ABSTRACT

Knowledge of the timing and sequence of permanent tooth eruption is essential for assessing occlusal development and providing population-specific reference standards to support diagnosis, prevention, and treatment planning. ***Aim:*** *To estimate mean age for eruption of permanent teeth, except third molars, in children in Buenos Aires City (CABA), compare means according to sex, jaw and laterality, and identify the eruption sequence per arch.* ***Materials and Method:*** *An observational, prospective, cross-sectional study was conducted (March–July 2024) on 4- to 15-year-old children who received care at Buenos Aires University's School of Dentistry and at four private offices in Buenos Aires City. A tooth was considered erupted when at least one cusp or incisal edge pierced the gingival epithelium. Mean ages were estimated using the Kärber method with 95 % confidence intervals (CI), and the sequence was established by placing those means in order.* ***Results:*** *We evaluated 1,063 children (532 female), mean age 9.9 ± 2.6 years. The first teeth to erupt were lower central incisors, and the last were upper second molars. No difference was observed between hemiarches. Teeth belonging to the lower incisor-canine group erupted significantly earlier than their homologous upper teeth. Girls presented earlier eruption than boys, with significant differences for all teeth except upper central incisors, and upper and lower first molars. Sequence for the upper jaw was 6–1–2–4–3–5–7 in girls, and 6–1–2–4–5–3–7 in boys. Sequence in the mandible was 1–6–2–3–4–5–7 in girls, and 1–6–2–4–3–5–7 in boys, with chronological proximity between canine and premolars in some comparisons.* ***Conclusion:*** *In this sample, eruption began with lower central incisors, was symmetrical, earlier in girls, and earlier in the lower incisor-canine group, with sequential stability in both sexes during the first transitional period, and variations in the second.*

Keywords: *tooth eruption - permanent dentition - child - cross-sectional studies*

Cronología y secuencia de erupción de la dentición permanente en niños de la Ciudad de Buenos Aires

RESUMEN

El conocimiento de la cronología y la secuencia de erupción de la dentición permanente es fundamental para evaluar el desarrollo oclusal y disponer de parámetros poblacionales de referencia que orienten el diagnóstico, la prevención y la planificación terapéutica. ***Objetivo:*** *Estimar la edad media de erupción de las piezas dentarias permanentes, excluyendo terceros molares, en niños de la Ciudad Autónoma de Buenos Aires (CABA), comparar las medias según sexo, maxilar y lateralidad, e identificar la secuencia eruptiva por arcada.* ***Materiales y Método:*** *Se realizó un estudio observacional, prospectivo y transversal (marzo–julio 2024) en niños de 4 a 15 años atendidos en la Facultad de Odontología de la Universidad de Buenos Aires y en cuatro consultorios privados de CABA. Se consideró erupcionada una pieza cuando al menos una cúspide o borde incisal atravesó el epitelio gingival. Las edades medias se estimaron mediante el método de Kärber, con intervalos de confianza (IC) del 95 %, y la secuencia se estableció ordenando dichas medias.* ***Resultados:*** *Se evaluaron 1.063 niños (532 mujeres) con una edad media de 9,9 ± 2,6 años. Los primeros dientes en erupcionar fueron los incisivos centrales inferiores y los últimos los segundos molares superiores. No se observaron diferencias entre hemiarcadas. Las piezas del grupo incisivo-canino inferior erupcionaron significativamente antes que sus homólogas superiores. Las niñas presentaron erupción más temprana que los varones, con diferencias significativas en todas las piezas excepto incisivos centrales superiores y primeros molares superiores e inferiores. La secuencia superior fue 6–1–2–4–3–5–7 en niñas y 6–1–2–4–5–3–7 en niños; en la mandíbula fue 1–6–2–3–4–5–7 en niñas y 1–6–2–4–3–5–7 en niños, con proximidad cronológica entre canino y premolares en algunas comparaciones.* ***Conclusión:*** *En esta muestra, la erupción inició con los incisivos centrales inferiores, fue simétrica, más precoz en niñas y más temprana en el grupo incisivo-canino inferior, con estabilidad secuencial en ambos sexos durante el primer período transicional y variaciones en el segundo.*

Palabras clave: *erupción dentaria - dentición permanente - niño - estudios transversales*

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INTRODUCTION

Tooth eruption is a long, complex physiological process, closely related to the growth and development of craniofacial structures. It is a dynamic process that includes tooth embryological development (odontogenesis), movement in the jaws, and fitting in the arches until occlusal functionality is attained. Three phases can be identified in this process: the pre-eruptive phase, from the beginning of odontogenesis until the crown is fully formed; the pre-functional eruptive phase, which begins with root formation and is completed when the tooth establishes contact with its antagonist; and the functional eruptive phase, which begins with this contact and continues during the masticatory function. The point at which the tooth pierces the oral mucosa and becomes visible in the oral cavity is called dental emergence. It occurs during the pre-functional eruptive phase, and is generally identified as "tooth eruption"[1].

Various factors influence the sequence and timing of tooth emergence. A strong genetic component has been identified in eruptive chronology[2], while longitudinal studies have shown associations between general corporeal growth and time of tooth emergence[3]. Moreover, recent research has reported that body mass index and nutritional status may be related to variations in eruptive chronology and sequence[4,5]. At population level, global evidence shows regional differences in mean ages of eruption, suggesting the possible influence of environmental and socio-economic factors[6].

Eruption chronology refers to the approximate time, expressed in years and months, at which the emergence of a permanent tooth is usually expected. Knowledge of eruption chronology enables the establishment of mean parameters and ranges of normality, facilitating identification of early or late eruption and the adoption of timely therapeutic measures[7]. Eruption sequence refers to the order in which teeth emerge in each jaw, and plays an essential role in harmonious jaw development and establishment of functional occlusion, being key to early orthodontic planning[7].

Although eruptive chronology suggests mean ages, classic studies have noted that each tooth presents a wide range of normality, wherefore moderately early or late eruption does not necessarily imply a pathological alteration[8]. In this context, clinical interpretation of tooth emergence requires consideration of both individual variability and populational patterns[6].

The timing and sequence of permanent tooth eruption have been widely studied globally due to their relevance to clinical diagnosis, therapeutic planning, and understanding populational variations in tooth development[6]. Both longitudinal and cross-sectional studies have been conducted with the aim of identifying reference patterns. Longitudinal studies enable direct observation of eruption timing, and provide greater precision, though they involve high costs and lengthy follow-up periods[3,9]. On the other hand, cross-sectional studies record the eruptive status at a given time of the pre-functional phase, and are the most often used in populational studies[10-15].

Inter-populational variations in mean ages of eruption have been recorded, even though eruptive sequence tends to be relatively stable among different regions[16]. Given that most available studies have been conducted in specific geographic contexts, and that each population has particularities conditioned by its biological and sociocultural setting, it is essential to generate updated local data upon which to base local parameters. In Argentina, recent information on eruptive chronology and sequence is limited, especially in urban populations. The aims of this study were to estimate mean age of eruption of all permanent teeth except third molars in children from Buenos Aires City (CABA); to identify the eruptive sequence for each jaw, and to compare mean age of eruption according to sex, jaw and laterality.

MATERIALS AND METHOD

A descriptive, prospective cross-sectional study was designed and submitted to the Ethics Committee of Buenos Aires University's School of Dentistry for approval (CETICA-FOUBA 003/2024). A non-probabilistic convenience sample was used, consisting of children of both sexes, aged 4 to 15 years, who received care at the Department of Comprehensive Children's Dentistry at Buenos Aires University's School of Dentistry and at four private offices in Buenos Aires City from March to July 2024. All permanent teeth except third molars were evaluated. Patients who provided assent and whose legal guardians signed informed consent were included.

Exclusion criteria were any systemic or local alterations that could modify the eruption pattern, such as syndromes, endocrine disorders, nutritional deficiencies, cleft palate, amelogenesis imperfecta, severe hypomineralization, history of dental trauma, premature loss of predecessor tooth, ongoing orthodontic treatment, agenesia, severe fluorosis, lack of space, presence of supernumerary teeth, odontogenic or non-odontogenic cysts or tumors.

Previously trained and calibrated researchers (Kappa index > 0.90) selected patients based on anamnesis, and clinical and radiographic (panoramic X-ray) examination. A tooth was considered erupted when at least one cusp or the incisal edge of the crown had pierced the gingival epithelium[17].

Patient sex (female, male) and chronological age were recorded. Age was calculated from date of birth to the time of the examination, and expressed in decimal format for analysis. Teeth were identified following the World Dental Federation (FDI) nomenclature.

Mean ages of dental eruption and standard error were estimated using Kärber's method, as described by Hayes and Mantel[18], based on data grouped into three-month age intervals. A 95 % confidence interval (CI 95 %) was calculated for each mean. Differences were considered statistically significant ($\alpha = 0.05$) when the 95 % confidence interval of a mean did not include the compared mean, following the criterion proposed by Hernández et al.[12]. Eruptive sequence was established by placing mean ages of eruption in ascending order.

RESULTS

The sample consisted of 1,063 children (532 girls and 531 boys), mean age 9.9 ± 2.6 years.

The first teeth to erupt were the lower central incisors, while the last to emerge were the upper second molars. There was a general trend towards mandibular teeth erupting before their homologous maxillary teeth. This difference was statistically significant only for the incisor and canine groups. No significant difference was found between left and right hemiarches on either upper or lower jaw (Table 1).

Comparison according to sex showed that girls presented earlier eruption than boys. In the upper jaw, this difference was statistically significant for all teeth except central incisors and first molars (Table 2). In the lower jaw, the difference was not significant only for first molars. The greatest difference between sexes was observed for lower canines, with a discrepancy of 9 months for the right hemiarch and 8 months for the left hemiarch (Table 3).

During the first transitional period, the eruptive sequence was similar in both sexes. In the upper jaw, the first molar preceded the central incisor and lateral incisor. In the lower jaw, the central incisor erupted before the first molar and the lateral incisor.

During the second transitional period, there were slight variations between sexes. In the upper jaw, girls presented eruption of the canine before the second premolar, while in boys, both erupted almost simultaneously.

In the lower jaw in girls, canine erupted before – though almost simultaneously to – the first premolar. In boys, the first premolar erupted before the canine. In both sexes, the eruptive sequence concluded with the upper second molars.

DISCUSSION

Permanent tooth eruption is a dynamic physiological process influenced by genetic, systemic, environmental and local factors[2,19]. The current study excluded any conditions with potential to modify the eruption pattern, enabling the results to be interpreted within physiological development parameters, reducing any potential biases associated to pathological alterations.

Many populational studies aimed at identifying patterns of eruptive chronology and sequence have used cross-sectional designs due to their methodological, logistic and economic feasibility[12,13,15,20-25]. Although longitudinal studies enable more direct individual monitoring of the eruption process, they require long observation periods and greater resources[10]. Moreover, exact determination of the time of tooth emergence is complex in both kinds of design[10]. In this context, it has been demonstrated that profiles of mean eruption age obtained by cross-sectional studies can provide estimates that are comparable to those from longitudinal studies, as long as appropriate statistical methods are employed[6,26].

Mean ages of eruption were estimated using Kärber's method, as described by Hayes and Mantel[18], which has been widely used in populational studies with grouped data. This procedure enables calculation of mean age of tooth emergence based on accumulated

Table 1. Mean age of eruption for upper and lower permanent teeth expressed in decimal years and in years and months, with their 95 % confidence intervals.

Upper jaw				Lower jaw			
Tooth	CI LL	Mean	CI UL	Tooth	CI LL	Mean	CI UL
1.1	6.87	7.01 (7 y)	7.16	**4.1**	5.71	5.88 (5 y, 10 m)	6.05
2.1	6.79	6.96 (6 y, 11 m)	7.13	**3.1**	5.79	5.95 (5 y, 11 m)	6.12
1.2	7.78	7.92 (7 y, 11 m)	8.06	**4.2**	7.03	7.18 (7 y, 2 m)	7.33
2.2	7.80	7.94 (7 y, 11 m)	8.08	**3.2**	6.97	7.12 (7 y, 1 m)	7.26
1.3	11.07	11.24 (11 y, 2 m)	11.42	**4.3**	10.25	10.40 (10 y, 4 m)	10.55
2.3	10.95	11.12 (11 y, 1 m)	11.29	**3.3**	10.29	10.45 (10 y, 5 m)	10.61
1.4	10.27	10.43 (10 y, 5 m)	10.59	**4.4**	10.22	10.37 (10 y, 4 m)	10.53
2.4	10.22	10.38 (10 y, 4 m)	10.53	**3.4**	10.22	10.37 (10 y, 4 m)	10.52
1.5	11.11	11.29 (11 y, 3 m)	11.46	**4.5**	11.00	11.17 (11 y, 2 m)	11.33
2.5	11.00	11.16 (11 y, 1 m)	11.32	**3.5**	11.00	11.17 (11 y, 2 m)	11.34
1.6	6.20	6.36 (6 y, 4 m)	6.52	**4.6**	6.12	6.27 (6 y, 3 m)	6.42
2.6	6.26	6.41 (6 y, 4 m)	6.57	**3.6**	6.19	6.34 (6 y, 4 m)	6.49
1.7	11.98	12.16 (12 y, 1 m)	12.34	**4.7**	11.79	11.98 (11 y, 11 m)	12.18
2.7	11.92	12.11 (12 y, 1 m)	12.30	**3.7**	11.74	11.94 (11 y, 11 m)	12.14

CI LL: lower limit of confidence interval; CI UL: upper limit of confidence interval.

proportions distributed in age intervals —three months, in this case— providing a reliable estimate without the need for complex statistical models. Its use in dentistry has been validated in multiple studies[12,13,27], supporting its applicability in the current study. Although some authors have used Probit regression models to estimate mean age of tooth emergence in cross-sectional studies[8,25,28,29], both methods provide consistent estimates in studies with dichotomic data, facilitating comparison between populations analyzed under different statistical approaches.

The results of the current study partially agree with the classic eruption tables described by Hurme[8], and Lo and Moyers[30], as well as with more recent studies conducted on Latin American and European populations[12-14]. Compared to classic references for the Caucasian population, the transitional period towards permanent dentition began and ended at similar mean ages; however, there was a difference in the first tooth to emerge. While Hurme[8] reported that the first tooth was the lower first molar, in our sample, the lower central incisor was the first to erupt. This finding agrees with Sturdivant et al.[31], who reported that the first permanent tooth to emerge may be a molar or an incisor, with predominance of the incisor in 64 % of cases.

Significantly earlier eruption in girls, bilateral symmetry and mandibular precedence were patterns that were consistent with those reported in the international literature[11-14,22-24,27,32]. Girls presented earlier mean ages of eruption for most tooth groups; nevertheless, no statistically significant difference was observed for upper central incisors or for upper and lower first molars.

Sexual dimorphism was more evident at the beginning of the second transitional period, at the time of canine and premolar emergence. This may be related to the earlier pubertal growth peak in females. The most outstanding finding was the

Table 2. Mean age of eruption for upper permanent teeth in girls and boys, expressed in decimal years and in years and months, with their 95 % confidence intervals.

Upper jaw - Girls				Upper jaw – Boys			
Tooth	**CI LL**	**Mean**	**CI UL**	**Tooth**	**CI LL**	**Mean**	**CI UL**
1.1	6.71	6.94 (6 y, 11 m)	7.17	**1.1**	6.90	7.09 (7 y, 1 m)	7.28
2.1	6.72	6.95 (6 y, 11 m)	7.18	**2.1**	6.77	6.98 (6 y, 11 m)	7.18
1.2	7.62	7.82 (7 y, 9 m)	8.01	**1.2**	8.00	8.20 (8 y, 2 m)	8.41
2.2	7.70	7.89 (7 y, 10 m)	8.09	**2.2**	7.98	8.18 (8 y, 2 m)	8.39
1.3	10.70	10.94 (10 y, 11 m)	11.18	**1.3**	11.24	11.54 (11 y, 6 m)	11.83
2.3	10.77	11.02 (11 y)	11.27	**2.3**	11.25	11.49 (11 y, 5 m)	11.73
1.4	10.04	10.26 (10 y, 3 m)	10.47	**1.4**	10.52	10.75 (10 y, 9 m)	10.98
2.4	9.99	10.21 (10 y, 2 m)	10.43	**2.4**	10.49	10.71 (10 y, 8 m)	10.92
1.5	10.97	11.26 (11 y, 3 m)	11.52	**1.5**	11.28	11.53 (11 y, 6 m)	11.77
2.5	10.83	11.11 (11 y, 1 m)	11.39	**2.5**	11.20	11.42 (11 y, 5 m)	11.64
1.6	6.07	6.33 (6 y, 3 m)	6.58	**1.6**	6.34	6.53 (6 y, 6 m)	6.72
2.6	6.13	6.39 (6 y, 4 m)	6.66	**2.6**	6.39	6.57 (6 y, 6 m)	6.76
1.7	11.81	12.00 (12 y)	12.20	**1.7**	12.03	12.28 (12 y, 3 m)	12.54
2.7	11.74	12.00 (12 y)	12.20	**2.7**	12.05	12.30 (12 y, 3 m)	12.55

CI LL: lower limit of confidence interval; CI UL: upper limit of confidence interval.

difference of approximately 8 or 9 months in the eruption of the lower canine, which was significantly earlier in girls.

The data also confirmed the tendency toward symmetrical bilateral eruption and earlier emergence of mandibular teeth than their homologous maxillary teeth, with statistically significant differences for the incisor and canine groups. Studies such as Almonaitiene et al.[28] and Bruna del Cojo et al.[15] also report significant differences between jaws for these groups, with precedence of lower teeth.

Most studies agree that the upper second molar is the last permanent tooth to erupt[11-13,23,24,33], a pattern that was confirmed in the current sample. In contrast, the first tooth to emerge is more variable. Some studies, like ours, describe the lower central incisor as the first tooth[28], whereas others report the lower first molar[21,22,32], sometimes with minimum differences in time between them[12,13]. These variations may be attributed to populational differences, secular changes, and the interaction of genetic, environmental and nutritional factors[34].

In relation to eruption sequence per arch, during the first transitional period, the pattern in the upper jaw matched reports by most authors[10-13,15,21,25,32,33,35].

For the second upper transitional period, Lo and Moyers[30] described the most prevalent sequence as 6–1–2–4–5–3–7 (48.72 %), followed by 6–1–2–4–3–5–7 (11.87 %). In the current study, the former matched the male sample, while the latter matched the female sample.

In the lower arch, the central incisor preceded the first molar in both sexes, in agreement with various authors[11-13,15,21,25,28]. In contrast, Taboada and Medina[33], in a Mexican population, and Diamanti and Townsend[36], in an Australian population, described simultaneous eruption of the central incisor and first molar in the male sample, while Ayala et al.[35] and Esan et al.[29] reported variations according

Table 3. Mean age of eruption for lower permanent teeth in girls and boys, expressed in decimal years and in years and months, with their 95 % confidence intervals.

Lower jaw - Girls				Lower Jaw - Boys			
Tooth	**CI LL**	**Mean**	**CI UL**	**Tooth**	**CI LL**	**Mean**	**CI UL**
4.1	5.38	5.70 (5 y, 8 m)	6.03	**4.1**	5.97	6.17 (6 y, 2 m)	6.37
3.1	5.65	5.86 (5 y, 10 m)	6.06	**3.1**	6.00	6.20 (6 y, 2 m)	6.41
4.2	6.87	7.10 (7 y, 1 m)	7.33	**4.2**	7.18	7.36 (7 y, 4 m)	7.54
3.2	6.85	7.07 (7 y, 1 m)	7.30	**3.2**	7.16	7.34 (7 y, 4 m)	7.51
4.3	9.91	10.12 (10 y, 1 m)	10.32	**4.3**	10.65	10.86 (10 y, 10 m)	11.07
3.3	9.96	10.19 (10 y, 2 m)	10.42	**3.3**	10.66	10.88 (10 y, 10 m)	11.09
4.4	9.99	10.20 (10 y, 2 m)	10.41	**4.4**	10.51	10.73 (10 y, 8 m)	10.95
3.4	9.99	10.20 (10 y, 2 m)	10.41	**3.4**	10.51	10.71 (10 y, 8 m)	10.92
4.5	10.82	11.04 (11 y)	11.26	**4.5**	11.19	11.42 (11 y, 5 m)	11.64
3.5	10.83	11.11 (11 y, 1 m)	11.39	**3.5**	11.16	11.40 (11 y, 4 m)	11.63
4.6	6.01	6.26 (6 y, 3 m)	6.50	**4.6**	6.23	6.41 (6 y, 4 m)	6.59
3.6	6.09	6.32 (6 y, 4 m)	6.55	**3.6**	6.23	6.41 (6 y, 4 m)	6.59
4.7	11.60	11.80 (11 y, 10 m)	12.03	**4.7**	11.92	12.18 (12 y, 2 m)	12.44
3.7	11.59	11.82 (11 y, 10 m)	12.05	**3.7**	11.97	12.23 (12 y, 2 m)	12.49

CI LL: lower limit of confidence interval; CI UL: upper limit of confidence interval.

to sex regarding the initial mandibular tooth. These discrepancies may be due to populational differences, methodological differences, and sexual dimorphism, in addition to the short chronological distance between the two teeth at the beginning of the first transitional period. During the second mandibular transitional period, the canine erupted slightly earlier than the first premolar in girls, while in boys, the first premolar preceded the canine, a pattern that agrees with the first and third most frequent sequences reported by Lo and Moyers[30] for said period.

A possible methodological limitation to this study is that the eruptive sequence was established by placing mean eruption ages in order. Lo and Moyers[30] reported that the standard deviation of mean times may vary considerably among teeth, which could lead to overlap among distributions and make absolute inferences difficult when the sequence is established based exclusively on means. Nevertheless, this approach has been widely used in cross-sectional populational studies and enables establishment of general trends that are comparable between studies.

Globally, the eruption sequence shows notable intercultural stability, despite some chronological variations between populations and cohorts. In European populations, a slightly earlier mean age for tooth emergence has been reported, particularly in more recent cohorts, with relative maintenance of the eruptive sequence between generations[9]. These observations highlight the need for availability of updated tables specific to each geographic and sociocultural context.

CONCLUSIONS

The findings of the current study provide updated reference values for eruptive chronology and

sequence in children evaluated in an urban clinical context in Buenos Aires City. This information may contribute to timely diagnosis of alterations in dental development and to interceptive therapeutic planning.

In this sample from Buenos Aires City, permanent tooth eruption presented a harmonious sequential pattern, beginning in both sexes with lower central incisors. Mean ages were symmetrical between hemiarches, earlier in the lower incisor-canine group and significantly earlier in girls than boys. During the first transitional period, the eruptive sequence coincided in both sexes, while in the second, there were some variations according to sex for canines and premolars, ending with the eruption of the upper second molars. These results provide local reference values for the eruptive chronology and sequence in this population of children.

CONFLICTS OF INTEREST

The authors declare no potential conflict of interest regarding the research, authorship and/or publication of this article.

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Penetration effect of sodium hypochlorite activated by the NSK Varios 370 ultrasonic system and the EQ-S sonic system in single-rooted premolars

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ABSTRACT

The effectiveness of endodontic disinfection largely depends on the ability of sodium hypochlorite (NaOCl) to penetrate deeply into dentinal tubules, particularly in the apical third, where the anatomy is more complex and mechanical access is limited. Sonic and ultrasonic activation techniques have been proposed to enhance this penetration by generating dynamic fluid movement. ***Aim:*** *To evaluate and compare the depth of penetration of 5.25% NaOCl into dentinal tubules using passive ultrasonic irrigation (PUI; NSK Varios 370) and sonic activation (SIM; EQ-S Metabiomed), in comparison to conventional needle irrigation.* ***Materials and Method:*** *thirty-six extracted human single-rooted premolars were instrumented to working length with ProTaper Gold F3. The teeth were randomly assigned to three groups (n = 12): PUI, SIM, and control. A standardized final irrigation protocol was performed, followed by the application of 1% methylene blue, activated or not, according to the group. Three transverse sections were obtained from each tooth (coronal, middle, and apical), and penetration depth was measured using ImageJ image analysis. Data were analyzed with non-parametric tests (Mann–Whitney U; $p < 0.05$).* ***Results:*** *Both PUI and SIM showed significantly greater penetration depths than the control group in all three thirds of the canal ($p < 0.05$). No significant difference was found between PUI and SIM ($p > 0.05$). The greatest penetration was observed in the coronal third, followed by the middle and apical thirds in all groups.* **Conclusions:** *Ultrasonic and sonic activation techniques produced greater marker penetration into dentinal tubules compared to conventional irrigation. Furthermore, the EQ-S system yielded similar results to those obtained with ultrasonic activation, with no statistically significant difference between the two systems.*

Keywords: *sodium hypochlorite - root canal irrigants - ultrasonic irrigation - sonic irrigation - dentin tubules*

Efecto de penetración del hipoclorito de sodio bajo sistema de activación ultrasónico nsk varios 370 y sónico eq-s, en premolares unirradiculares

RESUMEN

Introducción: *La eficacia de la desinfección endodóntica depende en gran medida de la capacidad del hipoclorito de sodio (NaOCl) para penetrar profundamente en los túbulos dentinarios, especialmente en el tercio apical, donde la anatomía es más compleja y el acceso mecánico limitado. Las técnicas de activación sónicas y ultrasónicas han sido propuestas para mejorar dicha penetración mediante la generación de flujo dinámico.* ***Objetivo:*** *Evaluar y comparar la profundidad de penetración del NaOCl al 5.25% en los túbulos dentinarios utilizando activación ultrasónica pasiva (PUI; NSK Varios 370) y activación sónica (SIM; EQ-S Metabiomed), frente a irrigación convencional con aguja.* ***Materiales y métodos:*** *Se utilizaron 36 premolares unirradiculares humanos, instrumentados a longitud de trabajo con ProTaper Gold F3. Los dientes se dividieron aleatoriamente en tres grupos (n=12): PUI, SIM y control. Se realizó un protocolo estandarizado de irrigación final y posteriormente se aplicó azul de metileno al 1%, activado o no según el grupo. Se obtuvieron tres secciones transversales por diente (coronal, medio, apical) y se midió la profundidad de penetración mediante análisis de imagen con ImageJ. Los datos fueron analizados con pruebas no paramétricas (U de Mann-Whitney; $p<0.05$).* ***Resultados:*** *Tanto PUI como SIM mostraron profundidades de penetración significativamente mayores que el grupo control en los tres tercios del conducto ($p<0.05$). No se encontraron diferencias significativas entre PUI y SIM ($p>0.05$). Las mayores penetraciones se observaron en el tercio coronal, seguidas del medio y apical en todos los grupos.* ***Conclusiones:*** *Las técnicas de activación ultrasónica y sónica mostraron una mayor penetración del marcador en los túbulos dentinarios en comparación con la irrigación convencional. Además, el sistema EQ-S arrojó resultados similares a los obtenidos con la activación ultrasónica, sin diferencias estadísticamente significativas entre ambos sistemas.*

Palabras clave: *hipoclorito de sodio - irrigantes - irrigación ultrasónica - irrigación sonica - túbulos dentinarios*

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INTRODUCTION

Endodontics focuses on the study of morphology, physiology, and pathology of the pulp cavity. Understanding the diagnosis and treatment of pulp and periapical diseases requires a broad knowledge of pulp biology. The goal of endodontic treatment is to eliminate infection from the root canal system and prevent or resolve apical periodontitis, while contributing to relieving dental pain associated with pulp and periapical inflammatory processes[1]. These inflammatory processes and apical periodontitis are caused by bacteria that penetrate the root canal system[1-3].

Biofilm consists of individual cells or mixed multicellular bacterial colonies attached to a surface and embedded in a highly hydrated extracellular polymeric matrix (EPM). Bacteria form biofilms on nutrient-rich surfaces, and within root canals, these can organize into multiple cell layers. The EPM plays a fundamental role in the development, organization, and survival of the biofilm[4,5].

Once bacteria reach the root canals, lateral canals, or dentinal tubules, strategies must be applied to eliminate them[5.] Studies show that bacteria can penetrate 300–500 micrometers into the dentinal tubules[6-8].

The main root canal is cleaned through chemo-mechanical action: manual or rotary instruments perform the physical removal of debris and shape the canal geometry to facilitate irrigant penetration. Irrigants play a crucial role in the chemical action by dissolving organic tissues, removing smear layer, reaching some lateral canals or apical deltas, and penetrating the dentinal tubules[2,3,6].

During canal shaping, 35–50% of root canal walls remain untouched by instruments due to anatomical irregularities, rendering those areas inaccessible to mechanical cleaning[9,10]. Several authors have reported that the apical region is the most anatomically complex, making debridement more challenging[7]. Apical cleanliness depends on the apical preparation size and the irrigation protocol employed; the apical size determines how closely the needle can reach the working length, influencing irrigant flow and volume. Some studies associate larger apical preparations with improved apical disinfection[7].

When irrigation is performed with a syringe and needle, greater canal enlargement becomes important. However, passive ultrasonic irrigation may achieve cleanliness comparable to that in canals prepared to larger apical sizes, meaning that canals with smaller preparations can be just as clean as larger ones[7,11]. Among irrigants, sodium hypochlorite (NaOCl) remains the gold standard due to its antimicrobial properties and ability to dissolve organic tissue[6,7,11,12].

Previous research has shown that 6% NaOCl can penetrate up to 300 μm into dentinal tubules[7,8].

Each irrigation technique influences how NaOCl penetrates the canal system and dentinal tubules. Another factor to consider is the air entrapment produced by a closed access cavity, due to the periodontal ligament and surrounding bone, which generates a vapor lock effect. This negatively affects irrigant flow in the apical region because an air bubble forms, preventing optimal irrigant penetration[13.]

Among the most commonly used activation techniques are: Dynamic Manual Irrigation using a conventional syringe; Passive Ultrasonic Irrigation (PUI), which uses a metallic file that vibrates at ultrasonic frequency; and Sonic Activation, which uses polymer tips vibrating at sonic frequency to enhance canal disinfection by removing debris and organic tissue. Sonic systems include EndoActivator (Dentsply Maillefer, Switzerland), Eddy tips (VDW, Germany), and EQ-S (Metabiomed, South Korea).

Given the availability of various systems that improve disinfection of the root canal complex, it is important to compare gold-standard systems with newer devices which generate apical-coronal motion in addition to sonic activation and lateral movement. The aim of this study was to evaluate the effect of sodium hypochlorite penetration using ultrasonic activation (NSK Varios 370, Dentsply Maillefer, Switzerland) and sonic activation (EQ-S, Metabiomed, South Korea) in single-rooted premolars.

MATERIALS AND METHOD

We used 36 recently extracted human single-rooted teeth with intact, straight roots and a single canal, obtained for orthodontic or periodontal reasons. These teeth were stored under controlled conditions in a sterile solution. Mesiodistal and buccolingual radiographs were taken to ensure the presence of a single canal and root lengths greater than 13 mm. Teeth with caries, root fractures,

open apices, calcified canals, previous root canal treatments, resorption defects, or posts were excluded.

Coronal access was prepared using a round diamond bur, after which the canal was located with a DG16 explorer (Hu-Friedy, Lyoner Frankfurt). Working length (WL) was determined using a size 15 K-file (Dentsply Sirona, Switzerland) inserted until the tip was visible at the apical foramen, and then reduced by 1 mm.

Teeth were divided into three activation groups:

Group 1: Ultrasonic activation (NSK Varios 370) Frequency: 28 – 32 KHz,

Group 2: Sonic activation (EQ-S Metabiomed) Frequency: 8 – 13 KHz,

Group 3: Control (methylene blue without activation).

Root canals were prepared to WL using ProTaper Gold F3 (size 30) (Dentsply Sirona, Switzerland). During instrumentation, canals were irrigated with 2 mL of 5.25% sodium hypochlorite (NaOCl) (Cloralex, Mexico) using a side-vented 30-gauge needle positioned 2 mm short of WL, applied between each instrument.

An apical barrier of LC Block-Out Resin (Ultradent Inc., USA) was placed to simulate a closed system, as in a non-extracted tooth. A layer of clear nail varnish was then applied to the external root surface to prevent external penetration of methylene blue.

Transverse grooves were made using a diamond disc (SYNDENT Tools Co., Ltd., China) at 2 mm (apical third), 5 mm (middle third), and 8 mm (coronal third) from the apex. The final irrigation protocol was standardized as follows:

1. NaOCl (5 mL, 1 min)
2. Distilled water (5 mL, 1 min)
3. 17% EDTA (5 mL, 1 min)
4. Distilled water (5 mL, 1 min)
5. NaOCl (5 mL, 1 min), activated for 30 s, followed by 30 s rest, and then 30 s additional activation.

To evaluate the penetration of 5.25% NaOCl into the dentinal tubules, the 36 teeth were randomly divided into three groups (n = 12) according to the activation system:

PUI Group: Passive Ultrasonic Irrigation (NSK Varios 370)

NaOCl (5 mL) was applied 2 mm from the working length (WL). A size 25/02 ultrasonic tip was positioned 3 mm from the WL and activated for 30 seconds, in three activation cycles. The canal was then irrigated with 5 ml of saline solution, after which 1% methylene blue was introduced as a penetration marker 1 mm from the WL and ultrasonically activated for 30 seconds.

SIM Group: Sonic Activation (EQ-S Metabiomed – 10,000 cpm)

NaOCl (5 mL) was applied 2 mm from WL. The sonic tip size 25/02 was positioned 2 mm short of WL and activated for 30 seconds, in three activation cycles. The canal was then rinsed with 5 mL of saline, followed by placement of 1% methylene blue 1 mm from WL and sonic activation for 30 seconds.

Control Group: Conventional Needle Irrigation (No activation)

The irrigant (5 mL) was applied 2 mm from WL. NaOCl was then replaced with an additional 5 mL of fresh solution without activation, repeated three times. The NaOCl was flushed with 5 mL of saline, after which 1% methylene blue was placed and left undisturbed for 30 seconds.

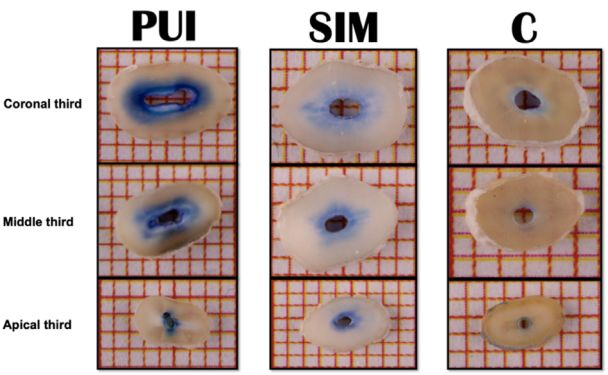

Fig. 1: Groups and their thirds.

Excess solution was removed using sterile paper points. The flowable resin was eliminated, the grooves were connected with a diamond disc, and any remaining irregularities were smoothed with a medium-grit polishing disc (3M Dental, USA). This procedure yielded one apical, one middle, and one coronal segment from each tooth, each 1 mm thick, providing a total 108 sections (Fig. 1).

The samples were examined under maximum magnification (Tokina AT-X 100 mm f/2.8 Pro D lens., Tokuyama Corporation, Japan) mounted on a camera (Nikon D3100, Nikon, Japan) to visualize the transverse plane of the canal walls. One image

was captured for each canal third—coronal, middle, and apical—together with a calibrated scale grid. All images were taken at the same focal distance of 0.3 meters, with fixed resolution and optimal focus, saved in TIFF format, and imported into ImageJ software (National Institutes of Health, Bethesda, Maryland, USA).

A calibrated straight line was drawn perpendicular from the internal canal wall to the deepest visible point of stain penetration. Twelve measurements were obtained from each image at equal angular intervals (clockwise direction). The mean NaOCl penetration depth for the coronal, middle, and apical segments of each group was calculated in micrometers (µm) (Fig. 2).

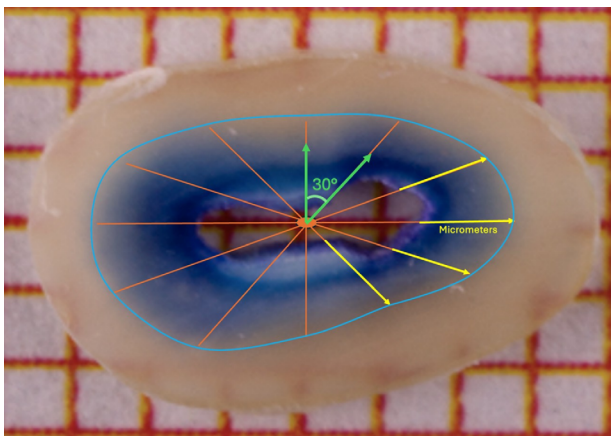

Fig. 2: Methodology for penetration depth. The blue area indicates methylene blue penetration.

The mean values and interquartile ranges (25–75%) were calculated for the full canal length within each group, as well as for the coronal, middle, and apical segments. A non-parametric statistical approach was employed due to the non-normal distribution of the data. Groups were compared using the Mann–Whitney U test, with significance level set at $p < 0.05$. All analyses were conducted using IBM SPSS Statistics, Version 27.0 (June 2020).

RESULTS

The penetration depth of sodium hypochlorite was evaluated in the apical, middle, and coronal thirds for each activation system (PUI and SIM). Both activation systems showed significantly greater penetration depths than the control group in all three canal thirds ($p < 0.05$). No statistically significant differences were observed between PUI and SIM in any of the evaluated thirds ($p > 0.05$). Overall, penetration depth increased from the apical to the coronal third in all groups. Descriptive statistics, including median values, are presented in Table 1, and the distribution of penetration depths is shown in Figure 3.

Table 1. The penetration depth of sodium hypochlorite into the dentinal wall of the root canal

Techniques	Root Canal Level	Penetration Depth
SIM	Coronal Middle Apical	1112.2 ± 588.72 799.39 ± 329.49 596.76 ± 157.98
PUI	Coronal Middle Apical	1110.88 ± 507.07 1019.34 ±254.12 346.13 ± 113.91
CONTROL	Coronal Middle Apical	549.62 ±263.01 407.01 ± 104.20 173.65 ± 27.07

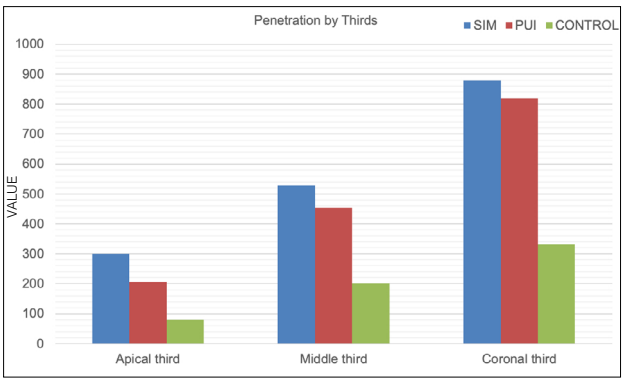

Fig. 3: Comparison of median values by thirds for each group.

Sodium Hypochlorite Penetration in the Apical Third

In the apical third, the minimum penetration depths, from lowest to highest, were 27.07 µm for the control group, 113.91 µm for PUI, and 157.98 µm for SIM (Fig. 4). Penetration depth was therefore markedly greater in both activated groups than in the control group. Statistical analysis confirmed a significant difference between the control group and both activation groups ($p < 0.05$), whereas no significant difference was observed between PUI and SIM ($p > 0.05$).

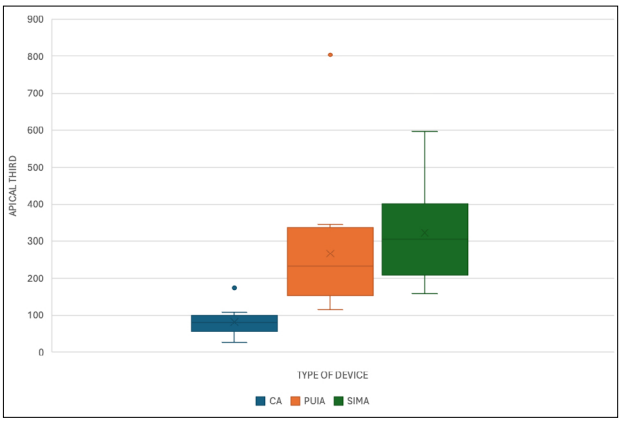

Fig. 4: Box-and-whisker plots of penetration depth for each activation device in the apical third.

Sodium Hypochlorite Penetration in the Middle Third

In the middle third, both PUI and SIM showed greater penetration depths than the control group (Fig. 5). Statistical analysis demonstrated significant differences between the control group and each activation system ($p < 0.05$), while no statistically significant difference was observed between PUI and SIM ($p > 0.05$).

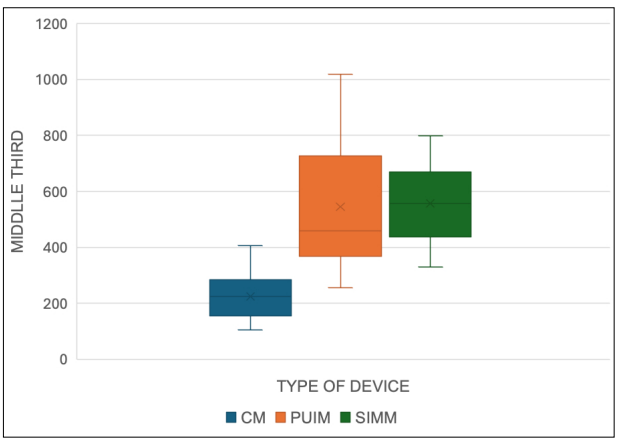

Fig. 5: Box-and-whisker plots of penetration depth for each activation device in the middle third.

Sodium Hypochlorite Penetration in the Coronal Third

In the coronal third, penetration depths were greater than those observed in the apical and middle thirds in all groups (Fig. 6). Both PUI and SIM showed significantly greater penetration depths than the control group ($p < 0.05$), with no statistically significant difference between the two activation systems ($p > 0.05$).

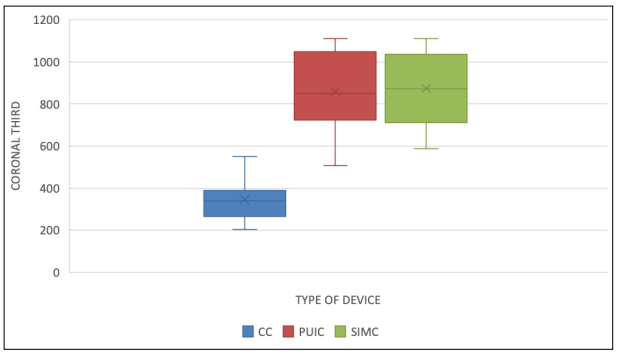

Fig. 6: Box-and-whisker plots of penetration depth for each activation device in the coronal third.

DISCUSSION

According to previous studies, sonic irrigation (EQ-S MetaBiomed) has shown a relevant role as a complementary method to improve root canal system cleaning. It enhances the penetration and efficacy of NaOCl by generating a dynamic flow (acoustic streaming) that facilitates irrigant access to irregularities, isthmuses, and flattened areas typical of oval canals. This effect promotes the removal of debris in areas unreachable by instruments, especially in the apical third. These findings are consistent with previous research indicating that sonic activation significantly reduces residual material in difficult-to-access regions[14]. Taken together, these results indicate that sonic activation can improve irrigant penetration and promote cleaning in hard-to-reach areas of the root canal. Furthermore, several studies have shown that the EQ-S system delivers results that are comparable or superior to conventional needle irrigation, especially in the apical third[15].

In many cases, although EQ-S improved cleaning compared to conventional irrigation, it did not completely remove all material (e.g., gutta-percha/sealer, calcium hydroxide, or smear layer), highlighting that complete canal cleanliness remains challenging, especially in oval, curved, or complex anatomy. Therefore, various studies suggest combining activation techniques or irrigation sequences to optimize apical cleaning[16, 17].

Furthermore, the effectiveness of any irrigant activation or complementary disinfection technique largely depends on its ability to enhance the penetration of agents into dentinal tubules. In this regard, both sonic and ultrasonic activation have been shown to significantly improve the diffusion of methylene blue into the dentinal tubules[18]. This finding suggests that activating the irrigant promotes

its penetration into the dentin microstructures, which could improve the distribution of solutions such as sodium hypochlorite and chelating agents within the root canal system.

Therefore, these results support the idea that activation methods, particularly sonic, not only optimize direct cleaning of the main canal but may also enhance disinfection in difficult-to-access areas such as dentinal tubules, reinforcing their usefulness as adjuncts in contemporary endodontic therapy.

Moreover, conventional irrigation (solution with syringe alone) is often insufficient, frequently failing to remove smear layer adequately or achieve complete disinfection[19,20.] These results reinforce the concept that sonic activation complements mechanical cleaning and enhances NaOCl infiltration into inaccessible areas, contributing to more thorough disinfection[21, 22].

Several studies have also demonstrated that complete removal of calcium hydroxide in canals with complex anatomy remains a clinical challenge, even when using activated irrigation protocols. Sonic activation with the EQ-S system has been shown to significantly improve $Ca(OH)_2$ removal compared to conventional irrigation, particularly in moderately and severely curved canals. This superior performance is attributed to the greater oscillation amplitude of its flexible tips, which promotes a more dynamic irrigant flow and more efficient agitation in the apical region. However, even with this technology, residual medication persists in hard-to-reach areas, consistent with evidence that no method achieves fully predictable removal in anatomically challenging canals. These findings underscore the importance of complementing mechanical preparation with activated irrigation protocols, especially in cases of high morphological complexity, to optimize cleaning prior to obturation[23,24].

Sonic activation using the EQ-S system has also been shown to significantly improve debris removal in conservatively instrumented canals, especially where irrigant penetration and flow are limited. Its vibratory action promotes greater debris removal and better irrigant contact with canal walls, supporting its use as an adjunct in minimally invasive instrumentation protocols, where preservation of original anatomy may compromise cleaning effectiveness without enhanced irrigation[25].

Additionally, EQ-S sonic activation facilitates smear layer removal, demonstrating adequate performance in anatomically complex areas and in the coronal and middle thirds of the canal. However, when compared to ultrasonic activation, the latter retains slight superiority in the apical region, where irrigant penetration and flow are more restricted. These results reinforce the idea that while ultrasonics remain more efficient in critical canal areas, EQ-S provides an effective, less aggressive alternative to optimize irrigation protocols in challenging anatomies[26,27]. Although sonic activation offers clinically relevant benefits, ultrasonic activation maintains overall superiority, particularly in apical regions where hydrodynamic demands are higher. Nevertheless, the difference between the two techniques is not always pronounced, and sonic activation can be considered an effective and safe alternative, especially when preserving internal anatomy or when clinical conditions limit ultrasonic use. Overall, the evidence supports sonic activation as a valuable adjunct in final irrigation protocols, contributing significantly to intracanal cleaning and potentially improving endodontic treatment outcomes[28-30].

CONCLUSION

Within the limitations of this study, efforts were made to standardize irrigation protocols and experimental conditions for each sample. Additionally, single-rooted teeth with wide canals and low anatomical complexity were selected. A limitation was the use of methylene blue as a penetration marker, since its physicochemical properties may differ from those of sodium hypochlorite; therefore, the results should be interpreted with caution. Based on the results obtained, the following conclusions are drawn:

Both the PUI and SIM techniques significantly increased irrigant penetration into the dentinal tubules compared to conventional needle irrigation. The greatest penetration was observed in the coronal third, followed by the middle and apical thirds in all groups evaluated.

No statistically significant difference was observed between the PUI and SIM systems in any of the analyzed thirds, indicating comparable behavior between both activation methods.

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CONFLICT OF INTERESTS
The authors declare no potential conflicts of interest regarding the research, authorship, and/or publication of this article.

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