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Micro-CT evaluation of the presence of voids in endodontic obturation

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ABSTRACT

Identifying the presence, size, type and location of voids in an endodontic obturation is of great clinical importance because it enables evaluation of the three-dimensionality of the sealing techniques, which can be related to the success of the endodontic treatment. **Aim:** To analyze by micro-CT the presence of voids in lower single-rooted premolar root canal obturations prepared using the single cone and ultrasound vibration technique. **Materials and methods:** Twenty extracted single-rooted lower premolars were selected, and the root canal prepared surgically and chemically. In GROUP 1 - Without Vibration, the canal was obturated with a single cone and bioceramic, without applying vibration. In GROUP 2 - With Vibration, the gutta-percha cone inside the root canal was held with a cotton plier to which ultrasound vibration was applied for 3 periods of 3 seconds each. A micro-CT scanner was used to acquire and reconstruct images for analysis. **Results:** No significant difference was found between obturation techniques, though there were differences between thirds, with the cervical third having a higher percentage of voids than the middle and apical thirds. **Conclusions:** The results suggest that the volume of closed, open and total voids does not differ between treatments with and without ultrasound vibration. In the cervical third, the highest volume of voids was related to oval geometry in the teeth evaluated.

Key words: endodontics - root canal obturation - x ray micro CT - voids

Evaluación microtomográfica de la presencia de vacíos en la obturación endodóntica

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RESUMEN

La presencia de vacíos en la obturación endodóntica, su tamaño y el tipo y localización tiene gran importancia clínica ya que permite evaluar la tridimensionalidad de las técnicas de sellado y relacionarlas con el éxito del tratamiento endodóntico. **Objetivo:** analizar mediante microtomografía la presencia de vacíos en la obturación del conducto radicular de premolares inferiores unirradiculares, utilizando la técnica de cono único y vibración con ultrasonido. **Materiales and Método:** se seleccionaron 20 premolares inferiores unirradiculares a los que se les realizó la preparación quirúrgica y química del conducto radicular. Se realizó la obturación con cono único y biocerámico GRUPO 1- sin vibración. En el GRUPO 2 - con vibración se aplicó vibración por ultrasonido, se tomó del cono de gutapercha colocado en el interior del conducto con pinza de algodón que fue vibrada durante 3 periodos de 3 segundos cada uno. Las mismas fueron adquiridas y reconstruidas en un microtomógrafo para posterior análisis de las imágenes obtenidas. **Resultados:** No se evidenciaron diferencias significativas entre ambas técnicas de obturación comparadas, pero si entre los tercios analizados, siendo el cervical el que mayor porcentaje de vacíos presenta en comparación a los cortes correspondientes al tercio medio y apical. **Conclusiones:** Los resultados sugieren que el volumen de vacíos cerrados, abiertos y total no varía en los tratamientos donde se aplica vibración por ultrasonido. En el tercio cervical, el mayor volumen de vacíos se relaciona con la geometría oval que presentaron las piezas dentarias evaluadas en este estudio.

Palabras clave: endodoncia - obturación endodóntica - microtomografía - vacíos



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INTRODUCTION

One of the main aims of endodontics is to provide obturations with three-dimensional sealing of the root canals as a basis for successful treatment¹⁻². However, three-dimensional sealing is not always easy to achieve. Surgical shaping cannot always adapt to the wide range of existing tooth anatomies³⁻⁸, and chemical preparation does not always eliminate 100% of the contents of the main, lateral, accessory and recurrent canals or apical deltas⁹⁻¹². Successful treatment requires extensive knowledge of materials and different techniques to achieve proper obturation of the entire volume of the root canal system.

For years, obturation techniques used cold-laterally compacted gutta percha cones as the main component, sealed with cements, usually of acid-base composition. Later, techniques were developed for gutta percha mechanical plasticization or direct injection into the system. These changes in canal-sealing techniques were mainly motivated by the need to ensure a 3D seal, i.e., an obturation mass without voids, with gutta percha and/or sealer filling any lateral and/or accessory canals¹³⁻¹⁵.

When 3D sealing is not successful, voids form. Voids are either closed, when they are within the obturation mass, or open, when in contact with the dentin wall. Open voids may connect to the periodontal space through the dentinal tubules. The presence of voids reduces the quality of the final obturation, promoting an environment in which microorganisms can survive, and connection between the periodontal space and the interior of the root canal system. This is why it is so important to ensure a three-dimensional seal, without voids or spaces in which bacteria may develop¹⁶.

Currently, with the advent of bioceramic sealers, the concept of obturation has changed. Gutta percha has ceased to be the main component, having been replaced by the sealer itself, which has excellent mechanical and biological properties¹⁷⁻¹⁸.

These new techniques usually use single, more tapered gutta percha cones that almost exactly fit the root canal that was prepared and shaped using rotary and/or oscillatory instruments¹⁹.

In 2018, Kim proposed an obturation technique based on the use of a bioceramic material with a single gutta percha cone, to which vibration was applied. The vibration transfers ultrasound mechanical energy through a cotton plier that is holding the gutta percha cone inside the root canal, thereby

transferring the energy to the bioceramic sealer. This eliminates voids or bubbles, and enhances sealer fluidity, enabling it to penetrate canals that are difficult to access. This technique provides three-dimensional sealing thanks to the sealer rather than the gutta percha²⁰.

X-ray micro-CT scanning is an imaging technique based on X-ray absorption by different types of tissues or materials. It can be used to observe and analyze *ex vivo* samples on a very small scale without causing deterioration²¹. Specimens are positioned in the micro-CT scanner with a holder. Scanning settings (voltage, amperage, rotation, filter, pixel size, grey-scale density, image format, among others) are established, and multiple 2D images are taken while the sample rotates 180° or 360° on its own axis, as programmed. Software is used to recognize the images and convert them into a three-dimensional volume. The volumes thus obtained enable observation, measurement and calculation of many specimen properties, such as volume, surface area, density, presence or absence of voids, etc²²⁻³³. Identifying the presence, size, type and location of voids in an endodontic obturation is of great clinical importance because it enables evaluation of the three-dimensionality of the sealing techniques, which can be related to the likelihood of healing periradicular lesions. Micro-CT scanning is the only technique that provides images in which the above parameters can be measured without destroying or cutting the specimens. It is important to avoid cutting specimens because doing so may smear the obturation material, preventing proper evaluation²². The possibility of observing voids as small as 7 µm enables different sealers and sealing techniques to be studied and compared with the aim of improving obturation three-dimensionality.

The aim of this study was to analyze by micro-CT the presence of voids in the root canal obturation of lower single-rooted premolars prepared using the single cone and ultrasound vibration technique.

MATERIALS AND METHOD

Specimen preparation

This study used 20 single-rooted lower premolars extracted for orthodontic and/or periodontal reasons, straight, with mature apices and caries-free root portion. Retroalveolar X-rays were taken of all specimens in the direction of proximal faces and free faces to verify that they had only one root canal.

Any specimens with more than one root canal were excluded from the sample.

Included specimens were opened at the level of the clinical crown using appropriately sized stone and round bur. Following root canal exposure, catheterization was performed with #10 K-file (Dentsply Maillefer, Switzerland), and opening rectification with EndoZ bur (Dentsply Maillefer, Switzerland) to eliminate any retentive angles. Accesses were prepared with the Sx instrument of the Protaper Gold system (Dentsply Maillefer, Switzerland), accessing the cervical and middle thirds of each sample. Next, working length was established by inserting a #15 K-file (Dentsply Maillefer, Switzerland) into the canal until the tip of the file could be seen to emerge from the apical end of the root. That length was measured, and 0.5 mm subtracted from it. The resulting value was recorded in a table as working length (WL).

Surgical preparation was performed with Protaper Gold System instruments S1, S2, F1 and F2, all at working length. Chemical preparation was performed by irrigating with 2.5% sodium hypochlorite between instruments, and final irrigation with 17% EDTA and again 2.5% sodium hypochlorite, alternating rinses with distilled water between the two solutions. Root canals were dried with sterile paper points.

Obturation was performed with size 25/04 gutta percha cones and bioceramic endodontic sealer BIOC Sealer (Angelus), using the single cone technique. The cones were selected and tested prior to obturation, to confirm that they reached the working length. In both groups, the bioceramic sealer was injected through its applicator tip, from the apical end towards the crown portion. As it filled the root volume, the tip was moved backwards towards the crown end up to the cervical third, where it was removed, and the previously selected gutta percha cone was inserted. In Group 1 (Without Vibration), the gutta percha cone was cut with a hot-tipped instrument and immediately compacted vertically. The pulp chamber toilette was performed and temporary obturation (Cavit 3) applied. In Group 2 (With Vibration), ultrasound vibration was applied by holding the gutta percha cone that had been inserted in the root canal with a cotton plier that was made to vibrate with ultrasound for 3 periods of 3 seconds each. Then, the cone was cut with a hot-tipped instrument and immediately compacted

vertically. The pulp chamber toilette was performed and temporary obturation (Cavit 3) applied.

The specimens were sent to the FOUBA Department of Diagnostic Imaging, Micro-CT scan Service, for scanning and analysis.

Acquisition and reconstruction

A SkyScan 1272 micro-CT scanner (Bruker-microCT, Kontich, Belgium) was used for image acquisition. The specimens were positioned with their major axis in vertical direction. Due to the size of the teeth, two or three scans were performed and then automatically combined during reconstruction. The following parameters were used for image acquisition: 90 kV, 100 μ A, pixel size 12 μ m, grey-scale density 0.5, rotation 180, filter Al 0.5 + Cu 0.038, TIFF format.

The images were reconstructed using Nrecon software version 1.7.3.1, adjusting the parameters to show the specimens as clearly as possible.

Image analysis

Images were analyzed using CTAN v1.17 software (Bruker, Kontich, Belgium). Coronal and apical limits were established on each specimen to mark the extent of the area to be studied, and three thirds were established: apical, middle and cervical, all with the same number of slices. The protocol followed was previously published by our working group⁽³⁴⁾.

Presence of voids in the whole tooth (global) was determined, after which the global volume of open and closed voids was analyzed in each tooth and each third.

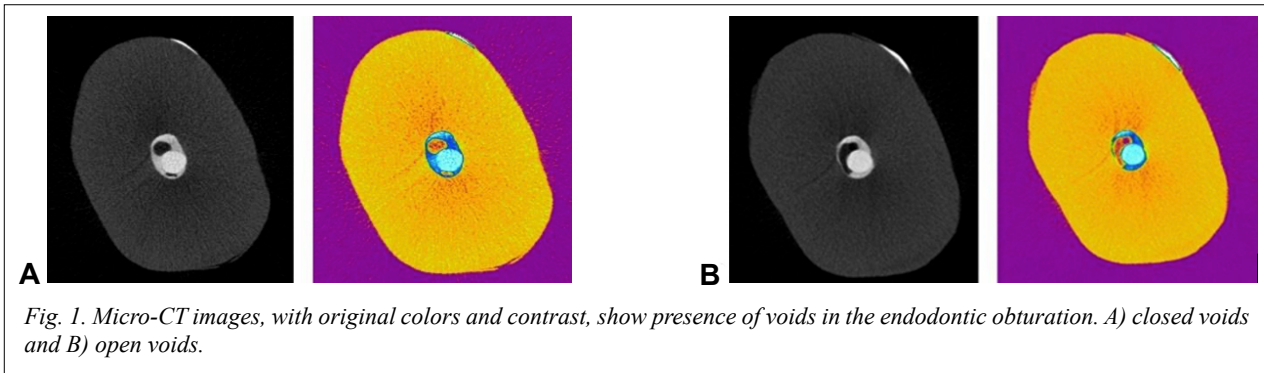
Statistical analysis

Numerical datasets were described by the following measures: minimum (min), maximum (Max), mean (Me), median (Md), standard deviation (SD), first quartile (Q_1) and third quartile (Q_3). To compare quantitative data between two groups, Student's t-test for grouped data (parametric) or Mann-Whitney's U-test (non-parametric) were applied, as appropriate. Quantitative data were compared between groups using the Kruskal-Wallis test (non-parametric), followed by *post-hoc* comparisons with the Conover method. Non-parametric tests were used when the conditions of normality or homoscedasticity were not met. To evaluate the assumption of normality, the D'Agostino-Pearson test was used. Homoscedasticity was analyzed by F or Levene tests when two or

three groups were compared, respectively. A p-value lower than 0.05 was considered significant. MedCalc software version 20.215 (MedCalc Software Ltd., 2023) was used.

RESULTS

Micro-CT scans showed presence of closed and open voids in both groups (Fig. 1 A-B).



Volume of closed voids

Comparison of the global volume of closed voids between groups showed no significant difference; Group 1 without vibration: 0.003 (0.0069) / Group 2 with vibration: 0.004 (0.006). Values expressed as mean (SD) (Fig. 2).

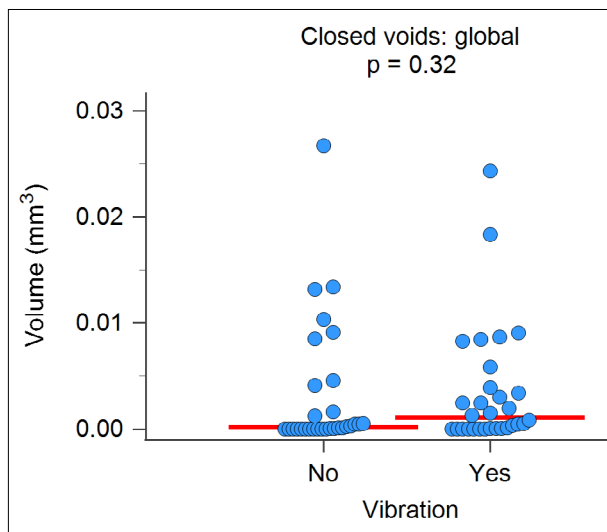


Fig. 2: Global volume of closed voids. Scatter diagram. Horizontal red line: median. p : Mann-Whitney's U-test.

In both groups, the volume of closed voids differed significantly between thirds ($p < 0.05$), with the cervical third having the highest volume of voids (Fig. 3 A and B).

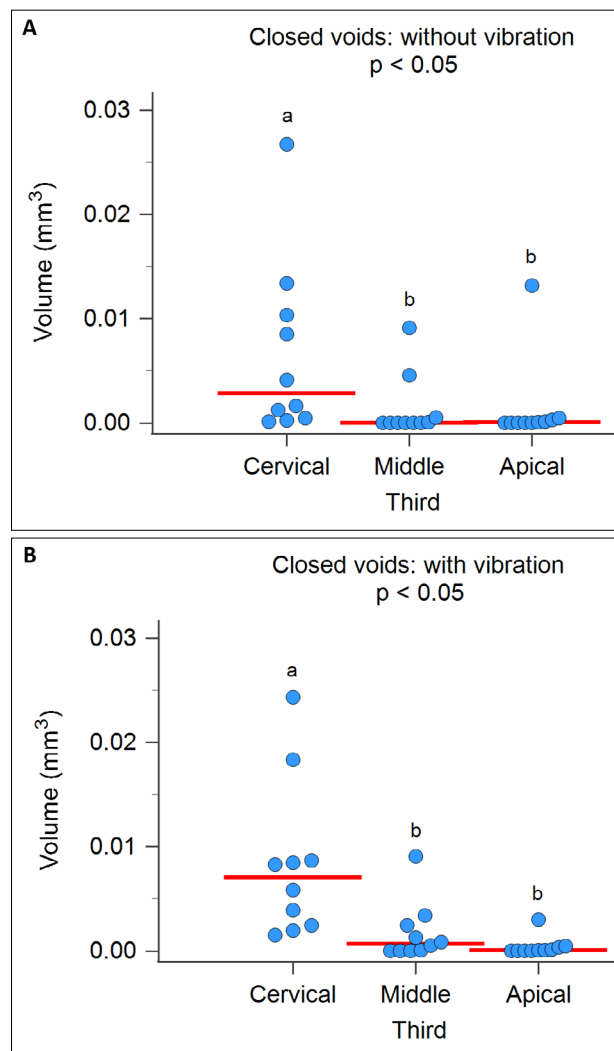


Fig. 3: Volume of closed voids according to third. A) Group 1, Without Vibration, and B) Group 2, With Vibration. Scatter diagram. Horizontal red line: median. p : Kruskal-Wallis test. Different letters indicate significant differences in post-hoc comparisons (Conover).

The volumes of closed voids per group, global and per third, are set forth in Table 1.

Table 1. Volume of closed voids in treatments without and with vibration, in each third and global.

Volume of closed voids (mm ³)															
Third	Vibration														p*
	No							Yes							
	N	min	Max	Me	Md	SD	Q ₁ - Q ₃	N	min	Max	Me	Md	SD	Q ₁ - Q ₃	
Cervical	10	0.000	0.027	0.007	0.003	0.008	0.000 - 0.010	10	0.002	0.024	0.008	0.007	0.007	0.002 - 0.009	0.39
Middle	10	0.000	0.009	0.001	0.000	0.003	0.000 - 0.001	10	0.000	0.009	0.002	0.001	0.003	0.000 - 0.002	0.35
Apical	10	0.000	0.013	0.001	0.000	0.004	0.000 - 0.000	10	0.000	0.003	0.000	0.000	0.001	0.000 - 0.000	0.69
Global	30	0.000	0.027	0.003	0.000	0.006	0.000 - 0.004	30	0.000	0.024	0.004	0.001	0.006	0.000 - 0.004	0.32

* *Mann-Whitney's U test*

N, sample size; min, minimum; Max, maximum; Me, mean; Md, median; SD, standard deviation; Q₁, first quartile; Q₃, third quartile.

The volumes of open voids per group, global and per third, are set forth in Table 2.

Table 2. Volume of open voids in treatments without and with vibration, in each third and global.

Volume of open voids (mm ³)															
Third	Vibration														p
	No							Yes							
	N	min	Max	Me	Md	SD	Q ₁ - Q ₃	N	min	Max	Me	Md	SD	Q ₁ - Q ₃	
Cervical	10	4.339	13.062	8.378	7.502	3.139	5.459 - 10.717	10	6.069	10.916	8.565	8.652	1.822	6.493 - 10.465	0.87 [#]
Middle	10	2.102	5.858	3.459	3.155	1.210	2.531 - 4.327	10	2.584	5.974	3.784	3.313	1.165	3.083 - 4.054	0.55 [#]
Apical	10	1.204	3.274	1.706	1.521	0.613	1.340 - 1.837	10	1.004	2.142	1.555	1.496	0.356	1.440 - 1.758	≈1*
Global	30	1.204	13.062	4.514	3.489	3.447	1.837 - 5.858	30	1.004	10.916	4.635	3.313	3.215	1.758 - 6.493	0.80*

[#]*Student's t-test for grouped data*; **Mann-Whitney's U test*

N, sample size; min, minimum; Max, maximum; Me, mean; Md, median; SD, standard deviation; Q₁, first quartile; Q₃, third quartile.

Global volume of open voids was 4.514 (3.447) in Group 1, and 4.635 (3.215) in Group 2; values expressed as mean (SD); without significant differences. (Fig. 4).

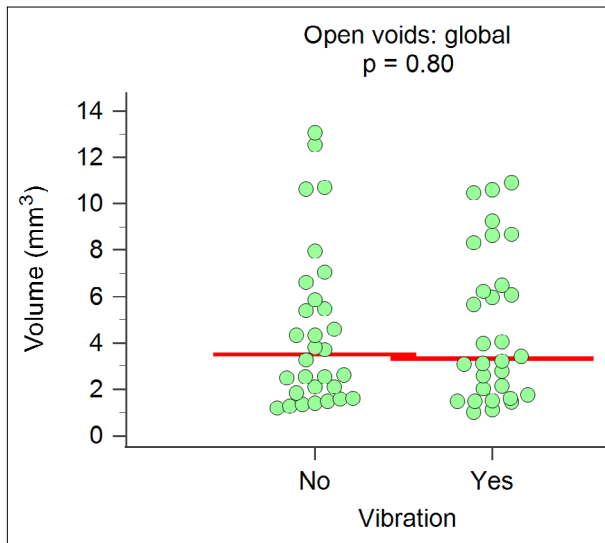


Fig. 4: Volume of open voids according to treatment. Scatter diagram. Horizontal red line: median. p Mann-Whitney's U-test.

The volume of open voids differed significantly between thirds ($p < 0.05$), both in the group without vibration (Fig. 5) and in the group with vibration (Fig. 5). In both cases, the cervical third had the highest volume of voids and the apical third had the lowest.

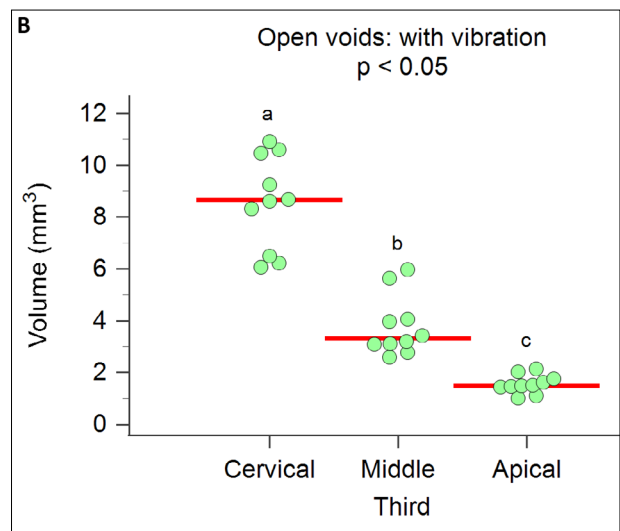
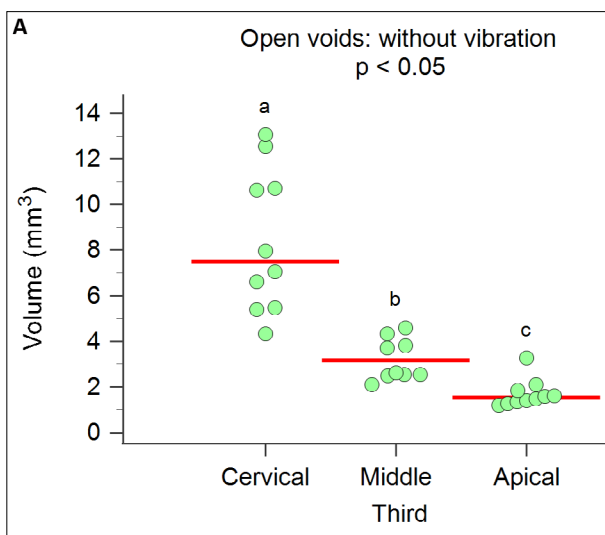


Fig. 5: Volume of open voids according to thirds. A) Group without vibration and B) Group with vibration. Scatter diagram. Horizontal red line: median. p Kruskal-Wallis test. Different letters indicate significant differences in post-hoc comparisons (Conover).

Total volume of voids

In Group 1, global volume of voids ranged from 1.204 to 13.076 mm³, with a median (Q_1 - Q_3) of 3.489 (1.850 - 5.867) and a mean (SD) of 4.517 (3.449). In Group 2, it ranged from 1.007 to 10.919 mm³, with a median (Q_1 - Q_3) of 3.313 (1.758 - 6.499) and a mean (SD) of 4.638 (3.218) (Fig. 6). There was no significant difference between the groups without and with vibration (Table 3), either within the thirds studied: cervical ($p = 0.87$), middle ($p = 0.55$) and apical ($p \approx 1$), or global ($p = 0.80$).

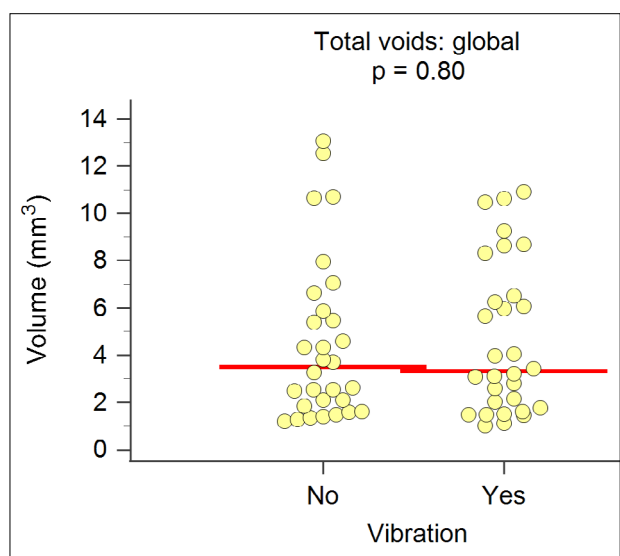


Fig. 6: Total volume of voids according to treatment. Scatter diagram. Horizontal red line: median. p Mann-Whitney's U-test.

The total volume of voids differed significantly between thirds ($p < 0.05$), both in Group 1 Without Vibration (Fig. 7 A) and in Group 2 With Vibration (Fig. 7 B). In both cases, the cervical third had the highest volume of voids, and the apical third had the lowest.

Table 3. Total volume of voids in treatments without and with vibration, in each third and global.

Total volumen of voids (mm ³)																
Third	Vibration															p
	No							Yes								
	N	min	Max	Me	Md	SD	Q ₁ - Q ₃	N	min	Max	Me	Md	SD	Q ₁ - Q ₃		
Cervical	10	4.340	13.076	8.384	7.508	3.139	5.463 - 10.717	10	6.077	10.919	8.573	8.655	1.820	6.499 - 10.483	0.87 [#]	
Middle	10	2.102	5.867	3.461	3.155	1.212	2.531 - 4.332	10	2.584	5.974	3.786	3.313	1.166	3.083 - 4.056	0.55 [#]	
Apical	10	1.204	3.274	1.707	1.521	0.614	1.340 - 1.850	10	1.007	2.143	1.555	1.496	0.355	1.440 - 1.758	≈1 [*]	
Global	30	1.204	13.076	4.517	3.489	3.449	1.850 - 5.867	30	1.007	10.919	4.638	3.313	3.218	1.758 - 6.499	0.80 [*]	

[#] Student's t-test for grouped data; ^{*}U de Mann-Whitney

N, sample size; min, minimum; Max, maximum; Me, mean; Md, median; SD, standard deviation; Q₁, first quartile; Q₃, third quartile.

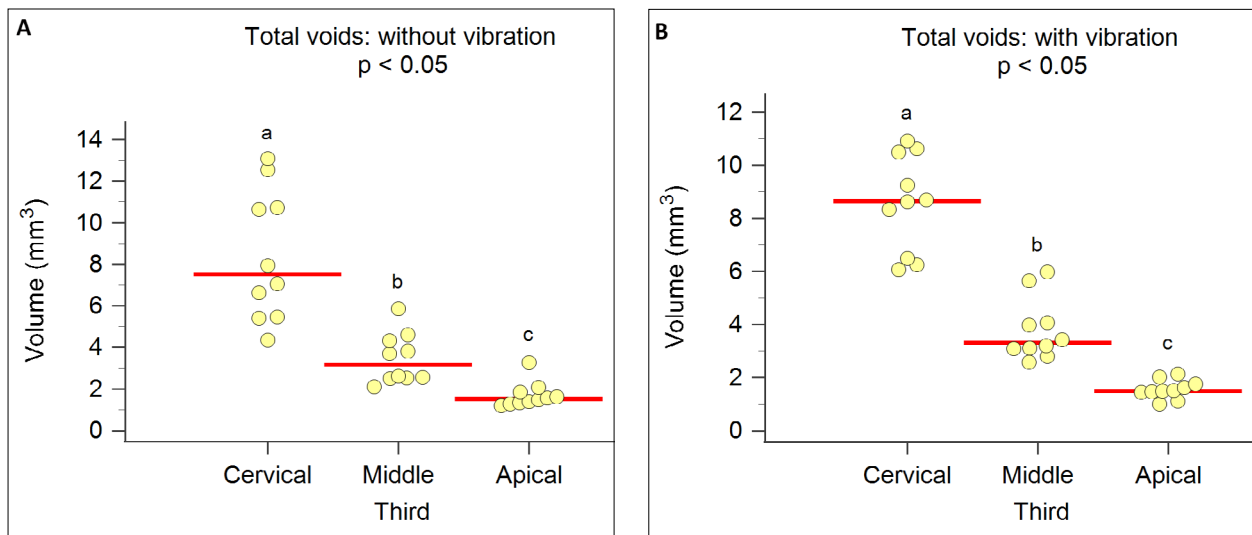


Fig. 7: Total volume of voids according to thirds. A) Group Without Vibration and B) Group With Vibration. Scatter diagram. Horizontal red line: median. p Kruskal-Wallis test. Different letters indicate significant differences in post-hoc comparisons (Conover).

DISCUSSION

Many different obturation techniques are currently in use, all of which seek to achieve a three-dimensional seal that will increase the likelihood of successful endodontic treatment, free from recontamination. One of the most popular techniques in recent years

uses a bioceramic as the main material, along with a more tapered gutta-percha cone that matches the last instrument used. In 2018, Kim introduced a variation of this technique by adding the use of ultrasound to make the gutta percha cone vibrate, which transmits

the waves to the sealer, thereby improving its flow towards the more irregular parts of the root canal²⁰.

The present study used micro-CT scans to evaluate 20 teeth obturated with bioceramic sealer and single, more tapered cone. Ultrasound was used to apply vibration to half the specimens. Statistical data showed no significant difference in sealing capacity between techniques. There were, however, differences between the thirds evaluated. As expected, the cervical third had the most voids, regardless of the obturation technique, because it contains more sealer due to the anatomical change in the canal, and the gutta percha cone has poorer fit. The data recorded in the current study are consistent with the report published by Kim et al. in 2018²⁰, which found no significant difference between groups with and without vibration, although many sealer-free spaces (voids) were observed, mainly in the cervical third, also in agreement with the current study.

Iglesias et al.²⁸ also reported similar results to the current study. They performed single-cone obturations and continuous wave obturations. Micro-CT analysis showed no difference between groups regarding the presence of voids, except in the cervical third.

Ho et al.³² conducted a study observing endodontically treated samples obturated using different techniques (cold lateral condensation, hot vertical condensation and thermoplasticization by ultrasound). The amount of gutta percha present in each sample was evaluated. The results showed that vertical compaction and ultrasound thermoplasticization achieved comparable obturation qualities with gutta percha in the canal system, with significantly higher density than the samples obturated using cold lateral condensation. This demonstrates better results with the use of ultrasound applied on the gutta percha, reducing voids in the mass, but the article does not specify results on the evaluation of the presence of voids in the endodontic sealer.

The data reported by Somma et al.³⁵ in 2011 on the evaluation of three different sealing methods also agree with the current study. They analyzed two thermoplasticization techniques (Thermafill and System B) and the single cone technique, by means of 19.1 μm pixel size micro-CT scan. All measurements found similar values for the quantity and distribution of voids and the percentage of occupation of the endodontic space, regardless of the technique.

In 2014, though without using ultrasound, Keleş³⁶ et al. measured the volumes of filling and voids in oval root canals by means of 12.5 μm micro-CT. They found that in the apical third, there was no difference between cold lateral compaction and hot vertical compaction techniques, but in the other two thirds, there was a difference in favor of hot compaction.

In 2021, Pérez-Alfayate et al.³⁰ analyzed the obturation of 200 lower molars with curved roots, using six sealers (AH Plus Thermafil, GuttaCore, GuttaFusion, Cold Side Compaction, AH Plus and EndoSequence BC sealer). Half of the samples from each group were activated with an Endoactivator sonic system tip size 25/06. Three sections were cut from each molar, representing the cervical, medium and apical thirds. Images taken with confocal laser microscope and operative microscope showed no statistically significant differences between sealers, techniques or thirds. It was concluded that the use of sonic activation was unrelated to the three-dimensional seal of the obturation, at least according to the data recorded under fluorescent laser and operative optical microscopes. These results are consistent with the present study.

Başer Can et al.²⁹ also used micro-CT to evaluate 30 single-rooted teeth obturated with EndoREZ, Activ GP or AHplus with gutta-percha. The images were acquired at 13.68 μm and showed significantly more voids in the apical third with Activ GP than with the other two techniques (EndoREZ or AHplus and gutta percha). As in the current study, micro-CT scans enabled determination of whether there were significant differences between different obturation methods.

Celikten et al.¹⁹ evaluated and compared the presence of voids in endodontic obturation with different sealers (EndoSequence BC Sealer, Smartpaste bio, Activ GP and AH Plus) by means of 13.47 μm pixel size micro-CT scans. A large quantity of voids was found in the samples obturated with bioceramics, but there was no statistical difference between them, or compared to the other sealers. This paper suggests that micro-TC scanning is essential for evaluating this type of parameters.

Huang et al.²⁷ evaluated two endodontic sealers (EndoSequence BC sealer and AH Plus) for obturation of lower premolars. Although the SEM study showed that both sealer were better adapted at crown level, micro-CT scans showed the presence of closed voids within the sealer mass, with no

difference between them. Moreover, the number of voids in the cervical third was significantly higher than in the other two thirds, in direct relation to the oval anatomy of root canals in these thirds. These results support the current study.

All of the above demonstrates the importance of using technology that enables parameters to be assessed at very high resolutions.

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CONCLUSIONS

The results suggest that the volume of closed, open and total voids does not differ between treatments with and without ultrasound vibration. In the cervical third, the highest volume of voids is related to the oval geometry in the teeth evaluated in this study.

DECLARATION OF CONFLICTING INTERESTS

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

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Clinical guidelines for herpes labialis: recommendations and quality evaluation according to AGREE II

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ABSTRACT

Cold sores require healthcare professionals to employ specific approaches for prevention and management, with the need for effective therapeutic guidelines and ongoing improvement in patient care. **Aim:** To evaluate the methodological quality of Clinical Guidelines (CG), clinical guides and manuals for care of the population affected by herpes labialis, to verify their compliance with evidence-based health standards. **Materials and Method:** A search was conducted for CG on labial herpes in the Medical Literature Analysis and Retrieval System Online (Medline) database, Google Scholar, Brazilian Virtual Health Library (BVS), and sites of institutions/professional categories, using the descriptors “herpes labialis” or “oral herpes”. Document quality was assessed using the Appraisal of Guidelines for Research & Evaluation Instrument (AGREE II). The Kappa test was used to avoid randomness or poor agreement between results. **Results:** Analysis of the 12 selected publications on the management of labial herpes revealed flaws in quality, as the publications did not follow a quality standard. The main quality flaws identified were in “rigor in development” and “applicability.” **Conclusions:** Priorities need to be redefined in the development of CG for clinical practice related to fever blisters to reduce the variability of the quality standard, and generate reliable, applicable recommendations.

Key words: pharmaceutical care - clinical guidelines - herpes labialis - evidence based practice - data validation.

Diretrizes clínicas para o herpes labial: recomendações e avaliação de qualidade de acordo com o AGREE II

RESUMO

A Herpes labial requer dos profissionais abordagens específicas para prevenção e manejo, com a necessidade de diretrizes terapêuticas eficazes e contínuo aprimoramento do cuidado ao paciente. **Objetivo:** avaliar a qualidade metodológica de documentos que abordaram Diretrizes Clínicas (DC), guias clínicos e manuais para o cuidado da população afetada pelo herpes labial, verificando sua conformidade com padrões de saúde baseados em evidências. **Materiais e Método:** As DC sobre herpes labial foram pesquisadas na base de dados Medical Literature Analysis and Retrieval System Online (Medline), Google Acadêmico, Biblioteca Virtual em Saúde (BVS) e em sites de instituições/categorias profissionais, utilizando os descritores “herpes labial” ou “herpes oral”. Utilizamos a ferramenta The Appraisal of Guidelines for Research & Evaluation Instrument (AGREE II) para a avaliação da qualidade. O teste Kappa também foi utilizado para evitar aleatoriedade ou baixa concordância entre os resultados. **Resultados:** Na análise das 12 publicações selecionadas sobre o manejo do herpes labial, foram identificadas falhas na qualidade dos documentos, que não seguiram um padrão de qualidade. As principais falhas de qualidade identificadas foram em “rigor no desenvolvimento” e “aplicabilidade”. **Conclusão:** é necessário um reenfoque para definir prioridades no desenvolvimento de DC para a prática clínica do herpes labial, a fim de reduzir a variabilidade do padrão de qualidade e gerar recomendações que possam ser confiáveis e aplicáveis.

Palavras-chave: cuidado farmacêutico - diretrizes clínicas - herpes labial - prática baseada em evidências - validação de dados

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INTRODUCTION

Evidence-based healthcare involves solving health problems by basing decisions on the best available evidence¹. Clinical Guidelines (CG) are based on systematically developed recommendations to mediate among health policies, best practices, government funding, local contexts, and patient choice^{2,3}. Thus, adopting CG helps qualify professional practices at a time when evidence-based health care has become consolidated as a standard to guide both continuing education and care⁴. The World Health Organization (WHO) recommends the development of national CG as one of the main strategies for promoting the rational use of medicines, with scientific guidance on diagnoses and treatments⁵.

The quality of guidelines is decisive regarding the potential benefits provided by their use^{4,6}. Key attributes of high-quality guidelines include validation, reliability, reproducibility, clinical applicability, adaptation to the healthcare setting, clarity, multidisciplinary, evidence review, and documentation⁶. Guideline quality is highly variable, often failing to meet basic methodological standards, and thereby discrediting a mechanism that is fundamental to the implementation of evidence-based health care^{2,4,6}. If numerous guidelines with different methods and objectives are developed for the care of the same disease, they can create unnecessary competition and a complex system of conflicting practices and interventions^{4,7}. Inadequate methodologies and inconsistent strategies in the CG development process can hinder the implementation of recommendations^{2,4}. This is particularly critical for conditions or diseases of considerable frequency and incidence that trigger organic reactions and tend to occur without harming the patient, including some infectious diseases such as herpes labialis⁸.

Cold sores are a common, highly contagious infectious disease that affects the orolabial region (most commonly the outer part of the lower lip), caused by Herpes Simplex Virus Type 1 (HSV-1), and less frequently, Herpes Simplex Virus Type 2 (HSV -2)^{9,10}. It is estimated that about 90% of the world population is exposed to HSV-1^{11,12}. The incidence of HSV is 70% to 80% in populations with low socioeconomic status, and 40% to 60% in those with higher status¹¹⁻¹⁴. Herpes labialis cases have increased globally over the past two decades, with over 23 million new cases per year,

becoming a significant public health problem, with 15 to 40% of the population experiencing recurrent symptomatic outbreaks^{12,14-16}. Most people with recurrent HSV-1 infection have fewer than two episodes per year, but 5% to 10% of affected people have at least six recurrences per year^{9,10,14,16}. HSV-1 recurrences appear to be precipitated by several factors that can compromise an individual's immune status, including prolonged exposure to ultraviolet (UV) light, use of immunosuppressive medications, HIV infection, stress, premenstrual tension, and surgery^{10,13,14,16}. Some researchers claim that a diet rich in arginine-containing foods (e.g., chocolate, nuts and seeds) may be associated with the reactivation of herpes labialis¹⁷⁻¹⁹.

Cold sores, a public health concern inherently linked to sexually transmitted infections (STI), constitute an incurable condition, requiring specific approaches for reducing complications and managing symptoms^{1,9,13,14,16,20}. The relevance of cold sores as a public health issue underscores the urgent need for continuous attention and education for proper prevention and management. The interconnection between cold sores and STI emphasizes the importance of comprehensive strategies encompassing prevention, diagnosis and treatment. It is crucial to recognize that, given their recurrent nature and high contagiousness, cold sores require essential therapeutic recommendations, especially considering the significant increase in HSV infections^{12,13,16-21}.

The scarcity of studies reviewing clinical recommendations for cold sore management underscores the need for additional research. There is a clear need for continuous improvement of patient care based on systematic, reliable, unbiased information^{2,3}. It is therefore crucial to adopt a comprehensive approach that considers public health perspectives to produce effective therapeutic guidelines to optimize cold sore management in the context of STI¹⁰⁻¹⁴. This study evaluated the methodological quality of clinical guidelines for the care of the population affected by fever blisters, checking their compliance with evidence-based health standards and guidelines.

MATERIALS AND METHOD

Identification and selection of guidelines

A search was made for CG on labial herpes from

June 2022 to March 2023, in the databases of the *Medical Literature Analysis and Retrieval System Online* (Medline), through Pubmed® (with filter for CG), Google Scholar, Biblioteca Virtual em Saúde do Brasil (BVS) (with filter for guidelines prepared by the Ministry of Health) and websites of institutions/professional categories, through the descriptors: “herpes simplex” or “herpes labialis” or “labial herpes” or “oral herpes” and their synonyms combined with Boolean operators, previously consulted on the websites *Descriptors in Health Sciences* (DeCS, from Brazil)²² and *Medical Subject Headings* (MeSH, from MEDLINE-PubMed)²³. Duplicate publications were excluded. There was no publication time restriction for identification in the databases consulted^{13,20}. The search was performed by peers, and any publication on whose inclusion they disagreed was included, given the limited number of specific CG for labial herpes.

Quality was assessed in documents containing guidelines for the care of individuals with fever blisters without other comorbidities. CG with interventions based on specific treatments (e.g., laser) and special care for specific patients (such as athletes, transplant patients, pregnant women, and newborns with or without the presence of comorbidities) were not included.

Guideline Evaluation Tool

The quality of the selected CG was assessed using the AGREE Instrument (*Appraisal of Guidelines for Research & Evaluation*), 2nd edition. AGREE is an assessment tool developed from reviews of more than 100 selected guidelines independently evaluated by more than 200 reviewers from different countries²⁴⁻²⁷. It is used as part of a protocol for quality assessment of CG to improve healthcare by WHO and several technology assessment agencies around the world^{13,25-27}. Its latest edition (AGREE II), used in this paper, contains 23 key items organized into six quality domains²². Four independent experts evaluated the Clinical Guidelines selected for this study according to the instructions in AGREE II^{3,22}.

Data extraction, management, and evaluation

The AGREE II Instrument includes six quality domains: (i) scope and purpose; (ii) stakeholders; (iii) development rigor; (iv) clarity of presentation; (v) applicability; and (vi) editorial independence^{3,24-26}. Data related to the six quality domains were collected

and recorded on a score sheet with ratings from 1 (strongly disagree) to 7 (strongly agree) for each of the 23 items provided by the instrument^{3,24}. Finally, a percentage of adequacy was calculated for each of the six domains, with values from 0% to 100%, in which the score obtained by each evaluator and the maximum possible score for the domain were used, following the suggestions in the AGREE II instrument²².

Although it is not suggested in the AGREE II instrument, the Kappa statistical test was used in the current study to analyze inter-rater agreement and avoid randomness or poor agreement^{3,21}. The Kappa coefficient of agreement describes the agreement between two or more researchers performing a nominal or ordinal evaluation of the same sample. Kappa coefficients of moderate agreement (Kappa>0.4) were considered preferable for this type of study^{3,27-28}. For the agreement analysis, the raters jointly decided that assessment scores of 1 and 2 would be considered “low,” scores of 3 to 5 would be “intermediate” and scores of 6 and 7 “high”.

The AGREE II instrument does not define a standard indicating whether the guideline should be recommended^{3,26}. Thus, to make the evaluation less subjective in this regard, it was agreed in the current study that domain (iii) “developmental rigor” would be the primary standard for the overall guideline evaluation. Following the criteria suggested by other authors^{3,19-21}, the evaluations defined 50% as the minimum score for “developmental rigor”. Thus, for a CG to be considered “recommended,” it should score above 50%. The CGs that scored between 30% and 50% were considered as “developmental rigor requiring modifications,” and any that scored less than 30% were considered as “not recommended”³.

RESULTS

Guideline Characteristics

The study included CG on labial herpes published in different countries. Initially, 168 publications were retrieved and screened by reading titles and abstracts. Application of the established inclusion and exclusion criteria led to the exclusion of 149. In the guideline pre-selection stage, a greater diversity of available publications on clinical management for treating and diagnosing genital herpes was observed, including an international guideline published by the WHO²⁹. After the CG pre-selection and selection processes, 12 publications remained,

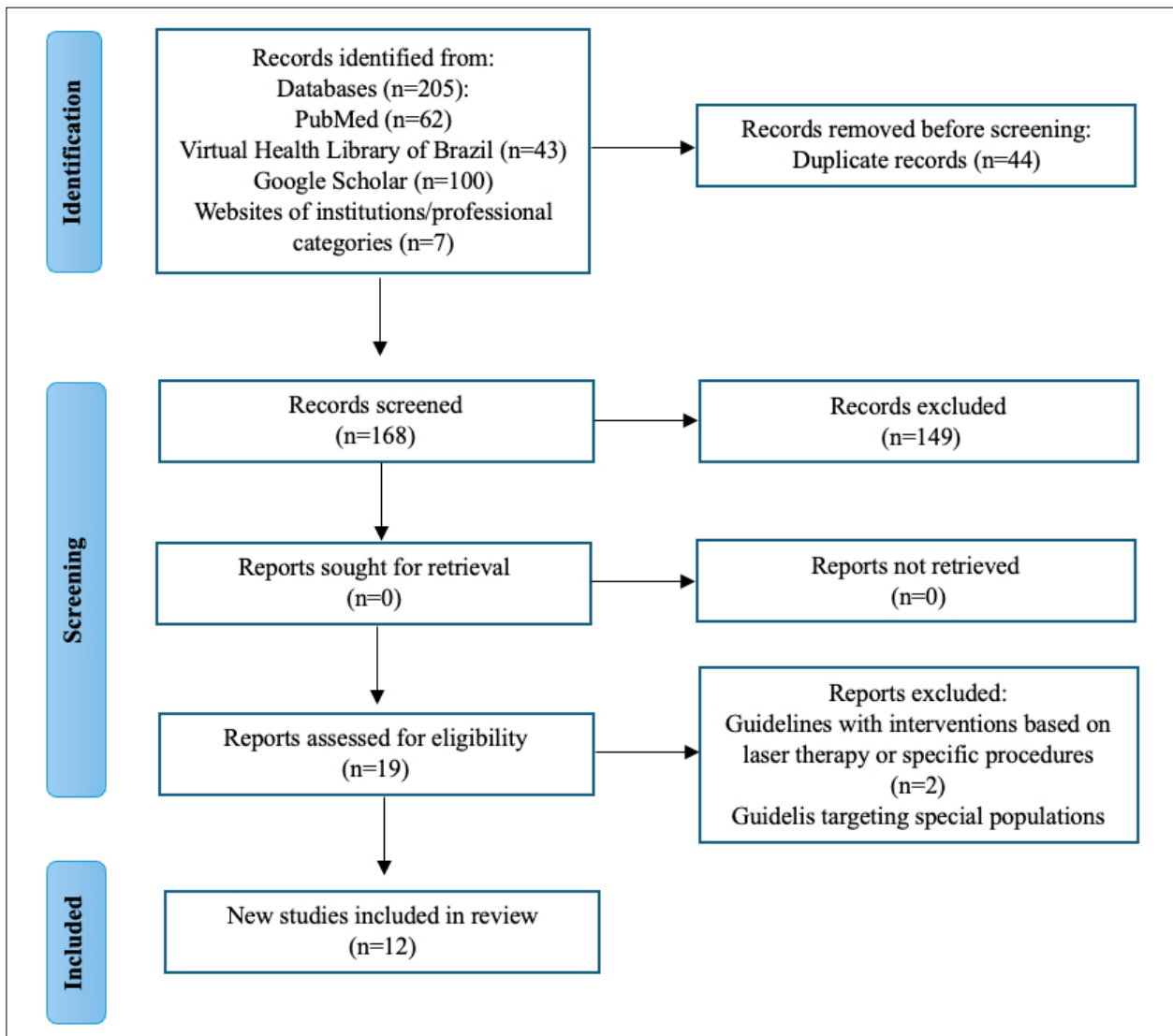


Fig. 1: Flowchart of the identification, selection, and inclusion of guidelines for evaluation. 2023.

which comprised the final sample in this study (Fig. 1, Table 1).

The most common type of recommendation in CG was treatment (62%), followed by recommendations on diagnosis (24%) and care (15%). Guidelines from the United States of America (USA), United Kingdom, Spain and Brazil agreed on the use of nucleoside antivirals (acyclovir, valacyclovir and famciclovir) as the drugs of choice in the treatment of herpes labialis (Table 2). In most guidelines, the oral route of administration was recommended more than the topical route. There was no consensus in the guidelines on the recommendation of topical antiviral therapy. CG 4, 9 and 10 listed some adjuvant treatments, such as oral analgesics and topical anesthetics, based on clinical severity,

cautioning to consider individual need³⁰⁻³³. The results of all primary studies and their combination characteristics addressed in the guidelines were summarized and systematized (Table 3).

Evaluation of Guideline Quality: Overall Assessment

Evaluation of the 12 CG in this study produced quality scores (0 to 100) for each domain (Table 4). The effectiveness of the method used in this study is demonstrated by the accuracy of the AGREE II methodology in identifying the weaknesses of different types of guidelines in the evaluation of comprehensive issues necessary for any type of care guidance²⁶.

There was a lack of transparency and methodological

Table 1. General characteristics of the clinical guidelines identified on herpes labialis. 2023.

Acronym	Selected guideline	Year of publication	Institution	Origin	Version	Population	Recommendation Rating System
CG 1	Oral Herpes simplex	2016	NICE	UK	Updated	Adult / Child	None
CG 2	Herpes simplex infections	2015	PCDS	UK	Original	Adult / Child	None
CG 3	Management guidelines for herpes simplex	2016	HVA	International	Original	Adult / Child	None
CG 4	Oral herpes - Clinical Guideline	2020	MSF	International	Original	Adult / Child	None
CG 5	Recurrent Herpes Simplex Labialis: Selected Therapeutic Options	2003	JCDA	CA	Original	Adult / Child	None
CG 6	Common Oral Lesions: Part I. Superficial Mucosal Lesions	2007	AAFP	USA	Original	Adult	SORT
CG 7	Congenital Herpes Simplex Virus	2010	AAFP	USA	Original	Adult	SORT
CG 8	Guidelines For The Management Of Community-Acquired Infections	2013	NHS	UK	Original	Adult / Child	CEBM
CG 9	Management of infection guidance for primary care for consultation & local adaptation	2014	PHE	UK	Original	Adult / Child	CEBM
CG 10	Pharmaceutical Indication Protocols and Derivation Criteria for Minor Symptoms	2008	GFS	ES	Original	Adult / Child	None
CG 11	Infectious and Parasitic Diseases	2010	MH	BR	Original	Adult / Child	None
CG 12	Dermatology In Primary Health Care	2002	MH	BR	Original	Adult / Child	None

CG: Clinical Guideline; SORT: Strength of Recommendation Taxonomy; CEBM: Centre for Evidence-based medicine; NICE: National Institute for Health and Care Excellence; MH: Ministry of Health; GFS: semFYC Drug Utilization Work Group; PHE: Public Health England; NHS: NHS Bolton Clinical Commissioning Group; AAFP: American Academy of Family Physicians; JCDA: Journal of the Canadian Dental Association; MSF: Médecins Sans Frontières; PCDS: Primary Care Dermatology Society, HVA: Herpes Viruses Association; BR: Brazil; ES: Spain; UK: United Kingdom; USA: United States of America; CA: Canada.

rigor in producing most of the CG, which made them inconsistent in reliability and reproducibility, and in turn may compromise decision-making by health professionals³⁰⁻³¹. Only two of the 12 CG evaluated (CG 1 and CG 5) declared conflict of interest. There is reason to believe that the production, dissemination and application of evidence in providing healthcare may often be influenced by conflicts of interest, especially financial²⁶.

Two of the 12 CG evaluated, “Oral Herpes simplex” (CG 1) and “Infection management guidance for primary care for consultation and local adaptation” (CG 9), were considered “recommended”, one,

“Non-genital Herpes Simplex Virus” (CG 7) was considered “recommended with modifications”, and nine (CG 2-6,8,10-12) were considered “not recommended”.

Inter-rater agreement square-weighted Kappa was 0.62, reflecting substantial agreement^{3,27-28}.

Evaluation of Guideline Quality: Domain 1 - Scope and Purpose

This was the domain with the highest scores. “Oral Herpes Simplex” (CG 1) had the highest score (93%). It defines the target population, actions and health context using an objective scientific essay of

Table 2. Pharmacological and non-pharmacological recommendations per clinical guideline. 2023.

Clinical Guidelines	Pharmacological Treatment					Non-pharmacological treatment				
	Acyclovir	Valacyclovir	Famciclovir	Penciclovir	Docosanol	Chlorhexidine solution	Hydrogen Peroxide	Copper Sulfate	Vaseline	Povidone iodine
CG1	YES	YES	YES	-	-					
CG 2	YES	YES	YES	-	-	-	-	-	-	
CG 3	YES	YES	YES	-	-	-	-	-	-	
CG 4	YES	-	-	YES	-	YES	-	-	-	YES
CG 5	YES	YES	YES	YES	-	-	-	-	-	
CG 6	YES	YES	YES		-			-		
CG 7	YES	YES	YES	YES	YES	-	-	-	-	-
CG 8	YES	-		YES	-	-	-	-	-	
CG 9	-	-	-	-	-	YES	YES	-	-	
CG 10	YES	-	-	-	-	-	-	YES	YES	
CG 11	YES	YES	-	-	-	-	-	-	-	
CG12	YES	YES	-	YES	-	-	-	-	-	

Legend: YES: Recommended use

Table 3. Scores of quality domains of the clinical guidelines for herpes labialis evaluated according to the AGREE II tool.

Acronym	Scope and Purpose	Stakeholder involvement	Rigor of development	Clarity of presentation	Applicability	Editorial independence
CG 1	93%	83%	91%	85%	82%	100%
CG 2	39%	7%	1%	35%	2%	0%
CG 3	46%	13%	3%	51%	10%	0%
CG 4	63%	24%	5%	47%	2%	0%
CG 5	75%	39%	21%	78%	11%	75%
CG 6	44%	28%	28%	71%	4%	0%
CG 7	57%	31%	36%	78%	39%	0%
CG 8	64%	19%	16%	32%	13%	0%
CG 9	82%	64%	61%	56%	15%	0%
CG 10	86%	64%	17%	60%	17%	0%
CG 11	83%	46%	15%	51%	21%	0%
CG 12	79%	43%	6%	61%	11%	0%

CG: Clinical Guideline.

Table 4. Synthesis of clinical studies present in the selected clinical guidelines of herpes labialis. 2023.

Study	Origin	Type of design	Interventions	Outcome	Adverse effects	Conclusions
Rooney JF et al. ³⁴	USA	RCT, double-blind, placebo-controlled, crossover (n = 22).	Acyclovir 400 mg (twice daily) orally or placebo for four months.	1. Number of relapses per patient. 2. Number of relapses with positive HSV culture per patient.	Not reported.	Treatment with acyclovir 400 mg (twice daily) resulted in a 53% reduction in clinical relapses and 71% in HSV-positive culture compared to placebo therapy.
Baker et al. ³⁵	USA	RCT, double-blind, placebo-controlled (n = 311)	Valacyclovir 500 mg (once daily) orally or placebo for four months.	1. Number of relapses per patient. 2. Average time to first relapse.	The most common adverse effect in both groups was a headache, reported five times among three patients in the valacyclovir group and twice in the placebo group.	Valacyclovir 500 mg orally once daily for four months is effective and well tolerated to prevent recurrent herpes labialis.
Sprauce et al. ³⁶	USA	RCT, double-blind, placebo-controlled, multicenter (n = 49)	Valacyclovir 500 mg (once daily) orally or placebo for four months.	1. Time to healing of the lesion. 2. Time to resolve pain and/or discomfort.	Not reported.	The time to heal the lesion and resolve pain and/or discomfort was statistically reduced with valacyclovir compared to placebo.
Sprauce et al. ³⁷	USA	RCT, double-blind, placebo-controlled (n = 701)	Famciclovir 1500 mg (once daily) or 750 mg (twice daily) for one day or placebo within 1 hour after the onset of prodromal symptoms of an episode of herpes labialis.	1. Healing time of the lesion.	Not reported.	The single dose of famciclovir reduced the healing time of herpes labialis lesions by approximately two days compared to placebo.
Raborn et al. ³⁸	USA, UK, and NA.	RCT, double-blind, placebo-controlled, multicenter (n = 4,273)	Penciclovir (1%) cream or placebo applied topically (six times a day) during the first day and every 2 hours for four consecutive days.	1. Healing time of the lesion. 2. Time to resolve pain and/or discomfort.	Not reported.	Penciclovir cream significantly outperformed placebo in healing classic lesions and resolving the pain of recurrent herpes labialis. The effectiveness was apparent if the therapy was started "early" (stage of prodrome injury or erythema).
Sprauce et al. ³⁹	USA	RCT, double-blind, placebo-controlled, multicenter (n = 699)	Acyclovir (5%) cream or placebo topically (five times a day) for four days, starting within 1 hour of the beginning of a recurrent episode.	1. Healing time of the lesion. 2. Time to resolve pain and/or discomfort.	Adverse effects were mild and uncommon.	Acyclovir cream did not prevent the development of classic lesions (progression to vesicles, ulcers and/or crusts).

Study	Origin	Type of design	Interventions	Outcome	Adverse effects	Conclusions
Sacks et al. ⁴⁰	USA	RCT, double-blind, placebo-controlled, multicenter (n = 370)	Docosanol (10%) cream or placebo (five times a day) until complete healing of the lesions.	1. Healing time of the lesion. 2. Time to resolve pain and/or discomfort.	Not reported.	Docosanol applied five times a day is safe and effective in treating recurrent herpes labialis.
Rahimi et al. ⁴¹	USA	Systematic review and meta-analysis	Topical and systemic antivirals	Prevention of recurrent herpes labialis.	Report of pain and nausea as the only adverse effects in 5% of patients using systemic antivirals.	Using acyclovir and systemic valacyclovir is safe and effective in preventing recurrent herpes labialis.

USA: United States of America, UK: United Kingdom, NA: North America, RCT: Randomized Clinical Trial.

questions answered with inclusion and exclusion criteria and is well updated²⁹.

Evaluation of Guideline Quality: Domain 2 - Stakeholder Engagement

Scores in this domain varied widely across guidelines (7%-83%). Overall, the evaluation identified two main weaknesses: little use of collaborative multidisciplinary practices, and lack of investigation of patients' opinions and preferences.

The guideline "Herpes Simplex Infections" (CG 02) had the lowest quality score in the domain (7%) because it was prepared by only one dermatologist, with no report of other health professionals or methodologists collaborating by supervising the search and analyzing evidence. Other CGs were authored and reviewed by infectious disease physicians, dermatologists, and epidemiologists, but did not include non-medical health professionals such as dentists, pharmacists and nurses.

Among the 12 guidelines evaluated in this study, only "Oral herpes simplex" (CG 1) reported active patient participation in its development through information obtained from the literature review on patients' experiences and individual and group consultations of stakeholders. Neither of the two guidelines evaluated by the Brazilian Ministry of Health (MS) was previously reviewed by the National Committee for the Incorporation of Technologies (CONITEC) or submitted for public consultation.

Evaluation of Guideline Quality: Domain 3 - Development Rigor

This domain was one of the most significantly

divergent from the quality standards assessed. Scores were lower than 36% in all guidelines except CG 1 and CG 9, which scored higher than 61%. Among the 12 guidelines evaluated, "Herpes simplex infections" (CG 02) had the lowest score in this domain (1%). It was found that most of the guidelines evaluated neglected some critical issues for quality assessment, such as a complete description of search methods, selection criteria and evidence, strengths and limitations of the evidence, consistency between the formulation of recommendations and evidence, consideration of benefits and drawbacks, and external review criteria for updating the guideline.

Evaluation of Guideline Quality: Domain 4 - Clarity of presentation

This was the domain with the second-highest scores. In general, all the evaluated CG were written in simple language with descriptions of the recommendations typed in bold or italics, presented in topics, flowcharts and/or summary tables.

Evaluation of Guideline Quality: Domain 5 - Applicability

This domain scored low in all the guidelines, with scores below 39%, except for "Oral herpes simplex" (CG 1), which scored 82%.

Evaluation of Guideline Quality: Domain 6 - Editorial independence

Of all the domains, this one diverged the most from the quality standards established by AGREE II. All guidelines scored 0%, except for "Oral Herpes

Simplex” (CG 1) and “Recurrent Herpes Simplex Labialis: Selected Therapeutic Options” (CG 5), which scored 100% and 75%, respectively.

Evaluation of Guideline Quality: International Comparison

In order to analyze the results from an international point of view, the quality scores per domain were compared to the findings of a systematic review of studies from around the world that gathered 625 different guidelines published since 2003³⁴. In general, CG for herpes labialis were not precisely aligned with international standards, as they had lower quality scores in all domains assessed by AGREE II. The main discrepancies were in the domains ‘developmental rigor’ and ‘editorial independence’, reinforcing the findings that these documents had problems of methodological rigor and transparency, since the methodological criteria were questions about evidence-based health, and the procedures did not describe an unbiased process²⁶⁻³¹.

Strength of recommendation and level of evidence of clinical guidelines for herpes labialis

In general, among the 12 CG for fever blisters, there was no grading of the level of evidence underlying the 83 recommendations identified. The strength of the recommendation rating system was covered by only four guidelines according to the SORT (*Strength of Recommendation Taxonomy*) and CEBM (*Center for Evidence-Based Medicine*) criteria. Only eight of the 83 recommendations were assigned a recommendation strength. Approximately 10% were based on recommendation strengths A and B (3.6% and 6.0%, respectively). The other recommendations were not ranked simply because the guidelines no longer use a recommendation ranking system.

DISCUSSION

Effective implementation of the recommendations requires the adoption of consistent methodologies in the development process^{3,4,6}. There is reason to believe that methodological information may be challenging to find because it appears in separate documents or appendices not specified in the CG. In the current study, relevant literature may have been overlooked since review procedures, including previous publications, do not work in many of the evaluated CG. The developmental approach

suggested presenting a rigorous evaluation by the authors and supervisors of the CG in examining the clinical conduct and transparency of the scientific evidence of the herpes labialis recommendations^{4,26}. There is often a normative character in the applicability/implementation of CG^{42,43}. This study revealed that most of the evaluated CG neglected to describe aspects of the potential implications for resources arising from the recommendations, validation procedures, follow-up criteria, and suggestions on how the recommendations can be put into practice. Furthermore, the evaluated guidelines showed a lack of a systematic, transparent approach to make judgments about the quality of evidence and the strength of recommendations in most of the unrated recommendations, which not only may impair care guidance for health professionals, population, and managers, but may also result in low reliability of the recommendations^{1,4,43}.

Regarding treatment, nucleoside antivirals (acyclovir, valacyclovir, and famciclovir), the main class of drugs recommended in CG, are synthetic analogs of acyclic purine (or guanine analogs), highly specific substrates for viral thymidine kinase and effective inhibitors of deoxyribonucleic polymerase (DNA) against HSV-1 and HSV-2⁹. It was also observed that few CG provided specific recommendations for diagnosing and screening labial herpes, which were grouped for cases of HSV infection. This promotes gaps in clinical relevance regarding differential diagnosis, risk stratification, and evolution of the patient with labial herpes. It is thus useful to have a classification that indicates the confidence level of the evidence quickly and practically. The results of the current study show that CG with adaptations of reliable graded recommendations on specific treatment and care for the management of fever blister are essential to fill the knowledge gaps⁴⁴⁻⁴⁶.

Regarding non-pharmacological measures, the development of CG for fever blisters could prioritize greater reliability of the scientific evidence of the recommendations provided to ensure greater methodological rigor, transparency, a classification system, and the participation of more oral health professionals and patients with the disorder¹⁵.

Furthermore, it was found that 10 of the total CG reviewed provided no clear, certified statement demonstrating that the opinions or interests of funders or competing bodies did not influence

the final recommendations. “Oral herpes simplex” (CG 1) was the only guideline that fully contemplated supplementary documents and terms that defined procedures and responsibilities for each stakeholder group and included declarations of no conflict of interest signed by all parties. CG should follow this procedure to contribute to unbiased final recommendations and authoritative publications^{3,28,30-32}.

The analysis revealed that the healthcare setting in most initial descriptions of CG for fever blisters was primary healthcare^{30-33,37}. Thus, patients seeking primary healthcare services are more likely to receive the actions recommended in the guidelines in that setting⁴³.

Including other health professionals such as dental surgeons and pharmacists in the CG development team may contribute not only to greater integration of professionals in the evidence review, but also to the diversity of target users qualified for caring for patients with the disorder⁶. It is essential to specify the professionals involved, as there is global difficulty due to issues related to the closed structure of the Health Technology Assessment (HTA) area and the suspicion that CG developers may be influenced by pharmaceutical companies^{3,31}.

It has been observed that including patients in developing the guidelines is also crucial to improving recommendation quality. According to

Van et al.³⁷ and Cluzeau et al.³⁸, doing so enables identification of points of disagreement between professionals and patients, priority needs from the users’ perspective, and aspects that are not well observed by guideline supervisors and can help improve guideline adherence and implementation strategies⁴⁴⁻⁴⁶.

One of the limitations of this study is the number of CG used, based on the inclusion and exclusion criteria. The study was nevertheless performed, and the results should be considered because they can be used to support the scientific community in developing and updating CG for labial herpes. Any further studies should use different tools to analyze the CG so that they can be compared to the results obtained in the current study.

The findings showed that there is consensus regarding the treatment of fever blister herpes using nucleoside antivirals – acyclovir, valacyclovir and famciclovir – as the drugs of choice. However, there are limitations in the vast majority of CG, especially concerning the methodology for diagnosis and screening. A multidisciplinary team should be involved in the preparation of CG for labial herpes, to ensure complementarity among the health knowledge areas. All recommended parameters for preparing CG should be followed in order to avoid publications of low quality and contradictions among the different CG that address the same subject.

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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Analysis of morphology and symmetry of the root canal system of incisors, premolars and mandibular molars using CBCT

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ABSTRACT

Knowledge of root canal internal anatomy and its variations is important for proper endodontic treatment. It is therefore necessary to investigate morphological aspects among different dental groups in the same patient to define the best protocol for the case. **Aim:** To evaluate the morphology and symmetry of homologous incisors, premolars and mandibular molars using cone beam computed tomography (CBCT). **Materials and Method:** Descriptive statistical analysis was performed for the frequency of categorical variables, and a chi-square test or Fisher's exact test was used to test whether gender and side were associated with number of roots, number of canals, and Vertucci's classification. Forty-five CBCT scans were evaluated, and 444 mandibular teeth were analyzed. The number of roots, number of canals, classification of the canals in each root according to Vertucci and presence of a symmetrical relationship between pairs of posterior teeth were analyzed. **Results:** The results showed that 74% of mandibular central incisors had type I root canal, 26% of mandibular lateral incisors had type I and, with a significant difference in the number of canals between males and females ($p < 0.05$). In mandibular first premolars, 70.5% had type I; and in mandibular second premolars, 98.5% had type I. Mandibular first molars had two roots in 98% of the cases. Second mandibular molars had two roots in 92.5% of the cases, one root in 6%, and three roots in 1.5%. Symmetry between central incisors was higher in females than in males. **Conclusion:** Teeth of the same group can have different morphologies in the same patient.

Keywords: endodontics - anatomy - permanent teeth.

Análise da morfologia e simetria do sistema de canais radiculares de incisivos, pré-molares e molares inferiores usando a TCFC

RESUMO

O conhecimento da anatomia interna e suas variações anatômicas é fator importante para o adequado tratamento endodôntico. Portanto, é necessário investigar esses aspectos morfológicos entre diferentes grupos dentários de um mesmo paciente para definir o melhor protocolo para o caso. **Objetivo:** Avaliar a morfologia e simetria de incisivos, pré-molares e molares inferiores homólogos por meio de tomografia computadorizada de feixe cônico (TCFC). **Materiais e Método:** Foi realizada análise estatística descritiva para a frequência das variáveis categóricas e foi utilizado o teste do qui-quadrado ou teste exato de Fisher para testar a relação entre sexo e lado em comparação com número de raízes, número de canais e classificação de Vertucci. Quarenta e cinco TCFC foram avaliadas e 444 dentes inferiores foram analisados. Foram considerados: o número de raízes, o número de canais, o tipo dos canais acordo com a classificação de Vertucci e a presença de relação simétrica entre pares de dentes posteriores. **Resultados:** Os resultados mostraram que 74% dos incisivos centrais inferiores tinham um canal radicular tipo I e 26% tinham dois canais; 73% dos incisivos laterais inferiores, 26% eram do tipo I, tinham um canal e 27% tinham dois canais, com diferença significativa no número de canais entre os grupos masculino e feminino ($p < 0,05$). Nos primeiros pré-molares inferiores, tipo I, um canal foi detectado em 70,5% e dois canais em 29,5%; nos segundos pré-molares inferiores, tipo I, um único canal foi detectado em 98,5%. O primeiro molar inferior foi observado com duas raízes em 98% e três raízes em 2%. O segundo molar inferior tinha duas raízes em 92,5% dos casos, uma raiz em 6% e três raízes em 1,5%. A simetria foi maior nas mulheres em comparação aos homens nos incisivos centrais. **Conclusão:** Pode-se concluir que dentes de um mesmo grupo podem apresentar morfologias diferentes no mesmo paciente.

Palavras-chave: endodontia - anatomia - dentes permanentes.

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INTRODUCTION

The goal of endodontic therapy is to disinfect and obturate the root canal system. One of the challenges is the internal morphology and the many anatomical variations of the different tooth groups¹.

For a long time, one of the main methods for assessing internal anatomy involved clearing, staining and making serial cross-sections of extracted teeth². Now, internal anatomy can be studied using microcomputed tomography (Micro-CT), which is considered the gold standard for this purpose. However, all these techniques are feasible only in *ex vivo* experiments using extracted teeth³.

Considering the anatomical aspects among dental types, one study found that root canals in first mandibular incisors were Vertucci type I (50%), II (62%) and III (28%)⁴, and another study found that root canals in lateral mandibular incisors were type I (30.2%) and III (18.9%)⁵. Mandibular first premolars are single-rooted teeth, well known for their anatomical variations, which can pose a challenge for endodontic treatment. Data from over 6,700 mandibular first premolars revealed that single canals were found in 75.8%, and two or more canals in 24.2% of the examined teeth⁶. A single apical foramen was found in 78.9% of the specimens evaluated, while 21.1% had two or more apical foramina⁷.

Mandibular molars, which may present several anatomical variations, have the highest frequency of endodontic treatment. The most frequent mesial root canal configuration in the first mandibular molar is type IV (46.2%), followed by type II (16.3%). In the distal canal, type I was found in 57% of cases, followed by V (27%)⁸. Another study on distal canals found 88.6% type I and other atypical configurations⁹. Despite the anatomical correlation between teeth, variations may occur, making it necessary to investigate their internal morphology. The clinician needs to be attentive to the presence of additional canals or configurations of the main root canals to avoid incomplete and inefficient shaping and disinfection that could lead to potential failure of endodontic treatment^{7,10}.

Cone Beam Computed Tomography (CBCT) can be used to assess anatomical aspects *in vivo*. Its main advantage over periapical radiographs is that it provides three-dimensional visualization of the tooth and can avoid superimposition of anatomical structures, enabling important aspects of internal tooth anatomy, such as the number of roots and

especially root canals, to be visualized with better definition¹¹. The study of internal anatomy is very important to the clinician. To date, no study has compared all these tooth groups in the same patient. Therefore, the aim of this study was to use CBCT scans to analyze the morphology and anatomical symmetry of molars, premolars, and mandibular incisors.

MATERIALS AND METHOD

This study was approved by the São Leopoldo Mandic Research Institute ethics committee through protocol #5.771.849. It was an observational study using images from CBCT scans to evaluate the morphology and symmetry of the roots and root canal systems of molars, premolars and mandibular incisors of patients from a multiracial subpopulation in southern Brazil for whom examinations had been requested for other purposes not related to this research. G*Power 3.1 software¹² was used to calculate the sample size. To test the difference in the number of canals in molars, premolars, and mandibular incisors in relation to symmetry (right side vs. left side), a sample size of 52 paired teeth was estimated for each of the three analysis groups, considering the use of a two-sided test, a small effect size (ES) ($d = 0.40$ Cohen, 1992), a power of 80%, and a 95% confidence level.

A total 45 CBCT scans of the mandibular region were analyzed, with 444 teeth evaluated: 120 molars, 148 premolars and 176 mandibular incisors. The study included exams from the mandibular region from patients aged from 18 to 60 years old. Inclusion criteria were teeth with complete root development, no endodontic treatment, no evidence of root fracture, no intraradicular retainers and no prosthetic crowns, no root resorption, and no calcifications. Exclusion criteria were images that did not meet quality standards and patients younger than 18 years.

CBCT images were acquired with a Vatech American (North South, Fort Lee, United States) Pax I3D Smart Pht 30 LFO CT scanner using the following acquisition protocol: *voltage 100-240 V and 2.2 Kva mA, FOV 100*85 voxel 10. MA and minimum exposure time 18 seconds.

The three-dimensional images were evaluated in a darkroom using the DICOM viewer HOROS, iCat Solutions Ltd, , Norwich, United Kingdom. The evaluation started with the number of roots, followed

by the analysis of the number and configuration of canals on the axial plane, followed by the sagittal and finally the coronal plane. For each group of teeth, the following information was recorded: gender, number of roots, number of canals, SCR configuration according to Vertucci's classification (Figure 1), and whether there was symmetry between each subject's right and left homologous teeth.

Statistical analysis

Image analysis was performed by two experienced calibrated examiners (an endodontist and a radiologist). The Kappa agreement test showed perfect agreement between the two examiners for all tooth groups examined (Kappa = 1.00; 95% CI: 1.00-1.00) and near-perfect agreement regarding the number and configuration of the root canal system. Descriptive statistics were performed with frequencies and percentages of categorical variables. To test whether sex and side variables were associated to the number of roots, number of canals, and Vertucci classification, the chi-square test (X²) or Fisher's exact test was used, as appropriate. Effect size was scored according to Cramer's V, using the following classification: small < 0.30; moderate 0.30-0.49; large ≥ 0.50. Analyses were performed using SPSS software (IBM SPSS Statistics, version 22.0; IBM Corporation). A p-value < 0.05 was assumed for statistical significance.

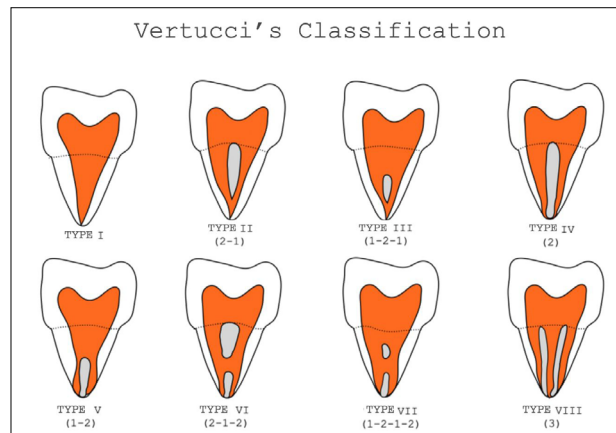


Fig. 1: Vertucci's classification. Type I: a single canal exits the pulp chamber and extends to the apex; type II: two canals leave the pulp chamber separately and unite into a single canal anterior to the apex; type III, a single canal leaves the pulp chamber, divides into two in the middle third, and unites into a single canal in the apical third; type IV, two canals leave the pulp chamber and extend to the apex; type V, a single canal leaves the pulp chamber and divides into two canals with two separate foramina; type VI, two canals exit the pulp chamber, reunite in the root body, and divide again into two separate canals at the apex; type VII, a single canal exits the pulp chamber, divides into two, then reunites into one canal and terminates in two separate canals; type VIII, three separate canals exit the pulp chamber and extend to the apex.

RESULTS

A total 444 teeth were assessed: 120 molars (60 F and 60 M), 148 premolars (73 F and 75 M), and 176 mandibular incisors (91 F and 85 M). No statistically

Table 1. Comparison of absolute frequencies (percentages) of the number of canals in incisors, premolars and molars for males and females.

Tooth	Sex	Number of canals					X ²	p-value	ES
		1C	2C	3C	4C	5C			
Central incisor	F	37(82%)	8(18%)	-	-	-	3.019	0.08	0.19
	M	27(66%)	14(34%)	-	-	-			
Lateral incisor	F	38(83%)	8(17%)	-	-	-	4.139	0.04*	0.21
	M	28(64%)	16(36%)	-	-	-			
1st premolar	F	28(74%)	10(26%)	-	-	-	0.358	0.55	0.07
	M	27(67%)	13(33%)	-	-	-			
2nd premolar	F	35(100%)	0(0%)	-	-	-	-	1.00	0.12
	M	34(97%)	1(3%)	-	-	-			
1st molar	F	-	-	14(48%)	15(52%)	-	0.488	0.48	0.09
	M	-	-	15(58%)	11(42%)	-			
2nd molar	F	-	6(19%)	24(77%)	1(3%)	0(0%)	5.340	0.08	0.29
	M	-	1(3%)	31(91%)	1(3%)	1(3%)			

(C: canals; X² chi-square test; *p < 0.05; ES: effect size assessed by Cramer's V).

Table 2. Comparison of the absolute frequencies (percentages) of the number of roots found in the incisors, premolars, and molars on the right and left sides.

Tooth	Side	Number of roots			p-value	ES
		1	2	3		
Central incisor	R	43(100%)	-	-	-	-
	L	43(100%)	-	-		
Lateral incisor	R	45(100%)	-	-	-	-
	L	45(100%)	-	-		
1st premolar	R	37(97%)	1(3%)	-	1.00	0.004
	L	39(98%)	1(2%)	-		
2nd premolar	R	33(100%)	-	-	-	-
	L	36(100%)	-	-		
1st molar	R	-	25(96%)	1(4%)	0.47	0.14
	L	-	29(100%)	0(0%)		
2nd molar	R	2(6%)	29(91%)	1(3%)	0.80	0.13
	L	2(6%)	31(94%)	0(0%)		

(R - right; L - left; p-value calculated by Fisher's exact test; ES: effect size evaluated by Cramer's V).

Table 3. Comparison of absolute frequencies (percentages) of the number of canals found in incisors, premolars and molars on the right and left sides.

Tooth	Side	Number of canals					X ²	p-value	ES
		1C	2C	3C	4C	5C			
Central incisor	right	32 (74%)	11 (26%)	-	-	-	<0.001	1.00	0.00
	left	32 (74%)	11 (26%)	-	-	-			
Lateral incisor	right	33 (73%)	12 (27%)	-	-	-	<0.001	1.00	0.00
	left	33 (73%)	12 (27%)	-	-	-			
1st premolar	right	28 (74%)	10 (26%)	-	-	-	0.358	0.55	0.07
	left	27 (67%)	13 (33%)	-	-	-			
2nd premolar	right	33 (100%)	0(0%)	-	-	-	0.930	0.33	0.12
	left	35 (97%)	1(3%)	-	-	-			
1st molar	right	-	-	13(50%)	13(50%)	-	0.147	0.70	0.05
	left	-	-	16(55%)	13(45%)	-			
2nd molar	right	-	3 (9%)	27(85%)	2(6%)	0(0%)	2.763	0.58	0.22
	left	-	4(12%)	28(85%)	0(0%)	1(3%)			

(C: Canals; X² chi-square test; ES: effect size assessed by Cramer's V).

In terms of root symmetry, number and shape of canals, the following percentages were observed: 92.9% in central incisors, 95.6% in lateral incisors, 84.2% in 1st premolar, 96.6% in 2nd premolar, 54.2% in 1st molars, and 76.7% for the 2nd premolar. Symmetry was greater in females than in males for the central incisors (X² = 7.108; p = 0.008; V = 0.29), lateral incisors (X² = 4.376; p = 0.036; V = 0.22), and 1st molar (X² = 5.185; p = 0.023; V = 0.33) (Figure 3).

significant difference was found in the number of roots in incisors, premolars and molars between men and women (p < 0.05).

The number of canals did not differ significantly between males and females, except in lateral incisors, where a higher proportion of teeth with two canals was observed in males than in females (Table 1) (P < 0.05).

Comparison of the right and left sides showed no

statistically significant differences in number of roots (Table 2) or number of canals (Table 3) in incisors, premolars and molars (p < 0.05).

According to Vertucci's classification, Type I was the most frequent in mandibular incisors and premolars. In central incisors, Type I was found in 82% of females and 66% of males. In lateral incisors, Type I was found in 83% of females and 64% of males. In first mandibular premolars, Type I was found in

74% of females and 73% of males, while in second mandibular premolars, the percentages were 100% for females and 97% for males (Table 4).

In molars, several Vertucci types were observed. In first molar mesial roots, the most frequent was type IV (59% for females and 65% for males), followed by type II (41% for females and 35% for males). In distal roots, type I was the most prevalent (52% for females and 65% for males), followed by type II (21% for females and 19% for males), type III (17% for females and 4% for males), and type V (10% for females). For the second mandibular molars, a high percentage of type II was found in mesial roots (52% for females and 51% for males), followed by type IV (26% for females and 43% for males), while types I, III, and V were found in lower frequencies.

The distal canal was type I in most cases (97% for both females and males) (Table 4).

The Vertucci frequencies on the right and left side can be found in Table 5.

DISCUSSION

This observational study analyzed CBCT scans of the mandible of men and women from a subpopulation in southern Brazil. Radiographic analysis is essential in endodontics for diagnosis, treatment planning and implementation, and preservation. Periapical radiography is the most important complementary examination. However, it has some limitations, such as compression of anatomical structures, conversion of three-dimensional structures into two-dimensional, and possible overlap of anatomical

Table 4. Comparison of the absolute frequencies of the Vertucci scale in incisors, premolars and molars between males and females.

Tooth	Root	Sex	Vertucci scale						p-value
			I	II	III	IV	V	VI	
Central incisor	B	F	37(82%)	2(5%)	6(13%)	-	-	-	0.22
		M	27(66%)	4(10%)	10(24%)	-	-	-	
	L	F	-	-	-	-	-	-	-
		M	-	-	-	-	-	-	
Lateral incisor	B	F	38 (83%)	2 (4%)	6 (13%)	-	-	-	0.04*
		M	28(64%)	9(20%)	7 (16%)	-	-	-	
	L	F	-	-	-	-	-	-	-
		M	-	-	-	-	-	-	
1st premolar	B	F	28(74%)	-	7 (18%)	-	3(8%)	-	0.30
		M	29 (73%)	-	4 (10%)	-	7(17%)	-	
	L	F	-	-	-	-	-	-	-
		M	2 (100%)	-	-	-	-	-	
2nd premolar	B	F	34(100%)	-	-	-	-	0(0%)	0.32
		M	34(97%)	-	-	-	-	1(3%)	
	L	F	-	-	-	-	-	-	-
		M	-	-	-	-	-	-	
1st molar	M	F	-	12(41%)	-	17(59%)	-	-	0.60
		M	-	9 (35%)	-	17(65%)	-	-	
	D	F	15(52%)	6 (21%)	5 (17%)	0	3(10%)	-	0.07
		M	17(65%)	5 (19%)	1 (4%)	3 (12%)	0	-	
2nd molar	M	F	4 (13%)	16(52%)	1 (3%)	8 (26%)	2(6%)	0(0%)	0.08
		M	0 (0%)	17(51%)	1 (3%)	14(43%)	0(0%)	1(3%)	
	D	F	28(97%)	-	1 (3%)	-	0(0%)	-	0,72
		M	32(97%)	-	0 (0%)	-	1(3%)	-	

Legend: B - buccal; L - lingual; M - mesial; D - distal; Source: authors' own.

No statistically significant difference was found in Vertucci's classification between the right and left sides (Table 5).

Table 5. Comparison of the absolute frequencies of the Vertucci scale in incisors, premolars and molars on the right and left sides.

Tooth	Root	Side	Vertucci scale						p-value
			I	II	III	IV	V	VI	
Central incisor	B	right	32(74%)	3(7%)	8(19%)	-	-	-	1.00
		left	32(74%)	3(7%)	8(19%)	-	-	-	
	L	right	-	-	-	-	-	-	-
		left	-	-	-	-	-	-	
Lateral incisor	B	right	33(74%)	6(13%)	6(13%)	-	-	-	0.92
		left	33(73%)	5(12%)	7(15%)	-	-	-	
	L	right	-	-	-	-	-	-	-
		left	-	-	-	-	-	-	
1st premolar	B	right	29(76%)	-	4(11%)	-	5(13%)	-	0.67
		left	28(70%)	-	7(18%)	-	5(12%)	-	
	L	right	1(100%)	-	-	-	-	-	-
		left	1(100%)	-	-	-	-	-	
2nd premolar	B	right	33(100%)	-	-	-	-	0(0%)	0.33
		left	35(97%)	-	-	-	-	1(3%)	
	L	right	-	-	-	-	-	-	-
		left	-	-	-	-	-	-	
1st molar	M	right	-	8(31%)	-	18(69%)	-	-	0.28
		left	-	13(45%)	-	16(55%)	-	-	
	D	right	14(54%)	7(27%)	3(11%)	1(4%)	1(4%)	-	0.77
		left	18(62%)	4(14%)	3(10%)	2(7%)	2(7%)	-	
2nd molar	M	right	2(6%)	18(56%)	2(6%)	9(28%)	1(4%)	0(0%)	0.55
		left	2(6%)	15(47%)	0(0%)	13(41%)	1(3%)	1(3%)	
	D	right	30(97%)	-	1(3%)	-	0(0%)	-	0.37
		left	30(97%)	-	0(0%)	-	1(3%)	-	

Legend: B – buccal; L – lingual; M – mesial; D – distal.

structures that may obscure the area of interest. CBCT provides a detailed, three-dimensional assessment of teeth and aids in diagnosis, planning, treatment, and follow-up after endodontic therapy¹³. In the present study, a single root was detected in 100% of the cases in central and lateral incisors, which agrees with the studies by Valenti-Obino et al.¹⁴ and Sroczyk-Jaszczyńska et al.¹⁵, in which 978 and 420 mandibular incisors were analyzed, respectively. Regarding the number of canals, a higher frequency of a single canal was observed in females (82% in central incisors and 83% in lateral incisors) than in males (66% in central incisors and 64% in lateral incisors). However, in absolute frequency, these data were 74% with one canal and 26% with two canals in central incisors, and 73%

with one canal and 27% with two canals in lateral incisors. Similar data were reported in the study by Sroczyk-Jaszczyńska et al.¹⁵, where the frequency of a single canal was about 65% in central incisors and 67% in lateral incisors, while the frequency of two canals was about 33% in central incisors and 31.76% in mandibular lateral incisors.

Regarding canal configuration according to Vertucci's classification, it was found that mandibular incisors were more frequently types I, II and III. Types I and III were even more prevalent for central incisors and types I (83%) and III (13%) for lateral incisors in females, and I (64%) and II (20%) in males, with statistical difference between the groups. Somewhat different data were reported by Valenti-Obino et al.¹⁴, who found type I canals in 57% of mandibular lateral

incisors and 55% of mandibular central incisors, type II in 35.7% and 34.3%, and type III in 9.3% and 6.9%. In this study, type IV and VII configurations were also found, although these differences can be explained by the sample size.

Regarding symmetry between the right and left sides of the same group of teeth, it was observed that females had higher symmetry than males. Symmetry of central and lateral incisors was present in 100% of females, while in males, symmetry was 85% for central incisors and 91% for lateral incisors. Similar data were reported by Sroczyk-Jaszczyńska et al.¹⁵, who found symmetry in 100% of mandibular incisors, while Valenti-Obino et al.¹⁴ found symmetry in 44.6% of central incisors and 44.8% of lateral incisors.

One- and two-rooted premolars were found: 100% of female patients had a single root in both first and second premolars, while two (5%) teeth with two roots were observed in first premolars in males. Similar data were found by Alfawaz et al.¹⁶ in a sample of 377 mandibular first premolars, of which 97.5% of female and 95.2% of male first premolars were found to have a single root. In terms of absolute data, a frequency of 97% first premolars with a single root and 100% second premolars with two roots were observed in this study.

Regarding the number of canals and canal configuration according to Vertucci's classification, more teeth with one canal than with two were observed. In second premolars, the most common configuration was Type I (100% in females and 97% in males), while only one type VI (3%) was found. In first premolars in females, 74% had type I configuration, 18% type III and 8% type V. Different data were reported by Alfawaz et al.¹⁶, where the frequency of type I was 87.8%, type II 3.4%, and type V 1.5%; other configurations were also found (types IV and VIII). In males, the current study observed a higher frequency of type I (73%), type III (10%) and type V (17%), in contrast to the data of Alfawaz et al.¹⁶, who found the occurrence of type I in 88.2%, type II in 3.8%, type III in 2.2%, type IV in 3.8%, type V in 6%, and type VI in 0.5% in male patients.

Symmetry was found in 84.2% of first premolars and in 96.6% of second premolars. These data are similar to those of Alfawaz et al.¹⁶, where symmetry was found in 96.7% of male patients and 97.3% of female patients.

Molars were found with one, two and three roots. In first molars, two roots were the most common (98%) and only one case with three roots was found on the right side. In second molars, there was one root in 6% of cases, two roots in 92.5%, and three roots in only one case of a second molar on the right side. Similar data were found by Kantilieraki et al.¹⁷, where in a sample of 478 first molars and 524 second molars, the presence of two roots was observed in 96.44% of first molars and 82.82% of second molars, with second molars being considered the teeth with the greatest variation in both canal configuration and morphology and symmetry.

Regarding the number of canals in mandibular first molars, three canals were observed in about 52.5% of cases and four canals in about 47.5% of cases, with two canals in the mesial root and two in the distal root. Different data were found by Plotino et al.³ in a Greek population, where three canals were found in 69.2% of mandibular first molars and four canals in 21.4%. The current study found greater differences in second molars, with a higher frequency of teeth with three canals (85%), but teeth with two, four, and five canals were also seen. The study by Plotino et al.³ found similar data, and 87.5% of teeth had three canals, two in the mesial root and one in the lingual root.

Regarding canal configuration according to Vertucci's classification, a higher frequency of type II (mean 38%) and type IV (mean 62%) was observed in the mesial root of the first molars, in both females and males. Similar data were found in a study by Gambarini et al.¹⁸, in which mandibular first molars were evaluated, finding in the mesial root a higher frequency of type IV (59%) and type II (41%). In the current study, distal root configurations was more variable, with the most frequent configuration being type I (58.5%), followed by type II, type III in females, while in males, the most frequent was type IV, followed by type V and type III. The study by Kantilieraki et al.¹⁷ found a higher frequency of type I in distal roots (81.7%) in a Greek population. The current study found higher incidence of type II in mesial roots and type I in distal roots (97%) in second molars, in agreement with the study by Kantilieraki et al.¹⁷, which also found a frequency of type I canal configuration in the distal roots of mandibular second molars.

Female molars were more symmetrical than male molars: 69% symmetry in female first molars and

36% in males, $p=0.023$, and 80% symmetry in female second molars and 73% in males. In general, symmetry was 54.2% in first molars and 76.7% in second molars. Different data were found in a study by Plotino et al.³, which reports a frequency of symmetry of 70.6% in first molars and 81% in second molars. These differences can be explained by the sample size.

Alghamdi and Khalil¹⁹ analyzed the morphology and symmetry of mandibular second premolars using CBCT in a Saudi population and concluded that there are several anatomical variations in the same population, suggesting that further studies should be conducted to investigate root canal anatomy in other regions of Saudi Arabia. Further studies

should be conducted to analyze the morphology and symmetry of the incisor, premolar, and mandibular molar canals of the same individual in different populations and subpopulations.

CONCLUSION

As of the date of completion of this work, this is the first *in vivo* CBCT study investigating the symmetry of homologous teeth considering several teeth groups in the same patient. The present results showed that there was no symmetry between the homologous teeth from the right and left sides, in the same patient, and the teeth showed different morphologies, considering number of roots and the number of canals.

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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Efficacy of WaveOne Gold and ProDesign RT systems in removing filling material: a micro-CT analysis

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ABSTRACT

The remaining filling material after retreatment can harbor bacteria and organic tissues that can influence the outcome of the therapy. **Aim:** The aim of this study was to evaluate, by micro-CT, the amount of filling material remaining in the root canal after its removal using WaveOne Gold or ProDesign RT. **Material and Method:** Forty human mandibular canines were instrumented with the ProTaper Next system up to the X2 instrument (25.06) and filled with gutta-percha cones and AHPlus. Teeth were divided into 2 groups (n=20): WaveOne Gold 25.07 (WOG) and ProDesign RT 25.08 (PRT) for filling removal, after which they were scanned in a micro-CT device to quantify the volume of remaining filling material. The data were subjected to log¹⁰ transformation, Student's t-test was performed to account for multiple observations per sample, significance was set at 5%. **Results:** Student's t-test showed that there was no difference between the two systems regarding the volume of remaining filling material in the thirds: apical (p = 0.392), middle (p = 0.065), or cervical (p = 0.918). **Conclusion:** Remaining filling material was present in all groups and both systems were similar in removing root filling material in mandibular canines.

Keywords: micro CT- root canal retreatment - reciprocal systems - rotary systems.

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Eficácia dos sistemas WaveOne Gold e ProDesign RT na remoção material obturador: uma análise micro-CT

RESUMO

A permanência de material obturador após o retratamento pode abrigar bactérias e tecidos orgânicos que podem influenciar o resultado da terapia. **Objetivo:** O objetivo deste estudo foi avaliar, por micro-CT, a quantidade de material obturador remanescente no canal radicular após a desobturação com WaveOne Gold e ProDesign RT. **Material e Método:** Quarenta caninos inferiores humanos foram instrumentados com o sistema ProTaper Next até o instrumento X2 (25.06) e obturados com cones de guta-percha e AHPlus. Os dentes foram divididos em dois grupos (n=20): WaveOne Gold 25.07 (WOG) e ProDesign RT 25.08 (PRT) e escaneados em micro-CT para quantificação do volume de material obturador remanescente. Os dados foram submetidos à transformação log¹⁰, o teste t de Student foi realizado para contabilizar múltiplas observações por amostra, a significância foi fixada em 5%. **Resultados:** O teste t de Student mostrou que não houve diferença no volume de material obturador remanescente entre os dois sistemas nos terços: apical (p = 0,392), médio (p = 0,065) ou cervical (p = 0,918). **Conclusão:** O material obturador remanescente estava presente em todos os grupos e ambos os sistemas foram semelhantes na remoção do material obturador radicular nos caninos inferiores.

Palavras-chave: micro CT - retratamento do canal radicular - sistemas reciprocantes - sistemas rotatórios.



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INTRODUCTION

Nonsurgical endodontic retreatment is the first choice when endodontic treatment has failed. The remaining filling material may harbor necrotic tissue and microorganisms responsible for endodontic failure¹. Efficient removal of filling material, new cleaning and shaping, irrigation, and access to the apical foramen are critical to the health of the periapical tissues².

Studies have evaluated the percentage of root canal filling material removed with rotary and reciprocating systems, with rates ranging from 59.4% to 96.9³⁻⁶. Due to the anatomical complexity of the root canal system and the limitation of instrument design, complete removal of the root canal filling material can be difficult or even impossible⁷⁻¹⁰, especially in the apical region.

The WaveOne Gold (WOG - Dentsply-Sirona, Ballaigues, Switzerland) reciprocating system, which is made of a heat-treated M-wire alloy, has shown good results in removing filling material¹¹ although it has not been recommended for use in retreatment. The ProDesign RT (PRT - Bassi / Easy Equipamentos Odontológicos, Belo Horizonte, Brazil) rotary system was developed for removing filling material from root canals. It is made of a CM alloy and has a triple helix cross-section. According to the manufacturer, PRT files remove approximately 90% of endodontic filling material without the use of solvents.

Micro-CT enables three dimensional (3D) evaluation of the amount of remaining filling material due to its precision for enabling visualization of morphological features with specimen preservation^{5,11,12}. The aim of this study was to use micro-CT to evaluate the amount of remaining filling material after retreatment of human mandibular canines using the WOG or PRT systems and the null hypothesis is that there is no difference between the systems in root canal filling removal.

MATERIAL AND METHOD

G*Power 3.1 software (Heinrich Heine College, Duesseldorf, Germany) was used to calculate power with a power of $\beta = 95\%$ and $\alpha = 5\%$, and a t test for independent samples was applied. The ideal sample size for each group should be at least 10 teeth, but 10 additional samples were added per group to compensate for possible loss.

Forty mandibular canines with moderately curved roots (10° to 20°) were selected from a pool of 432 teeth. All specimens were scanned in micro-

CT (SkyScan 1173, Bruker, Kontich, Belgium) at 70 kV and 114 mA, with a 1-mm-thick aluminum filter, exposure time 320 milliseconds, pixel size 12.1 μm , rotation step 0.8° , and rotation 360° along the vertical axis. The files were then reconstructed into a three-dimensional data set using the software NRecon v1.6.1.0 (Bruker micro-CT), with beam hardening correction 50%, ring artifact correction 10, and smoothing 5. The volume of interest extended from the cemento-enamel junction to the root apex, resulting in the acquisition of 600 to 700 axial slices per sample. CTAn v.1.14.4 and CTVol v.2.2.1 software (Bruker Micro-CT) were used to evaluate root canal morphology in terms of volume, surface area and 3D configuration. Teeth were then paired based on the anatomical similarities of preoperative canal volume, canal length of approximately 18 mm, canal surface area and 3D configuration.

This study included teeth with a single canal and complete root formation, and excluded teeth with pulp calcifications, resorptions, root fractures or canals whose patency was not achieved. Teeth were washed under running water, the root surface was cleaned ultrasonically, and kept hydrated in saline solution until the start of the experiment. Tooth length was standardized to 18 mm by partially crowns removal with a diamond disk (FKG Dentaire, La Chaux-de-Fonds, Switzerland). Each tooth was radiographed in the mesiodistal and buccolingual directions to verify for presence of a single canal and the degree of curvature of the roots. Periapical radiographs were performed with an X-ray unit (Procion, Ribeirão Preto, Brazil) coupled to a digital sensor (Elite - Schick, São Paulo, Brazil), with a distance of 10 cm between tooth and film, using the following exposure parameters: 0,08s; 70 kV, 8 mA. To measure root canal length, a K#10 hand file (Dentsply Maillefer, Ballaigues, Switzerland) was inserted in the tooth under an optical microscope at $20\times$ magnification until the tip of the file was visible through the apical foramen. Working length (WL) was determined by subtracting 1 mm from this measurement.

The canals were instrumented at WL using the ProTaper Next system (Dentsply-Sirona) up to the X2 instrument (25.06). Patency was maintained with a #10 K instrument extended 1 mm beyond WL. Root canal instrumentation was performed by a single operator according to the manufacturer's

recommendations for speed and torque, using an electric motor (Dentsply-Sirona) under a surgical microscope with 8x magnification. At each instrument change, irrigation was performed with 5 mL of 2.5% NaOCl (Fórmula e Ação, São Paulo, Brazil). The smear layer was removed at the end of instrumentation with 17% EDTA (Fórmula e Ação, São Paulo, Brazil) agitated for 3 minutes with the EasyClean instrument (Bassi / Easy).

The teeth were dried with absorbent paper and filled using Tagger Hybrid Technique¹³, with an X2 cone (Dentsply-Sirona) and AHPlus (Dentsply-Sirona). To assess the quality of the filling, new radiographs were taken with the same parameters in the mesio-distal and bucco-lingual directions. The crowns were sealed with Coltosol (Whalent, Cuyaho, OH) and stored at 100% humidity and 37 °C for 30 days. After this period, the teeth were paired with micro-CT for specimens based on the volume of the original filling material. The teeth were divided into 2 groups according to the system. In the WOG 25.07 group (n = 20), the filling material was removed with the instrument in a reciprocating motion using the WaveOne ALL program driven by a 6:1 contra-angle handpiece (Sirona, Bensheim, Germany) powered by an electric motor (VDW Silver - VDW GmbH, Munchen, Germany). In the PRT 25.08 group (n = 20), the Easy SI endodontic motor (Bassi/Easy) was used to remove the filling material in a rotary motion at a speed of 900 rpm and torque 4N.

In both groups, the instruments were used with reciprocating motion with an amplitude of 3 mm and with a brushing motion until the WL was reached. Patency was maintained with a K #15 hand file. After each of the three in-and-out movements of the automated instruments, they were removed and cleaned with sterile gauze soaked in 70% alcohol. Each instrument was used only once and then discarded. Irrigation was performed with 20 mL of 2.5% sodium hypochlorite (Fórmula e Ação), using a 21 mm Navitip 30G needle (NaviTip, Ultradent, South Jordan, USA). The final irrigation protocol consisted of 5mL of 2.5% NaOCl using a disposable syringe (Injex Indústrias Cirúrgicas Ltda) and a Navitip 30 G needle.

Micro-CT analysis

After obturation and removal of the filling material, a new microtomographic scan was performed to evaluate the amount of filling material remaining in

the canals along the entire length with a SkyScan 1173 X-ray device (Bruker micro-CT) with the same parameters as described above.

Images were reconstructed using the CTAn v.1.15 software (Bruker micro-CT), and the volumes of obturation material (mm³) were determined according to the obturation and retreatment procedures. All measurements were performed for the entire canal volume and for each third: cervical, middle, and apical. Subsequently, 3D models were created and visualized using the software CTVol v.2.3 (Bruker microCT).

Statistical analysis

The percentage of the volume of filling material remaining after instrumentation was determined for each third and the entire root canal. The data were subjected to log¹⁰ transformation before statistical analysis. The data obtained were analyzed for their coefficient of variation and passed the D'Agostino & Pearson normality test, thus assuming a homogeneous distribution of the data. Student's t-test was performed to account for multiple observations per sample. All analyses were performed using IBM SPSS version 25.0 software (IBM Corp. Armonk, USA), with $p < 0.05$.

RESULTS

Microtomographic images showed residual filling material in all specimens (Fig. 1). Student's t-test showed that there was no statistical difference in the volume of remaining filling material between the two systems, either in the apical ($p = 0.392$), middle ($p = 0.065$) or cervical ($p = 0.918$) thirds (Table 1) but in the intragroup evaluation, differences were found ($p=0.013$).

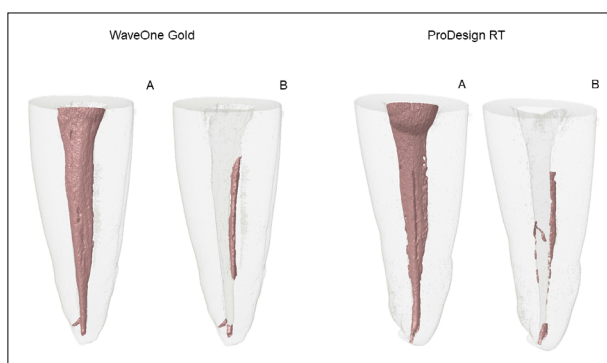


Fig. 1: Images of representative micro-CT reconstructions of the root canals in mesiodistal view showing the remaining filling material before (A) and after retreatment (B).

Table 1. Mean value (standard deviation) of the volume (mm³) of initial filling material (IFM) and remaining filling material (RFM), and mean value (standard deviation) of the percentage (%) of the volume of the filling material remaining after treatment with the PRT and WOG instruments.

Thirds	WaveOne Gold 25/07			ProDesign RT 25/08		
	IFM	RFM	%	IFM	RFM	%
Apical	0.98(0.23)	0.03(0.06)	2.82(6.44)	1.12(0.37)	0.04(0.05)	3.65(4.13)
Medium	2.30(0.42)	0.02(0.04)	0.74(1.52)	2.56(0.65)	0.04(0.05)	1.56(2.39)
Cervical	4.39(1.00)	0.02(0.04)	0.86(1.24)	4.38(0.98)	0.04(0.05)	2.07(2.12)

Notes: Student's t test accounting for multiple observations per sample was performed. * p > 0.05.

DISCUSSION

Efficiency in removing filling material from root canals may depend on variables such as diameter and taper of the endodontic instrument, type of alloy, heat treatment, cross-section, and kinematics of movement¹⁴. Therefore, the objective of this study was to evaluate the ability to remove filling of two systems with different characteristics and kinematics: Wave One Gold and ProDesign Logic RT.

ProDesign Logic RT is a rotary system with multiple instruments (#30.10; #25.08 and #20.06). It has a CM-wire treatment and a triple helix section, and fixed taper that gives it a greater volume of metallic mass, favoring contact with the canal walls¹⁵. Wave One Gold is a reciprocating single file system (#20.07; #25.07; #35.06 and #45.05) made of NiTi with special gold heat treatment, with parallelogram-shaped cross-section and variable taper that decreases in the cervical direction⁵. Despite the differences between the instruments in terms of manufacturing process, kinematics and taper, no significant difference in the removal of filling material in the cervical, middle, and apical thirds was found in the present study ($p > 0.05$). Therefore, the null hypothesis was accepted.

Although ProDesign Logic RT (25.08) has a larger taper than Wave One Gold Primary (25.07), there was no difference in the amount of filling material removed ($p > 0.05$) between the tested systems. This result confirms previous studies showing that Wave One Gold Primary (25.07) has the same effectiveness in removing filling material as reciprocating systems with a larger taper such as Reciproc (25.08) and Reciproc Blue (25.08), and rotary systems such as Protaper Gold F2 (25.08)¹⁴. One reason that may explain why the Wave One Gold instrument and the ProDesign Logic RT have similar ability to remove filling material is that the WOG has a parallelogram cross-section with two

cutting edges and asymmetric motion, which may have reduced the percentage of remaining filling material¹⁶. The absence of significant differences between the systems for extrusion and intracanal filling material removal may also be associated with the standardized conditions of root canal anatomy, irrigation, and working length^{3,6,8,9,23}. In these conditions, studies have shown that differences in taper, tip and cross-sectional shape, as well as the operation mode and the number of instruments used, fail to promote significant differences in filling removal^{3,6,8,9,23}.

The difference in kinematics between the two systems studied did not affect the effectiveness of filling material removal. Previous studies have also found no difference in ability to remove filling material between rotary and reciprocating systems¹⁷⁻¹⁹. Moreover, a systematic review²⁰ concluded that reciprocating and rotary systems have similar retreatment capabilities. However, some studies have shown better performance with reciprocating systems^{21,22}, while others have shown better performance with rotary systems^{23,24}. This divergence in results could be due to the method used to evaluate the removal, the endodontic sealer, and/or the filling technique used.

Although the ProDesign Logic RT system provides a sequence of three instruments, only the 25.08 instrument was used in the current study, in order to approximate the diameter of the WOG 25.07 instrument and improve the comparison parameters. The PRT 30.10 instrument could be more effective because instruments with a larger tip diameter may remove more filling material than instruments with smaller diameters¹⁴.

Some limitations regarding the methodology of this study should be highlighted. Straight roots were used to facilitate specimen standardization and adequate specimen pairing. Oval canals were

selected because this type of anatomy presents challenges for chemical-mechanical preparation and nonsurgical retreatment. AH-Plus epoxy-based endodontic sealer was used due to its excellent sealing ability, dimensional stability and flowability. Micro-CT is the assessment method commonly used in non-surgical endodontic retreatment studies^{5,22}. Studies show that additional irrigation with agitation techniques improves the removal of filling material^{23,25,26}. A previous study using oval canals of mandibular premolars compared the efficacy of the XP-Endo Finisher R and R1-Clearsonic inserts in removing filling material remnants from oval canals using micro-CT and concluded 82.1% and 64.6% in the entire canal for XP-Endo Finisher R and R1-Clearsonic ($P < 0.05$)⁹. Another study showed similar results with Photon-induced photoacoustic streaming (PIPS) using XP-Endo Finisher and Passive ultrasonic irrigation (PUI) to remove residual root canal filling material from oval canals. Filling

material was removed up to size X4 using ProTaper Next system, and the supplementary procedures were performed. The authors demonstrated that PIPS could reduce residual root canal filling materials to a similar extent as PUI and XPF²⁷. Therefore, the results of the current study should be evaluated with caution as only conventional irrigation with a syringe was used, which is the most common irrigation system worldwide.

CONCLUSIONS

The ProDesign RT and WaveOne Gold systems significantly reduced filling material in single-rooted canines with oval canals with similar results, although a residual amount of filling material remained following the use of both instrument systems. Further studies are needed to evaluate instruments that can compensate for the limitations of mechanical root canal preparation.

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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Açaí juice stains a glazed resin-modified glass-ionomer cement

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ABSTRACT

Coloured compounds (anthocyanins) in açai can stain resin-modified glass-ionomer cement (RMGIC) due to its low staining resistance. **Aim:** The aim of this study was to assess whether açai compromises the surface colour and roughness of RMGIC in vitro. **Materials and Method:** Disc-shaped specimens (2 mm thick, 8 mm in diameter) of Vitremer™ (3M ESPE, St Paul, MN, USA) were prepared according to the manufacturer's instructions. The mixture was inserted into a silicone mould placed between two mylar strips, and light cured. Specimens were randomly divided into three groups (n=25) according to the solutions to be used for chemical degradation: artificial saliva (control), açai sorbet and açai juice. A spectrophotometer CM-2600d/2500d (Konica Minolta, Tokyo, Japan) was used to analyse the colour (CIE $L^*a^*b^*$ scale). Surface roughness (Ra, mm) was measured using the profilometer Surfcomer SE 1700 (Kosaka Corp, Tokyo, Japan). The specimens were subjected to three daily soaks (6 ml, 15 minutes) for 14 days at 37°C. They were washed in distilled water and placed in fresh saliva (30 minutes in the interval). After the third soak in a day, they were stored in fresh saliva overnight. Outcomes were analysed at baseline (L^* , a^* , b^* , Ra) and after degradation (L^* , a^* , b^* , Ra). **Results:** The pH values of saliva, sorbet, and juice were 7.0, 3.8, and 4.9, respectively. ΔE^* values were 6.6 for saliva, 6.9 for sorbet and 7.8 for juice. There was a significant ΔE^* difference between saliva ($p=0.005$) and juice ($p=0.002$), and between juice and sorbet ($p=0.019$), but none between saliva and sorbet ($p=0.401$). There was no significant Δb^* difference between the solutions. No difference between juice and sorbet was observed for Δa^* , but they were significantly different from saliva ($p<0.001$). Brightness (L^*) changed significantly. Juice showed the highest ΔE^* (7.8) and ΔL^* (7.7). No significant change was observed for roughness and there was no difference between the solutions for ΔRa . **Conclusions:** Açai and saliva led to unacceptable staining, but no significant roughness changes in the resin-modified glass-ionomer cement.

Key-words: euterge - glass ionomer cements - colour - in vitro techniques

Suco de açai provoca o manchamento do glaze do cimento de ionômero de vidro modificado por resina

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RESUMO

As antocianinas presentes no açai podem manchar o cimento de ionômero de vidro modificado por resina (CIVMR) devido a baixa resistência ao manchamento do material. **Objetivo:** O objetivo desse estudo foi avaliar se o açai compromete a cor e a rugosidade de superfície de um CIVMR in vitro. **Materiais e Método:** Amostras (2 mm de espessura, 8 mm de diâmetro) de Vitremer™ (3M ESPE, St Paul, MN, USA) foram preparadas de acordo com as instruções do fabricante. O material foi espatulado, inserido em um molde de silicone colocado entre duas tiras de poliestireno e fotopolimerizado. Após, as amostras foram randomizadas e alocadas em três grupos (n=25) de acordo com as soluções usadas para a degradação química: saliva artificial (controle) e sorbet de açai e suco de açai. Utilizou-se o espectrofotômetro CM-2600d/2500d (Konica Minolta, Tokyo, Japan) para a análise da cor (escala CIE $L^*a^*b^*$) e o rugosímetro Surfcomer SE 1700 (Kosaka Corp, Tokyo, Japan) para a rugosidade de superfície (Ra, mm). As amostras foram submetidas a três imersões diárias (6 ml, 15 minutos) em cada solução por 14 dias a 37°C, tendo sido lavadas em água destilada e mantidas em saliva fresca (30 minutos) nos intervalos. Após a terceira imersão no dia, as amostras foram mantidas em saliva renovada até o dia seguinte. As variáveis foram analisadas antes (L^* , a^* , b^* , Ra) e depois da degradação química (L^* , a^* , b^* , Ra). **Resultados:** Os valores de pH da saliva, sorbet e suco foram, respectivamente 7,0, 3,8 e 4,9. Houve diferença significativa para ΔE^* entre saliva ($p=0.005$) e suco ($p=0.002$) e entre suco e sorbet ($p=0.019$), mas não entre saliva e sorbet ($p=0.401$). Não foi observada diferença significativa para Δb^* entre as soluções. Não houve diferença significativa para Δa^* entre suco e sorbet, mas eles foram significativamente diferentes da saliva ($p<0.001$). A luminosidade (L^*) mostrou alteração significativa. O suco mostrou os maiores valores de ΔE^* (7,8) e ΔL^* (7,7). Não houve mudança significativa para a rugosidade e não foi observada diferença significativa entre as soluções para ΔRa ($p>0.05$). **Conclusão:** O açai e a saliva causaram manchamento inaceitável do glaze do CIVMR e insignificante alteração da rugosidade.

Palavras-chave: euterge - cimento de ionômero de vidro - cor - técnicas in vitro.

INTRODUCTION

Consumption of açai (*Euterpe oleracea*) is popular among Brazilian children, teenagers and adults, especially in the summer. The fruit is rich in compounds with variable colours¹ (green, red, and purple) and the pH of commercial açai pulps is low². These characteristics might be a source of potential adverse effects for aesthetic dental materials, particularly conventional glass ionomer cement³.

The anticaries properties⁴ and substantial evidence confirm the outstanding performance of resin modified glass ionomer cement (RMGI) for sealing⁵ and restoring⁶ primary teeth. However, some level of discolouration and increase in roughness are likely⁷, even though it is more resistant to physical and mechanical changes than its conventional counterpart. The hydrophilic monomers in RMGI composition, such as HEMA (hydroxyethyl methacrylate), BIS-GMA (bisphenol A-glycidyl methacrylate), and TEGDMA (triethylene glycol dimethacrylate), may be prone to discolouration and increased roughness, shortening the material's longevity^{8,9}.

Although paediatric patients care about aesthetic issues, it is not reasonable for dentists to spend time polishing or even replacing restorations because of açai-related stains (particularly in low-compliance children). Given the lack of evidence on RMGI and açai interaction, information on it is relevant. Thus, the aim of this study was to assess the effect of açai on the colour and surface roughness of an RMGIC in vitro. The null hypothesis tested was that açai would not affect these variables.

MATERIAL AND METHOD

Sample Size Calculation

The sample size of 66 specimens (22 per group) was calculated in terms of the difference among the three

groups (Test F; one-way ANOVA) for colour change magnitude (ΔE^*), α error level of 5%, effect size of 0.4, and β error level of 20% (software GPower 3.1.9.2, University of Düsseldorf). Given potential losses during specimen preparation, 10% (25 per group) was added.

Experimental Design

Seventy-five disc-shaped specimens (2 mm thick, 8 mm in diameter) of Vitremer, PEDO shade (3M ESPE, St Paul, MN, USA; Batch 1927700210) (Table 1)^{10,11,12} were randomly distributed into three groups (n=25) corresponding to the tested solutions: artificial saliva (control), açai sorbet and açai juice. The chemical degradation protocol was based on Ozera et al. (2019)¹³. CIE L*a*b* and surface roughness (Ra, mm) values were analysed at baseline (L*, a*, b*, Ra) and after degradation (L'*, a'*, b'*, Ra'). The mean values of each specimen were recorded.

Material and Specimen Preparation

A trained operator hand mixed Vitremer's powder/liquid (1:1 ratio) following the manufacturer's instructions. Silicone moulds (2 mm deep, 8 mm internal Ø) (Adsil Silicone Addition, Coltene, Vigodent, Rio de Janeiro, Brazil) were filled slowly with the material using a Centrix syringe (Dentsply Ind. E Com. Ltda., Petrópolis, RJ, Brazil). The mould was placed between two mylar strips, and the top was pressed by hand with a glass plate to make the specimens flat and smooth¹⁴. The glass was removed, and the mixture was light-cured with Valo (Ultradent, USA) under 1,000 mW/cm² for 20 s, following the manufacturer's instructions. After the material had set and the strips had been removed, a thin layer of glaze (the finishing gloss included

Table 1. RMGIC composition.

Material	Vitremer
Manufacturer	3M ESPE (St Paul, MN, USA)
Batch #	1927700210
Powder content	Fluoroaluminosilicate glass; redox system. Mean Filler Size 3.0 µm.
Liquid content	Copolymer of Acrylic and Itaconic Acids, water, HEMA, Ethyl Acetate, Diphenyliodonium Hexafluorophosphate.
Glaze content	Triethylene Glycol Dimethacrylate (TEGDMA), Bisphenol A Diglycidyl Ether Dimethacrylate (BISGMA), Triphenylantimony, 4-(Dimethylamino)-Benzeneethanol, Hydroquinone.
Content information obtained from manufacturer information ^{10,11,12}	

in the Vitremer kit) was applied to protect the specimens, followed by light curing, as described above¹⁵. A radiometer (Hilux Dental Curing Light Meter, Benlioglu Dental Inc., Demetron, Ankara, Turkey) was used to monitor the irradiance before and after three measurements. The specimens were not subjected to finishing and polishing procedures. Finally, all 75 specimens were placed in deionized water at 37°C for 24 h to set (manufacturer's recommendation)¹⁴.

Colour and roughness assessments

A CM-2600d/2500d spectrophotometer (Konica Minolta, Tokyo, Japan) was used to record the mean values of each colour coordinate (measured in triplicate). The ΔE^* calculation followed the formula $\Delta E^* = [\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}]^{1/2}$ ¹³. Placing the specimens on a white background prevented potential absorption effects. Surface roughness was measured with a Surfcomer SE 1700 instrument (Kosaka Corp, Tokyo, Japan) to record the mean values (Ra, mm) from three successive measurements at the centre of each specimen in different directions, covering 1.25 mm with a cut-off length of 0.25 mm at a tracing speed of 0.1 mm/s¹⁵.

Solutions and Chemical Degradation Protocol

Table 2 provides the composition, manufacturer, batch number and pH of the solutions (artificial saliva, sorbet, and juice). The decrease in the pH of açai observed after 7 days in the pilot study required the use of fresh solutions. The specimens were subjected to three daily soaks (6 ml, 15 minutes) for 14 days at 37°C. After these soaks and a wash with distilled water, the specimens were placed in fresh saliva for 30 minutes. After the third soak, the specimens were placed in fresh saliva, where they remained until the next day¹³. A SC06 electrode

(Sensoglass, SensopH Ind. E Comércio de Sensores, São Paulo) coupled to an ion 450 M analyser (Analyser Analytical Instrumentation, São Paulo, Brazil) was used to check the pH before beginning the test.

Statistical analysis

Statistical analysis was performed with Jamovi 1.2. software (5% significance). The Shapiro–Wilk and Levene tests were used to assess data normality and homogeneity. The variables L^* , a^* , b^* , b'^* , ΔL^* , and ΔE^* were subjected to ANOVA followed by Tukey's test. The other variables (R_a , R_a' , ΔR_a , L'^* , a'^* , Δa^* and Δb^*) were subjected to the Kruskal–Wallis test followed by the Dwass–Steel–Critchlow–Fligner test (Table 3).

RESULTS

Saliva, sorbet, and juice pH values were 7.0, 3.8 and 4.9, respectively. Table 3 shows the roughness and colour ordinates baseline and final values. ΔE^* values were 6.6 for saliva, 6.9 for sorbet, and 7.8 for juice. All specimens showed clinically significant pigmentation ($\Delta E > 3.3$) regardless of the solution. There was a significant ΔE^* difference between saliva ($p=0.005$) and juice ($p=0.002$), and between juice and sorbet ($p=0.019$), but none between saliva and sorbet ($p=0.401$). No difference between juice and sorbet was observed for Δa^* , but they differed significantly from saliva ($p < 0.001$). Brightness (L^*) changed significantly. Juice had the highest ΔE^* (7.8) and ΔL^* (7.7).

DISCUSSION

This study shows that açai might discolour RMGIC, although there is no change in roughness. Specimen standardization was used to manage bias. For instance, saliva/açai was removed from the freezer/

Table 2. Tested solutions.

Solution	Composition	Manufacturer/Batch No.
Artificial saliva	Calcium (0.1169 g of calcium hydroxide/L of deionized water); 0.9 mM of phosphorus and potassium (0.1225 g potassium phosphate monobasic/L of deionized water); 20 mM TRIS buffer (2.4280 g TRIS buffer/L of deionized water)	Pharmaderm, Cascavel-PR, Brazil.
Sorbet	Açai pulp, water, sugar, guar gum, carboxymethyl cellulose, tara gum, natural guarana extract, citric acid acidulant, natural guarana aroma identical, glucose, artificial dye amaranth and brilliant blue FCF, xanthan gum.	Polpa Norte, Japurá-PR, Brazil; 0136(TB)
Juice	Medium açai pulp (100 g) blended with water (100 ml)	Polpa Norte, Japurá-PR, Brazil; 0430

Table 3. Colour parameters and roughness of RMGIC at baseline and after chemical degradation with the three solutions.

	Treatment			p values
	Saliva	Sorbet	Juice	
Ra	0.15 (0.10) A	0.18 (0.10) A	0.19 (0.10) A	0.159 ^{KW}
Ra'	0.15 (0.15) A	0.15 (0.10) A	0.20 (0.18) A	0.241 ^{KW}
ΔRa	-0.0008 (0.13) A	-0.03 (0.12) A	0.00 (0.18) A	0.660 ^{KW}
L	69.1 (2.1) A	68.5 (2.3) A	69.2 (1.7) A	0.502 ^{OwA}
L'	62.5 (1.6) A	61.7 (1.7) A	61.4 (1.7) A	0.073 ^{KW}
ΔL	-6.5 (1.3) A	-6.8 (1.7) AB	-7.7 (1.1) B	0.003 ^{OwA}
a*	-1.7 (0.4) A	-1.6 (0.3) A	-1.5 (0.4) A	0.367 ^{OwA}
a'*	-1.9 (0.2) A	-1.1 (0.8) B	-1.0 (0.7) B	<.001 ^{KW}
Δa*	-0.3 (0.2) A	0.5 (0.5) B	0.5 (0.5) B	<.001 ^{KW}
b*	11.7 (1.2) A	10.9 (1.1) A	11.2 (1.0) A	0.087 ^{OwA}
b'*	11.9 (1.1) A	11.4 (0.8) A	11.5 (1.1) A	0.316 ^{OwA}
Δb*	0.2 (0.8) A	0.5 (0.7) A	0.2 (0.9) A	0.229 ^{KW}
ΔE*	6.6 (1.2) A	6.9 (1.6) A	7.8 (1.1) B	0.002 ^{OwA}

Mean (standard deviation), n= 25. The symbol (') means after chemical degradation. Different letters indicate significant differences by row. One-way ANOVA (OwA)/ Tukey's test. Kruskal Wallis (KW)/Dwass-Steel-Critchlow-Fligner's test (p<0.05).

refrigerator immediately before checking pH and the beginning of the protocol.

The three solution ΔE* values (>3.3) supported rejection of the null hypothesis. Açai juice made the specimens darker with lower lightness coordinates (ΔL)⁷, and the positive Δa* indicates a shift towards red. These results suggests that the coloured compounds in the fruit and the liquid have stronger staining potential than those in the sorbet. Moreover, staining could be critical for other fruit colours. We speculate that staining was limited to the glaze applied to prevent surface irregularities¹⁵. The BIS-GMA (glaze) and HEMA (matrix and liquid content)¹⁶ may have increased pigment transportation during the test. Glaze removal by brushing machine and a long-term protocol would show the possibility of body discolouration. The colour results for the saliva group might have been related to carboxymethyl-cellulose (a thickening

agent), which can cause discolouration. Even composite resins showed noteworthy ΔE* values (7.8 to 10.6) when immersed in artificial CMC-based saliva¹⁷. The negative Δa* and ΔL values indicate a shift towards green colour and darker specimens, respectively.

A polyester strip provided a smooth surface¹⁴, which is why the samples were not polished¹⁵. The surface roughness variation (ΔR<0.00) was not significant (null hypothesis accepted). Despite the conflicting reports, experimental dissimilarities may explain disagreements¹⁸. The longer the time in coloured acid solution, the more colour would change. Scanning electron microscopy could provide information about the specimen surfaces. Inter-RMGIC comparison is still under investigation. The authors conclude that açai and saliva led to unacceptable RMGIC staining, but no significant change in its roughness.

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CONFLICT OF INTEREST

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Periodontal status and mandibular biomechanics in rats subjected to hyposalivation and periodontitis

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ABSTRACT

Xerostomia emerges as a consequence of salivary gland hypofunction, and seriously compromises the integrity of hard and soft oral tissues, while periodontitis is an infectious disease characterized by biofilm accumulation, inflammation and alveolar bone resorption. **Aim:** The aim this study was to compare the deleterious effects caused by experimental hyposalivation, periodontitis, and the combination of both on periodontal tissues and mandibular biomechanics in rats. **Materials and Method:** Hyposalivation (group H) was induced through bilateral submandibulectomy. Periodontitis (group EP) was induced by injecting LPS (1 mg/ml) into the gingiva of the first lower molars. A third group was subjected to both conditions (group H+EP). Alveolar bone loss was evaluated by micro-computed tomography and histomorphometric analysis, and gingival inflammatory mediators were assessed by specific techniques. Biomechanical properties were evaluated in mandible. **Results:** Alveolar bone loss increased similarly in groups H, EP and H+EP compared to control. Metalloproteinase (MMP2 and MMP9) activity was similar in H and control, but higher in groups EP and H+EP (MMP2: C 9644±2214, EP 34441±3336, H 5818±1532, H+EP 42673±3184; MMP9: C 5792±961, EP 14807±861, H 9295±520, H+EP 4838±1531). The rest of the inflammatory mediators evaluated increased in groups H, EP and H+EP to a greater or lesser extent with respect to the control, although in most cases, they were higher in groups EP and H+EP than in group H. The biomechanical properties of the mandible increased in group H compared to the other three groups. **Conclusions:** Both hyposalivation and periodontitis cause periodontal damage, but hyposalivation also produces biomechanical alterations, causing more extensive deleterious effects than periodontitis.

Keywords: jaw - xerostomia - periodontal diseases - gingiva - inflammation

Estudio del estado periodontal y la biomecánica mandibular en ratas sometidas a hiposalivación y periodontitis

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RESUMIO

La xerostomía surge como consecuencia de la hipofunción de las glándulas salivales y compromete seriamente la integridad de los tejidos orales duros y blandos, mientras que la periodontitis es una enfermedad infecciosa caracterizada por la acumulación de biofilm, inflamación y reabsorción ósea alveolar. **Objetivo:** El objetivo del presente estudio fue comparar los efectos deletéreos causados por la hiposalivación y la periodontitis experimental, y la combinación de ambas sobre los tejidos periodontales y la biomecánica mandibular en ratas. **Materiales y Método:** La hiposalivación (H) se indujo mediante una submandibulectomía bilateral. Por otra parte, la periodontitis (PE) se indujo mediante la inyección de LPS (1 mg/ml) en la encía de los primeros molares inferiores. Otro grupo se sometió a ambas condiciones (H+PE). La pérdida ósea alveolar se evaluó mediante tomografía microcomputarizada y análisis histomorfométrico, mientras que los mediadores inflamatorios gingivales fueron determinados mediante técnicas específicas. Se evaluaron las propiedades biomecánicas en la mandíbula. **Resultados:** La hiposalivación aumentó la pérdida ósea alveolar en comparación con el control de forma similar a la PE y H+PE. La actividad de las metaloproteinasas (MMP2 y MMP9) fue similar en los grupos H y control, pero resultó mayor en los grupos PE y H+PE (MMP2: C 9644±2214, PE 34441±3336, H 5818±1532, H+PE 42673±3184; MMP9: C 5792±961, PE 14807±861, H 9295±520, H+PE 4838±1531). El resto de los mediadores inflamatorios evaluados aumentaron en mayor o menor medida en los grupos H, PE y H+PE respecto al control, aunque en la mayoría de los casos fueron superiores en los grupos PE y H+PE respecto al grupo H. Sin embargo, las propiedades biomecánicas de la mandíbula aumentaron en el grupo H con respecto a los otros grupos. **Conclusiones:** Tanto la hiposalivación como la periodontitis causan daño periodontal, pero la hiposalivación también produce alteraciones biomecánicas, provocando efectos deletéreos más extensos que la periodontitis.

Palabras clave: mandíbula - xerostomía - enfermedad periodontal - encía - inflamación

INTRODUCTION

Periodontal disease is an infectious disease characterized by accumulation of dental biofilm, inflammation of tooth supporting tissues, and formation of periodontal pockets. It can lead to alveolar bone resorption and loss of periodontal attachment tissue¹. In most cases, periodontitis entails chronic progression, and under certain circumstances, advanced stages of the disease can cause tooth loss. Destruction of periodontal tissues is mainly due to complex interactions between pathogenic bacteria toxins and host-derived mediators generated during immunoinflammatory response^{2,3}. Exposure to bacterial products such as lipopolysaccharide (LPS) can trigger an inflammatory sequence of events^{1,4}. LPS is known to stimulate the production of cytokines and other inflammatory mediators, and to promote the release of matrix metalloproteinases (MMP) from the host tissues, which are destructive for the extracellular matrix and alveolar bone^{5,6}. In addition to degrading type I collagen (which is required for the access of osteoclasts to the resorption site) from the extracellular matrix, MMPs modify the biological activity of cytokines, chemokines and growth factors, leading to a proinflammatory pathway^{7,8}. Nitric oxide (NO) is a free radical produced by the endothelium, which is involved in various physiological processes such as a protective role in the microvasculature. However, excessive NO production is associated with tissue injury⁹⁻¹⁰. The ingress of inflammatory cells into sites of infection promotes the release of eicosanoids such as prostaglandin E₂, which is known to be a potent stimulator of bone resorption associated with loss of periodontal attachment tissue¹¹. LPS also stimulates host immune cells to produce interleukins, TNF α , acute-phase proteins, chemokines and adhesion molecules. These processes occur due to the interaction with toll-like receptor 4 (TLR4) expressed on the target cell membranes⁵, via CD 14 protein, and to the activation of the nuclear factor kappa-B (NF- κ B). These proinflammatory molecules are believed to induce several cellular reactions, which eventually lead to the inflammatory response of periodontal tissue and a consequent imbalance between alveolar bone formation and resorption, by favoring the latter. This resorption is mediated by receptor activator of nuclear factor-kappa B ligand (RANKL), its receptor RANK, and a decoy receptor

osteoprotegerin (OPG)^{12,13}. Furthermore, it is likely that bacteria contribute to the pathogenesis of periodontal disease by producing enzymes and other substances that could alter the tissues surrounding the periodontium and produce a toxic effect on their cells.

Saliva is a fluid constituted by 99% water, while the remaining 1% is formed by electrolytes, mucins, enzymes, growth factors and other organic molecules. All these components give saliva a vital role in the oral cavity, enabling initial digestion, swallowing, modulation of taste, speech, involvement in immunity, wetting and diluting substances, and maintenance of mucosal and hard tissue integrity¹⁴⁻¹⁶. Consequently, hyposalivation compromises the integrity of hard and soft oral tissues, enhancing new bacterial and fungal infections and the occurrence and progression of dental caries¹⁷, as well as causing ill-fitting dentures and taste alterations. It can also alter speech, eating and swallowing, with serious harm to life quality¹⁸. Furthermore, the submandibular gland is involved in immune and inflammatory responses in both local and systemic disorders¹⁹.

Xerostomia is a sensation of dryness in the oral cavity produced mainly by insufficient saliva secretion from salivary glands. It affects millions of patients around the world, ranging from 1% to 30% of the population, or perhaps more, depending on the features of the population studied²⁰⁻²¹. Xerostomia prevails in individuals over 65 years of age. Its causes may be local, such as sialadenitis, sialolithiasis, infections or neoplasm of the oral cavity and damage to the salivary gland tissue caused by radiotherapy; or systemic, including chronic autoimmune diseases such as Sjögren syndrome²², psychiatric or neurological disorders²³, diabetes²⁴, HIV²⁵ and drug or medicine intake²⁶, among others. Studies on rat pathophysiology have implemented some experimental models of xerostomia, such as X-ray-induced xerostomia²⁷. However, those models have seldom been studied in combination with experimental periodontitis²⁸, and have never been used with LPS-induced periodontitis. Since submandibular glands (SMG) and sublingual (SLG) glands combined contribute to whole saliva volume by approximately 65% of stimulated saliva and almost 50% of resting to whole saliva volume²⁹, submandibular-sublingual complex ablation provides

a useful model to assess the impact of reduced salivary flow on oral structures. We hypothesize that periodontal disease or hyposalivation individually produce deleterious effects on periodontal tissues and mandibular bone, and that coexistence of both exacerbates the deterioration. The aim of this study was to compare the deleterious effects caused by hyposalivation, periodontitis, and the combination of both on mandibular bone and periodontal tissues in rats subjected to such experimental conditions.

MATERIALS AND METHOD

Animals

Three-month-old adult male Wistar rats (300-350 g) from the authors' own laboratory colony were kept in group cages in an animal room with a 12-hour light photoperiod (0700-1900), room temperature maintained at 22°C-25°C, and free access to rat chow and tap water. The experimental procedures performed were approved by the Animal Care Committee of the Dental School of the University of Buenos Aires, Argentina (CICUAL-ODON/FOUBA N° 013/2016), and conducted according to the European Communities Council Directive of 24 November 1986 (86/609/EEC). All animal experiments complied with the ARRIVE guidelines.

Design

Twenty-eight rats were distributed into the following four groups containing seven rats per group: 1) control rats, 2) rats subjected to experimental periodontitis (EP), 3) rats subjected to hyposalivation (H), and 4) rats subjected to hyposalivation and experimental periodontitis (H+EP). On day 0, groups Hand H+EP underwent bilateral submandibulectomy (SMx) under anesthesia consisting of intraperitoneal injection of ketamine hydrochloride (Holliday-Scott SA, 70 mg/kg body weight) and 2% xylazine hydrochloride (König Laboratories SA, 10 mg/kg body weight), based on a previously described method²⁸. A midline incision of 15 mm was made through the skin and fascia of the ventral surface of the neck, the excretory ducts and main blood vessels were tied off, and submandibular–sublingual salivary complexes were dissected free from their supporting connective tissue and removed. They were excised with preservation of the surrounding neural structures, including the marginal mandibular branch of the facial nerve and the hypoglossal and facial nerves.

In groups EP and H+EP, on day 7, periodontitis was induced by injecting 20 µl of LPS (1 mg/ml) from *Escherichia coli* (serotype 055-B5, Sigma-Aldrich) into the vestibular and lingual gingiva of the right and left mandibular first molars and into the interdental space between the first and the second mandibular molars (60 µl of LPS per tooth and 120 µl per rat each time of treatment) under inhalational sevoflurane anesthesia, while control and H rats received no injections during the experiments. This protocol of injections was executed for a period of 6 weeks on days 1, 3, and 5 of each week, based on a previously described method³⁰. Gingival injections were placed with a 13-mm 27-gauge microfine insulin syringe. All the experiments were performed at least twice. The graphs represent the results obtained from one of them.

Micro-computed tomography (µCT) assessment

After a 48 hour-period of fixation in 4% paraformaldehyde solution and 10% 0.1M phosphate buffer (pH 7.4), hemimandible samples were scanned using the Skyscan 1172 (Bruker microCT) at 90 kV, 111 µA, with a 0.5 aluminum + 0.038 copper filter and 36% beam hardening correction, ring artifacts reduction 13, 180 degrees of rotation and exposure range of 0.4 degree. Images were captured with 1304x1024 pixels and a resolution of 13.2 µm pixel size. Projection images were reconstructed using the NRecon software (Bruker N Recon 1.7.1.0) and three-dimensional images were obtained by the CT-Vox software. Morphological parameters of trabecular bone microarchitecture were assessed employing the CTAn software following the guidelines recommended by Bouxsein et al.³¹.

One region of interest was confined to the first molar-interradicular trabecular bone and limited by its four roots. Tomograms were oriented in the axial direction, considering the most coronal part of the interradicular bone and the last tomogram which showed the four first molar roots, as the upper and lower borderlines, respectively. The working area was drawn among these four roots and redefined as tomograms progress. Measurements of axial images included bone volume fraction (bone volume/total volume, BV/TV, %), total bone porosity (Po-tot, %), trabecular thickness (Tb.Th, mm), trabecular separation (Tb.Sp., mm), trabecular number (Tb.N, 1/mm) and height of the periodontal space. Interradicular bone was also assessed through

tomograms oriented in the parasagittal direction. Images for this evaluation were selected so that whole mesial and distal roots and bone located between them appeared in tomograms, while lingual and buccal roots did not. In the parasagittal images, the height of periodontal space was evaluated by finding the average of equidistant lines between bone surface and dental surface (BS-DS) in millimeters. Buccal and lingual cortical plates were studied by means of coronal images. Distances were measured between the end of the mesial root and the beginning of the distal root, as the anterior and posterior references. This was used as an approach to the first lower molar-cortical bone. Distance between the cement-enamel junction and alveolar crest (CEJ-AC) was measured, eliciting separately measurements from buccal and lingual faces of the first lower molar in ten tomograms per side. Results were expressed in millimeters.

Histomorphometric analysis

Hemimandibles were extracted and fixed in formalin buffer. After 3 days, they were decalcified in 10% EDTA pH 7 for 45 days, then dehydrated with ethyl alcohol and clarified with xylene. The sector containing the first molar of each decalcified hemimandible was embedded in paraffin at 56°C to 58°C. Sections oriented mesial-distally of each mandibular first molar were cut from the paraffin blocks under astereomicroscope with a Jung microtome (Leica Biosystems). Sections 5 µm thick were stained with hematoxylin and eosin (H&E), and a histomorphometric evaluation was performed on digitized microphotographs using imaging software (University of Texas Health Science Center). Bone histomorphometric analysis was conducted by determining bone formation and bone resorption surfaces on the coronal half of the interradicular bone.

Measurements of inducible nitric oxide (iNOS) activity

The activity of iNOS was measured in gingival tissue (GT) and SMG by modifying the method described by Bredt and Snyder³², as follows: GTs were homogenized in 500 mL of cold 20 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES, Sigma-Aldrich, pH 7.4), with ethylene glycol-bis(2-aminoethylether)-N,N,N',N'-tetraacetic acid (Sigma-Aldrich, 2 mM) and DL-

dithiothreitol (DTT, Sigma-Aldrich, 1mM). A similar procedure was used for the SMG from control and EP groups, but it was homogenized in 600 mL of HEPES. After tissue homogenates were achieved, nicotinamide adenine dinucleotide phosphate (Sigma-Aldrich, 120 mM) and 200,000 dpm of [¹⁴C]-arginine monochloride (PerkinElmer, 297 mCi/mmol) were added to each tube and incubated for 10 minutes at 37°C in a Dubnoff metabolic shaker (Thermo Fisher Scientific 50 cycles per minute; 95% O₂/5% CO₂) at 37°C. The tubes were then centrifuged at 10,000 g for 10 minutes at 4°C. Supernatants were applied to individual 1-mL resin columns (Dowex AG 50W-X8 Na⁺ form mesh 200-400, Bio-Rad Laboratories) and washed with 2.5 mL of double-distilled water. The collected effluent fluid from each column was counted as activity of [¹⁴C]-citrulline in a Tri-Carb 2800TR Liquid Scintillation Analyzer (PerkinElmer). Since NOS converts arginine into equimolar quantities of NO and citrulline, data were expressed as picomoles of NO produced per minute per milligram of protein.

Determination of TNF-α

After extraction, gingival tissue was immediately homogenized in phosphate buffered saline containing protease inhibitor cocktail (Sigma-Aldrich) for mammalian tissue extracts to obtain the TNF-α preservation. Concentration of TNF-α was determined using a sandwich enzyme-linked immunosorbent assay according to the manufacturer's instructions. Briefly, in a 96-well ELISA plate, capture antibody, standards and samples, detection antibody, streptavidin-peroxidase (SAV-HRP) (enzyme) and tetramethylbenzidine (TMB, enzyme substrate), purchased from BD Pharmingen, were added sequentially. Data were expressed as picograms TNF-α per milliliter.

Radioimmunoassay of PGE₂

To determine PGE₂ content, gingival tissue was homogenized in 500 ml of absolute ethanol, and after centrifugation, supernatants were dried in a centrifugal vacuum concentrator (Speed Vac, Thermo Fisher Scientific) at room temperature. Residues were resuspended in buffer; and antiserum (sigma-Aldrich) was used as described in Mohn et al. (2011)³³. Sensitivity of the assay was 12.5pg per tube. Cross-reactivity of PGE₂ and PGE₁ was 100%, but cross-reactivity of other prostaglandins

was 0.1%. The intra- and inter-assay coefficients of variation for PGE₂ were 8.2% and 12.0%, respectively. Results were expressed in picograms of PGE per milligram of wet weight.

Metalloproteinases by gelatin zymography

MMP-2 and MMP-9 enzymatic activity from gum were assessed using gelatin zymography. Briefly, a homogenate of gingival tissue from unilateral upper and lower first molar was obtained and placed in a 7.5% polyacrylamide gel containing 0.1% sodium dodecyl sulfate (SDS) and 1 mg/ml purified gelatin (G8150, Sigma-Aldrich), as a MMP substrate. This gel was submerged in an electrophoretic cell along with a running buffer. After this step, gels were washed and then included in an appropriate buffer for 24 hours at 37°C to allow proteases to degrade the gelatin. Gelatin was stained with Coomassie blue solution R-250 (Imperial Chemical Industries plc) in order to recognize the clear bands related to enzymatic activity, and subsequently subjected to a bleaching solution. MMP-2 and MMP-9 were identified through the corresponding molecular weight of proteolytic bands, by comparison with commercial standards (Rainbow Marker, Amersham ECL). Stain band intensity was determined by densitometry, using Image J software, and results were expressed in arbitrary densitometry units. Protein concentration was determined by the Bradford method³⁴. Salivary gland homogenates were used as positive controls because they have a high concentration of MMP-2 and MMP-9.

Salivary secretion assessment

Rats were removed from cages 1 week before euthanasia and anesthetized with an intraperitoneal injection of ketamine hydrochloride (Holliday-Scott SA, 70 mg/kg body weight) and 2% xylazine hydrochloride (König Laboratories, 10 mg/kg body weight). Then, a dose of pilocarpine (Sigma-Aldrich, 0.5 mg/kg body weight) was administered intraperitoneally to induce salivation, and a cotton ball was immediately placed under the tongue of the rat to take up the total salivary secretion. This was determined as the difference in weight of the cotton ball before and after collection. Saliva was collected for 90 minutes after the administration of pilocarpine.

Biomechanical assessment

The mandibles of each rat were cleaned of adherent soft tissue and stored at -20°C wrapped in gauze soaked in Ringer's solution. Mechanical properties of right hemimandibles were determined by a three-point bending mechanical test on an Instron Universal Testing Machine Model 4442 (Instron Corporation) to obtain the load/deformation (W/d) curves. The head used to load the bone was a rectangle of 12 mm x 2 mm. Each bone was placed on two lower supports, with the lateral side facing down and centered along its length. The supports were equidistant from the bone ends and separated by a constant distance L (distance between supports = 11 mm), equivalent to not more than two fifths of the bone length. Loads were applied transversally to the bone axis at a point immediately posterior to the posterior surface of the third molar at a rate of 5.00 mm/min, which is useful to describe the static properties of the bone structure. Responses to the loading obtained in the curve enabled analysis of the structural mechanical properties of the bones as a whole³⁵, which are described below.

- a) Load at the yielding point or elastic limit (Wy): represents the end point of the elastic deformation of the bone (yielding point) and defines a threshold about which unrecoverable permanent deformation occurs.
- b) Structural stiffness or bone rigidity (Wydy): represents the slope of the elastic phase of the W/d curve and is a measure of the resistance of bone to deformation.
- c) Structural strength (Wf): represents the value of the load at fracture and expresses directly the resistance of the whole bone to fracture.

Masseter muscle and hemimandible weights

After euthanasia, the superficial head of the masseter muscle and the hemimandible were identified, cleaned and weighed using a precision balance (Pioneer Ohaus). Results were expressed as relative weights: masseter muscle/animal weight or hemimandible weight/animal weight (mg/g).

Statistical analysis

Data were expressed as the mean of 6 determinations ± SEM per group. Results were evaluated by Two-Way ANOVA followed by the Tukey's multiple comparisons test for unequal replicates. The sample size was set to enable the treatment differences

with a similar magnitude to the experimental error with 80% power in the ANOVA test. All analyses were conducted with Prism software (GraphPad Software). Differences with P values <0.05 were considered statistically significant. Columns with distinct letters indicate a significant difference between groups.

RESULTS

Micro-computed tomography (μ CT) assessment

In the interradicular analysis of the alveolar bone, groups H, EP and H+EP had lower values of bone volume fraction (BV/TV) and higher values of periodontal space height and total porosity (Po-Tot) than the control group. In turn, H+EP had lower bone volume fraction and higher total porosity value than group H, while these parameters did not differ significantly between groups H and EP (Table 1). The height of the periodontal space in the interradicular area increased in groups subjected to H, EP and H+EP, with respect to controls, but differences were not significant (Fig. 1A). Groups H and EP had a reduced trabecular number (Tb.N) compared to controls, although this reduction was not statistically significant when H+EP was compared with the control group. Trabecular thickness (Tb.Th) and trabecular separation (Tb.Sp.) did not differ among the experimental groups, with the exception of the lower trabecular thickness in H+EP than in the other three groups (Table 1).

In the analysis of the cortical bone, rats subjected only to H or EP had longer CEJ-AC distance than controls on both lingual and buccal sides, which indicates higher alveolar bone loss. The same detrimental effect was observed in the H+EP group on the lingual side, but not on the buccal side. Group H had greater distance than control on both the buccal and the lingual side, but it was lower than in groups EP and H+EP on the lingual side (Figs. 1B-C).

Histomorphometric analysis

Histomorphometric analysis of the interradicular area, showed smaller formation surfaces in groups H, EP and H+EP than in the control. No statistical differences were found among the three experimental groups. Bone resorption surface values were higher in groups EP and H+EP than in group H and control. Mean resorption surfaces were very similar in control and H, as well as in EP and H+EP (Figs. 2A-C).

Measurements of inducible nitric oxide (iNOS) activity

iNOS activity in the gingival tissue increased in groups H, EP and H+EP with respect to control. It was higher in group H+EP than in groups H and EP (Fig. 3A).

Determination of TNF- α

TNF- α level in gingival tissue was much higher in groups EP and H+EP than in the control group. Group H showed a lower level of TNF- α than the other two experimental groups, but it was significantly higher than in the control group (Fig. 3B).

Radioimmunoassay of PGE₂

Like other inflammatory mediators, gingival PGE₂ content level was higher in groups H, EP and H+EP than in controls, with no significant differences between groups H and H+EP (Fig. 3C).

Metalloproteinases by gelatin zymography

Extracellular matrix metalloproteinases 2 and 9 (MMP2 and MMP9) were measured in gingival tissue next to the first molar. Both groups with periodontitis, EP and H+EP, showed greater activity of MMP2 and MMP9 with respect to control and H groups. No differences were found between control and H groups. The gingival tissue of group H+EP

Table 1. Micro CT evaluation of interradicular bone.

Group	BV/TV %	Tb.Th mm	Tb.N 1/mm	Tb.Sp mm	Po(tot) %	Height of the periodontal space
Control	74.5±2.1 ^A	0.15±0.01 ^A	4.9±0.3 ^A	0.08±0.01	26.0 ±2.2 ^A	0.144±0.02 ^A
EP	65.2±2.6 ^{BC}	0.16±0.02 ^A	4.1±0.5 ^B	0.10 ±0.01	34.8±2.7 ^{BC}	0.260±0.01 ^B
H	69.0±2.8 ^B	0.17±0.02 ^A	3.9±0.2 ^B	0.09±0.00	31.0±2.9 ^B	0.221±0.02 ^B
H+EP	61.5±1.7 ^C	0.12±0.01 ^B	4.8±0.2 ^A	0.09±0.00	38.5±1.8 ^C	0.229±0.02 ^B

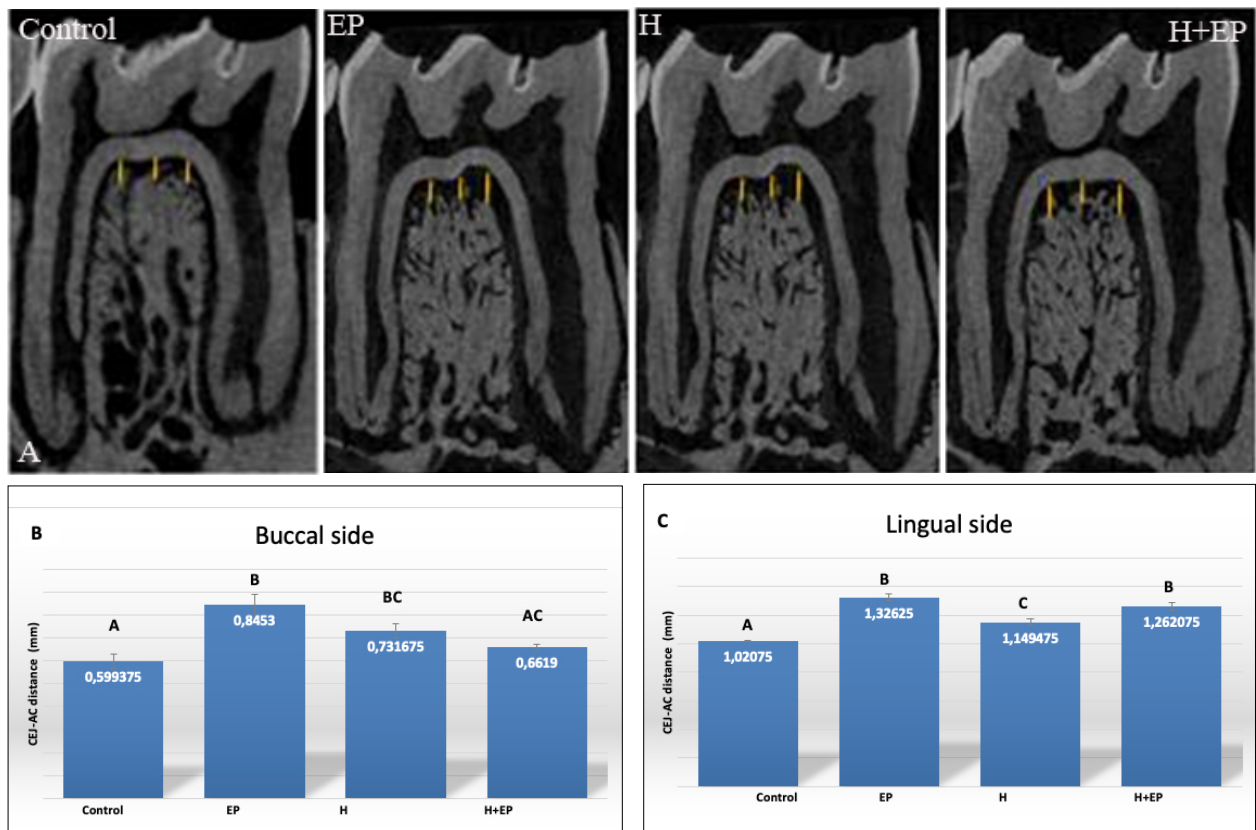


Fig. 1: A) Mesiodistal microtomographic images of the first lower molar, with special focus on the interradicular bone. Micro-computed tomography (μ CT) evaluation of alveolar bone loss in cortical plates: B) buccal side and C) lingual side. Results are presented as mean \pm SEM.

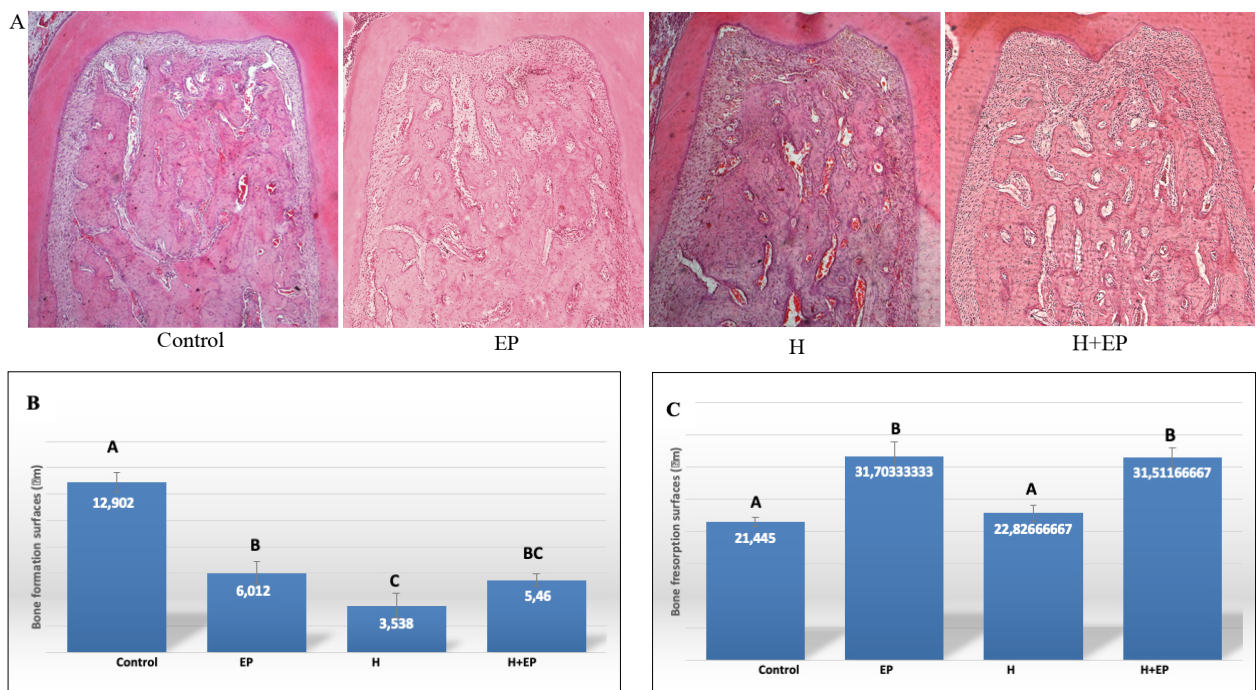


Fig. 2: A) First molar-interradicular bone. Histomorphometric evaluation showing bone formation (B) and bone resorption (C) surfaces. Results are presented as mean \pm SEM.

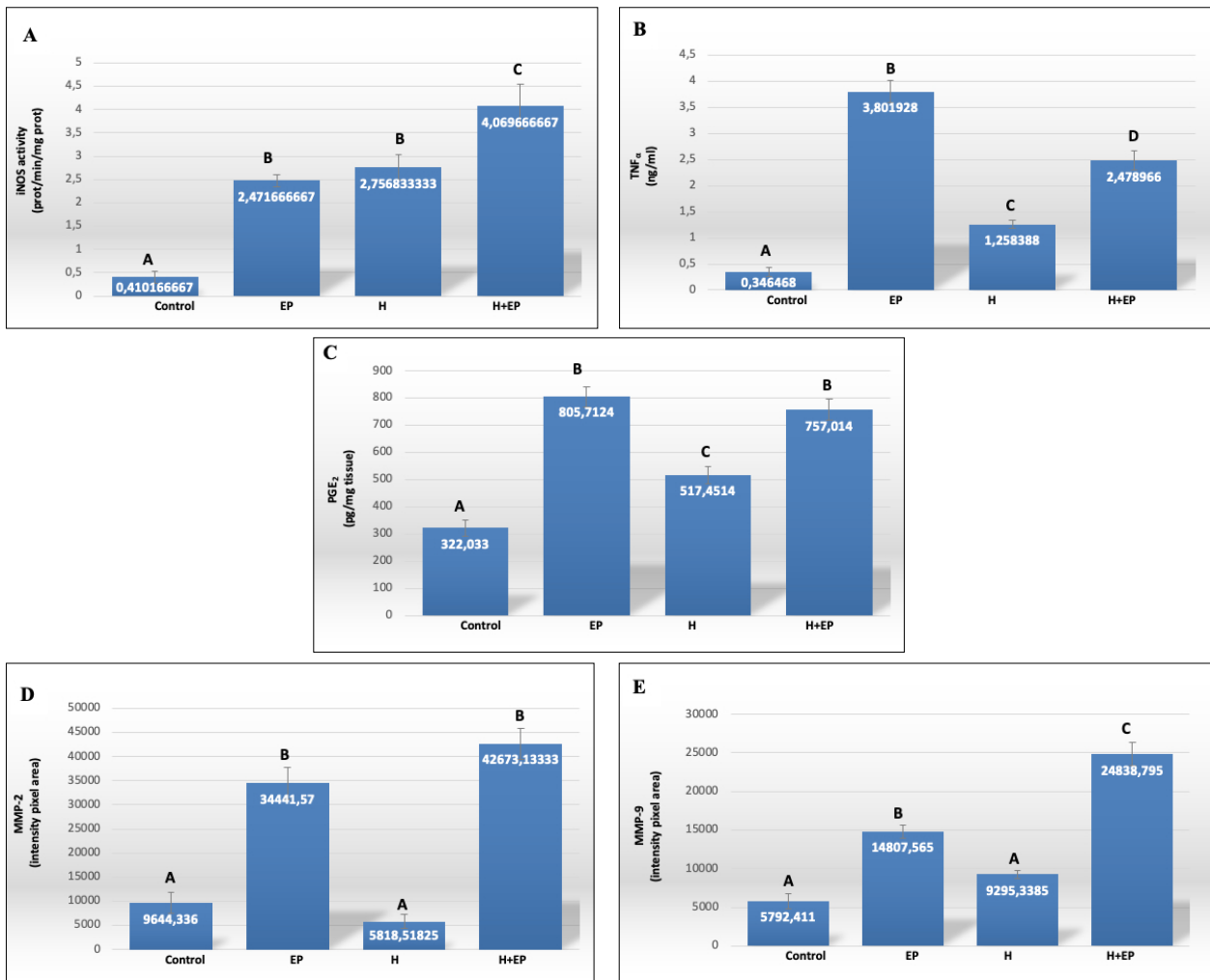


Fig. 3: Inflammatory mediators in gingival tissue: A) iNOS activity, B) TNF α level, C) PGE $_2$ content, D) extracellular matrix metalloproteinase 2 activity (MMP2) and E) extracellular matrix metalloproteinase 9 (MMP9) activity. The unit of measurement is the intensity of the pixel area. Results are presented as mean \pm SEM.

showed higher MMP9 activity than group EP, but not higher MMP2 activity (Figs. 3D-E).

Salivary secretion assessment

Total collected saliva was significantly reduced in animals subjected to experimental periodontitis as compared to controls, after 90 minutes of pilocarpine administration. As might be expected, the two groups subjected to submandibulectomy, H and H+EP, experienced a strong reduction of salivary secretion in comparison to controls, and even with respect to group EP (Table 2).

Biomechanical analysis

Load at fracture, load at yielding and rigidity were higher in group H than in control, EP and H+EP. These biomechanical parameters did not differ

Table 2. Salivary secretion

Group	Saliva (mg)
Control	795.8 mg \pm 23.4 ^A
EP	498.8 mg \pm 34.3 ^B
H	82.8 \pm 72.7 ^C
H+EP	83.5 \pm 106.3 ^C

significantly between groups EP and H+EP, or between them and the control group (Figs. 4A-C).

Masseter muscle and hemimandible weights

Superficial head of masseter muscle weight did not differ between control and EP groups. However, groups H and H+EP had greater masseter muscle weight than the two groups without submandibulectomy, which did not

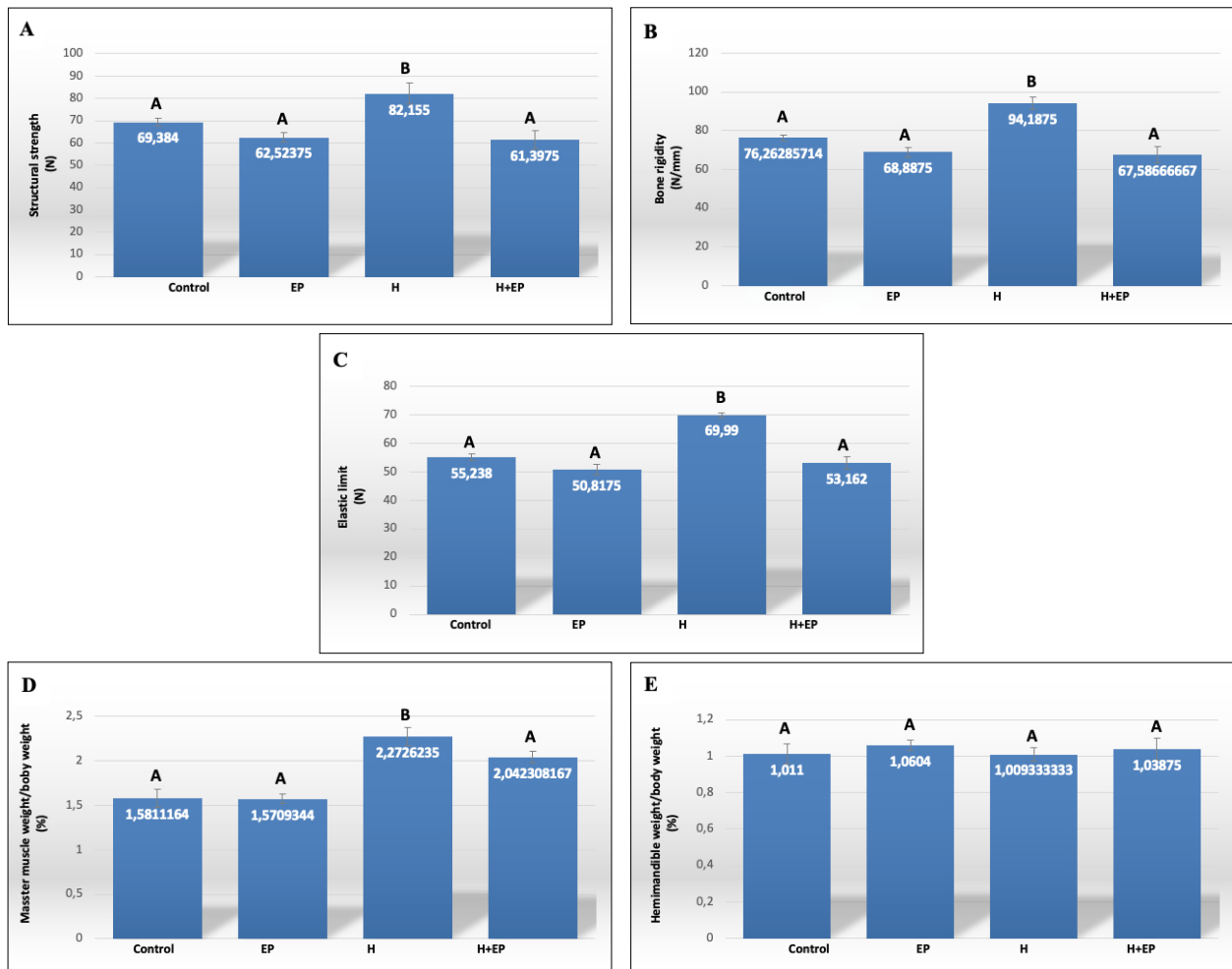


Fig. 4: Biomechanical properties of hemimandible. A) Structural strength or load at fracture; B) structural stiffness or bone rigidity, and C) load at the yielding point or elastic limit. Weights of masseter muscle (D) and hemimandible (E) as a percentage of body weight. Results are presented as mean \pm SEM.

differ significantly from each other. None of the experimental groups presented variations in the weight of the hemimandible with respect to the control group (Figs. 4D and E).

DISCUSSION

Restorative effects of saliva have been widely demonstrated both in mucosa and dental tissues, though they are still currently under study^{36,37}. In another study published by our laboratory, Mohn et al. demonstrated that wound repair after early post-tooth extraction is delayed in rats subjected to SMx-induced hyposalivation while the local inflammatory process is longer in those sockets³⁸. The present study analyzed changes in oral tissues of rats subjected to the same kind of experimental hyposalivation and compared them to those produced by experimental LPS-induced periodontitis.

Micro-computed tomography assessment showed that animals in group H had higher periodontal space height and total porosity, but lower bone volume and trabecular number in the interradicular bone than controls. On the buccal and lingual cortical plates, also known as marginal bone, the distance from CEJ to AC was higher in group H than in control. These changes in the interradicular bone area and external cortical plates evidenced increased alveolar bone loss caused by SMx-induced hyposalivation. Similarly, the group of animals subjected to EP also showed an increase in the height of the periodontal space, total porosity and CEJ-AC distance on buccal and lingual cortical plates, and a decrease in bone volume and trabecular number with respect to control. However, no differences were found between groups H and EP, with the exception of the lingual side, where alveolar bone loss was higher in group EP. One

interesting point is that in the animals subjected to hyposalivation and periodontitis concomitantly, bone damage caused by both insults individually did not exhibit a clear additive effect. Instead, values of alveolar bone loss in the interradicular area and external cortical plates did not differ greatly from those caused by the induction of hyposalivation and periodontitis separately, with the exception of trabecular thickness, which was lower, and total porosity, which was higher in group H+EP than in control, H and EP.

Our results partially agree with Vacas et al. (2008) regarding two parameters of interradicular bone loss, since in both studies bone volume fraction and height of periodontal space were higher in rats subjected to hyposalivation or to experimental periodontitis than in controls, although no additive effects were observed in a third group combining both experimental conditions. However, results in marginal bone differ between the studies, since Vacas et al. reported an increase of marginal alveolar bone loss on the lingual side, but not on the buccal side, in first molars of rats subjected to both hyposalivation and periodontitis compared to the individual experimental conditions²⁸. Such differences could be explained by the different periodontitis models used in each study (ligature versus LPS-induced periodontitis). Additionally, Vacas et al. (2008) used contralateral side as a control of ligature-induced periodontitis, which they call a sham²⁸. By contrast, in our study, control of LPS-induced periodontitis was represented by a different group of rats, exposed to the same conditions as the others, without either H or EP induction.

It should be underlined that in preliminary studies, we worked with two additional groups called sham and sham+EP, where sham underwent simulated surgery in which the neck skin was cut as in submandibulectomy, but the submandibular and sublingual gland remained intact after suturing. These preliminary studies showed that sham surgery did not produce any additional change to that produced by submandibulectomy alone on alveolar bone and inflammatory parameters (data not shown). Based on these results and considering the rational use of animals recommended by CICUAL, we decided not to proceed with further studies using sham groups.

It should also be considered that the expected additive deleterious effect of hyposalivation and

periodontitis on alveolar bone loss, which is not reflected in the results obtained for H+EP group, may already exist in H and EP groups. This could be a consequence of the reciprocal induction of these two pathological conditions, as it has been reported that hyposalivation causes periodontitis-like bone loss, while periodontitis induces hyposalivation^{6,39}. Furthermore, periodontitis-induced hyposalivation was also demonstrated in the present study. Therefore, the additive effect of hyposalivation and periodontitis would be masked in the H+EP group, since it does not differ significantly from the H and EP groups regarding most of the bone loss parameters assessed. In other words, hyposalivation and periodontitis may both be present in each group with these individual conditions, as a result of a direct or indirect induction. Another possibility is that the concomitance of the actions of LPS and the low volume of saliva that alters oral homeostasis evokes compensatory effects that prevent the harmful additive effects, as a protective response of the organism.

The histological examination of the interradicular bone showed smaller formation surfaces in groups H, EP and H+EP in comparison to control, with group H value being the lowest, while groups EP and H+EP had larger resorption surfaces than either group H or control. Images showing increased bone resorption in experimental groups included an increase in the number of osteoclasts (data not shown). These microscopic changes in the alveolar bone agree with previous studies by our group and others, which particularly reported alveolar bone histomorphometric damage originated by experimental periodontitis^{40,41}. On another note, this finding related to the increased resorption surfaces and decreased formation surfaces in groups H, EP and H+EP is consistent with μ CT results, in which the three experimental groups displayed changes with respect to the control. Conversely, some parameters evaluated by μ CT, such as trabecular thickness and total porosity, showed an apparent additive effect when H and EP were induced concomitantly, while no additive effect was noticed in reference to the bone formation and resorption surfaces.

In order to analyze the mandible bone performance under hyposalivation and LPS-induced periodontitis conditions, we evaluated its biomechanical properties. Group H had higher load at fracture, load at yielding and rigidity than control, EP and

H+EP rats, suggesting higher strength, lower elastic deformation and higher stiffness. The values did not differ significantly among control, EP and H+EP. The effects of hyposalivation on the biomechanical properties of the mandible recorded in the current study are similar to those reported by Mohn et al. (2018), who also demonstrated the increase of bone mass in the mandible after sumandibulectomy, working with a model of tooth extraction⁴². This might be related to the fact that the lower saliva production in group H causes dry mouth, making chewing difficult, which would lead to an increase in bone biomechanical properties, as a result of an adaptation to the greater requirement. This hypothesis could be supported by the augmented masseter muscle weight in rats subjected to submandibulectomy in the present study. Another possible hypothesis is that there could be mediation by signaling of transforming growth factor beta (TGF- β), a cytokine involved in the resorption phase and in bone organic matrix disorders⁴³. Since TGF- β has been found as a constituent of saliva⁴⁴, hyposalivation could imply reduced promotion of its pathway, leading to altered bone properties in the oral cavity⁴⁵. Moreover, the absence of biomechanical changes in mandible bones of group EP in the current study had been also reported in a previous study by of our group⁴⁶. In addition, biomechanical properties did not increase in the group of animals exposed to both conditions, H+EP, which exhibited similar values to those of group EP. This could be related to the imbalance of bone metabolism revealed by histological studies, since groups EP and H+EP showed larger bone resorption surfaces and smaller bone formation surfaces than the control, while group H showed bone resorption surfaces similar to those of the control and the smallest bone formation surfaces with respect to the other three groups. These results suggest that when hyposalivation and periodontitis coexist, the biomechanical effect of hyposalivation fades, perhaps as a compensatory phenomenon.

Inflammatory mediators were evaluated in gingival tissues around first molars. Groups exposed to hyposalivation, periodontitis or both conditions together exhibited higher levels of inflammatory mediators such as iNOS, TNF α and PGE₂, compared to control. Moreover, rats subjected to experimental periodontitis mostly showed increased inflammatory parameters with respect to group

H, except in iNOS activity, which only surpassed group H levels when experimental periodontitis and hyposalivation developed together. These results suggest that, although hyposalivation causes an increase in gingival inflammatory mediators, they increase more markedly in response to LPS-induced periodontitis. However, the pro-inflammatory effect provoked by experimental periodontitis could be at least partially promoted under hyposalivation conditions. Hori et al. (2021) recently reported that xerostomia increases mucosal inflammation and bone resorption in a model of ligation-induced peri-implantitis, although xerostomia does not cause osteoclastogenesis or bone resorption around implants not subjected to ligature⁴⁷. On the other hand, in the current study, MMP2 and MMP9 also had higher activity in groups EP and H+EP than control and H groups. MMPs belong to a protein family involved in the degradation of extracellular matrix compounds, associated with the progression of dental caries, apical lesions, periodontal diseases and orthodontic treatments⁴⁸. MMP2 and MMP9 act on several forms of collagen and their activation can be initiated by bacterial species such as *Treponema denticola* and *Porphyromonas gingivalis*⁴⁹⁻⁵⁰. Tissue imbalance resulting from molecule alteration, through the increase of inflammatory mediators plus the increase in MMP production, can lead to a complex tissue response characterized ultimately by bone deterioration.

The limitations of this study might be related to the experimental models used. LPS-induced periodontitis is an appropriate model for studying the chronic deleterious effects induced by the immune response during the disease; however, the damages induced by bacterial toxins do not occur due to the lack of biofilm accumulation on the teeth. Submandibulectomy consisting of submandibular and sublingual ablation does not exactly reproduce the hyposalivation frequently observed in patients, in whom secretory activity is reduced in both submandibular and parotid glands. However, both models used in this study are supported by the scientific community, as demonstrated by the large number of published works based on them^{28, 30, 38, 40, 42, 46}.

In summary, the present study demonstrated that SMx-induced hyposalivation increased gingival inflammatory parameters and caused damage to both alveolar bone and soft periodontal tissues. EP generated higher levels of inflammatory mediators

than H, leading to greater damage to periodontal tissues. The concomitance of H and EP did not show a clear additive effect, in slight divergence from one of our hypotheses. Further studies could be conducted to elucidate mechanisms through which deleterious effects of induction of periodontitis and hyposalivation do not add up as might be expected,

considering that when H and EP coexist, instead of producing an additive result, the effect of one of them seems to prevail. Finally, hyposalivation seemed to produce more extensive deleterious effects on the oral cavity than periodontitis, since biomechanical alterations were added to periodontal damage.

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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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Apical periodontitis in endodontically-treated teeth: association between missed canals and quality of endodontic treatment in a Colombian sub-population. A cross-sectional study

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ABSTRACT

A high prevalence of post-treatment apical periodontitis associated to variables such as endodontic treatment quality and missed canals has been reported. **Aim:** The aim of this study was to evaluate the quality of endodontic treatment and the frequency of missed canals associated with teeth with apical periodontitis (AP) through CBCT in a Colombian sub-population. **Material and Method:** This was a cross-sectional study assessing 318 cone beam computed tomography (CBCT) scans of endodontically treated teeth from Colombian individuals. The scans were taken using J Morita X550 (J Morita Corporation, Osaka, Japan), with voxel size 0.125 to 0.20 mm. All endodontically treated teeth were assessed for quality of treatment, presence of missed canals and AP. All samples were analyzed by two endodontics specialists and a radiology specialist. Chi-square or Fisher's test and odds ratio were calculated to identify the association and risk relationship between the presence of AP and the study variables. **Results:** Missed canals were found in 18.61% (86/462), and 95.3% were associated with AP. The frequency of AP was 62.34% (288/462) for all the evaluated teeth. AP was found in 27.43% (79/288) of the teeth with adequate endodontic treatment, in contrast to 72.57% (209/288) of the teeth with inadequate treatment ($P < 0.01$). The frequency of missed canals was highest in maxillary molars, with 55.23% (58/105), with 96.55% presenting AP. The second mesiobuccal canal was the most frequently missed canal, 88.52% (54/61), with AP in 90.74% (49/54) of the cases. **Conclusion:** There was a high frequency of teeth with missed canals and PA. More than half of the teeth with missed canals were maxillary molars, with MB2 being the most common canal, commonly presenting apical periodontitis.

Keywords: apical periodontitis - cone-beam computed tomography - cross-sectional study - endodontically treated teeth.

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Periodontite apical em dentes tratados endodonticamente: associação entre canais não localizados e qualidade do tratamento endodôntico em uma população colombiana: Um estudo transversal

RESUMO

Uma alta prevalência de periodontite apical pós-tratamento associada a variáveis como qualidade do tratamento endodôntico e fracasso do tratamento é relatada na literatura. O objetivo deste estudo foi avaliar a qualidade do tratamento endodôntico e a frequência e fracasso do tratamento associados a dentes com periodontite apical (PA) por meio de tomografia computadorizada de feixe cônico (TCFC) em uma subpopulação colombiana. **Material e Método:** Este foi um estudo transversal que avaliou 318 tomografias computadorizadas de dentes tratados endodonticamente de indivíduos colombianos. Os exames foram realizados utilizando o tomógrafo J Morita X550, com tamanho de voxel de 0,125 a 0,20 mm. Todos os dentes tratados endodonticamente foram avaliados quanto à qualidade do tratamento, presença de canais não localizados e AP. Todas as amostras foram avaliadas por dois especialistas em endodontia e um especialista em radiologia. Foram calculados o teste qui-quadrado ou de Fisher e a razão de chances para identificar associação e relação de risco entre a presença de PA e as variáveis do estudo. **Resultados:** Foram encontrados canais não localizados em 18,61% (86/462) e 95,3% estavam associados à PA. A frequência de AP foi de 62,34% (288/462) para todos os dentes avaliados. AP foi encontrada em 27,43% (79/288) dos dentes com tratamento endodôntico adequado, em contraste com 72,57% (209/288) dos dentes com tratamento inadequado ($P < 0,01$). A frequência de canais não localizados foi maior nos molares superiores, com 55,23% (58/105), sendo que 96,55% apresentavam PA. O canal méso-palatino (MB2) apresentou maior frequência de canal não localizado (88,52% - 54/61), com PA em 90,74% (49/54) dos casos. **Conclusão:** Houve alta frequência de dentes com canais não localizados e com PA. Mais da metade dos dentes com canais não localizados eram molares superiores, sendo o MB2 é o canal com a maior frequência, comumente apresentando periodontite apical.

Palavras-chave: periodontite apical - tomografia computadorizada de feixe cônico - estudo transversal - dentes tratados endodonticamente.



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INTRODUCTION

Apical periodontitis (AP) is an inflammatory condition of periapical tissues, and one of the most common infectious diseases in the world¹⁻³. When the root canal treatment fails, post-treatment AP may set in or persist and lead to tooth loss. Persistent, secondary, or extra-radicular infections are the leading causes of endodontic treatment failure^{2,3}.

Epidemiological studies have traditionally used periapical and panoramic radiographs to follow up endodontic treatment and determine possible variables associated with post-treatment AP. However, these radiographic examinations have limitations such as the superimposition of the overlying anatomy, the two-dimensional nature of the image, and the geometric distortion⁴. These deficiencies have been partly overcome by cone-beam computed tomography (CBCT) due to its high sensitivity to assess three-dimensional changes in dental tissues and supporting structures⁵, the quality of endodontic fillings, and hypodense bone lesions. It can also be used to evaluate different visual planes with slices as thin as 0.5 mm or less⁶.

Recent epidemiological studies using CBCT in Scotland, Brazil, France, Belgium, Germany, and Portugal confirmed the high prevalence of post-treatment AP, ranging from 40.8% to 55.5%⁷⁻¹². In addition, a systematic review and meta-analysis of cross-sectional studies showed that 41.3% of endodontically treated teeth had post-treatment AP, on average. AP was significantly more frequent in teeth with both inadequate endodontic treatment and poor coronal restoration¹³.

A risk factor for post-treatment AP is the inability of the clinician to locate all the root canals. Missed canals may harbor enough bacteria to maintain or lead to apical periodontitis. Even when initially uninfected, a missed canal becomes a susceptible site for infection¹⁴. Studies conducted in North America, Brazil, Portugal, and Saudi Arabia have reported a strong association between a missed canal and the presence of AP, with frequencies ranging from 12% to 23%, and 2.57 to 6.25 times more risk of presenting AP than teeth in which all canals have been treated¹⁴⁻¹⁸. However, these studies focused mainly on the missed canal variable, which could be considered a selection bias, given the greater number of teeth with AP in which all canals were treated¹⁹.

It is not clear whether the presence of a missed canal would remain statistically significant in a multivariate

analysis with a large set of variables, including those known to have a significant impact on treatment prognoses, tooth type, and quality of endodontic treatment¹⁹. Considering this context and the limited number of studies in the Latin American population, the present study evaluated the relation between the quality of endodontic treatment and the frequency of missed canals associated with teeth presenting AP through CBCT in a Colombian sub-population. The frequencies were determined for all tooth types.

MATERIALS AND METHOD

This research was conducted under the principles established by Resolution 08430 of the Government of Colombia and approved by the Ethics Committee of Santo Tomás University.

A cross-sectional study was conducted. A database of 1100 CBCT scans of Colombian individuals (n=318: 190 females and 128 males) was analyzed. Scans showing at least one endodontically treated tooth and voxel size between 0.125 and 0.20 mm were selected (594), while scans not showing endodontic treatment (472) or with voxel size greater than 0.20 mm (34) were excluded. Of the 594 selected scans, 276 were discarded due to artifacts, teeth without coronal restoration, or teeth with intra-radicular retainer, apical surgery, root fracture, perforations or resorption, leaving 318 CBCTs for study (Fig. 1). The CBCTs were obtained from two private radiology centers, one in Bucaramanga and another in Medellin. The scans were taken between June 2017 and June 2020, using two J Morita X550 CBCT Scanners (J Morita Corporation, Osaka, Japan): 127V, 50/60 Hz and 4 A panoramic X-ray,

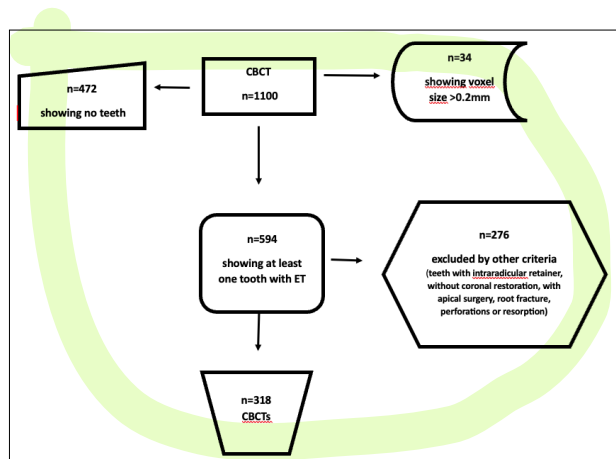


Fig. 1 | Flowchart of the sample selection process.

and three different FOV sizes: small (up to 5 cm), single arc (5 to 7 cm), and inter-arc (7 to 10 cm)²⁰. The I-Dixel One volume viewer software (J. Morita) was used. The tomographic images included in the database were requested for reasons beyond the scope of this research.

All dental types were evaluated. In multirooted teeth, each root was evaluated independently, centered, and aligned in sagittal and coronal planes, obtaining the most centered image to determine treatment quality based on the following criteria: Adequate Endodontic Treatment (AET), apical obturation limit between 0-2 mm from the root apex, and homogeneous compaction of filling mass; or Inadequate Endodontic Treatment (IET), apical obturation ending more than 2 mm from the apex or extruded gutta-percha, or poorly compacted root canal filling²¹. Missed canals were considered when there was a hypodense image, similar to a root canal, but without evidence of filling material, crossing the tooth root vertically, from the cervical region to the root apex¹⁵, and they were also classified as IET¹¹.

AP was defined according to the cone-beam computed tomographic-endodontic radiolucency index (ERI), as a hypodense zone associated with the root apex greater than 0.5 mm in a coronal-sagittal view; length measured in millimeters in the vestibule-palatine or lingual direction and the mesial-distal direction, tracing a perpendicular from the root apex to the upper limit of the radiolucent area in its greatest extension⁶.

Two endodontic specialists independently analyzed all samples. An oral and maxillofacial radiology specialist decided on discordant cases to identify the quality of the ET, the presence of missed canal, and the presence of AP. The observers' accuracy in detecting missed canals and AP was initially gauged with a pilot test of 100 images and using Cohen kappa intra and inter-evaluator reliability tests²², the kappa concordance coefficient obtained was higher than 90% intra-evaluator and between 95.34 and 96.1% inter-evaluator for both evaluators, a satisfactory data reliability result.

STATISTICAL ANALYSIS

The data were recorded using the Microsoft Excel software database (Microsoft Corp., LA, USA) and entered into the STATA 14.2 software (StataCorp LP, College Station, TX, USA). Absolute frequencies and percentages were calculated for qualitative

variables. The proportion of AP in each group was calculated and expressed with a 95% confidence interval (CI). The presence of AP was related to the explanatory variables (quality of the filling, presence of missed canal, and tooth type) using the chi-square test or Fisher's exact test for proportions and analysis of group differences. P-value ≤ 0.05 was considered significant. The odds ratio (OR) was calculated to analyze the odds that AP occurs in teeth with missed canal or IET compared to teeth with all canals treated or AET, respectively.

RESULTS

The frequency of AP was 62.34% (288/462); 27.43% (n = 79) of teeth with AET, and 72.57% (n = 209) of teeth with IET, a statistically significant result (p < 0.01, Table 1).

The 288 teeth with AP and 174 without AP were distributed as follows: for teeth with IET, 60.76% had AP without missed canal, compared to 39.23% with AP and missed canal, with a statistically significant difference (p < 0.01, Table 2). In this study, 18.61% of endodontically treated teeth had a missed canal, of which 95.3% had AP compared to a prevalence of AP of 54.7% for teeth without a missed canal (Table 3). Concerning dental groups, multi-rooted teeth had the highest frequency of

Table 1. Frequency of apical periodontitis according to the quality of endodontic treatment.

Endodontic Treatment	Quality	N (%)	Apical Periodontitis			
			Yes (n)	%	No (n)	%
Adequate (AET)		207(44.81)	79	27.43	128	73.56
Inadequate (IET)		255(55,19)	209	72.57*	46	26.43
Total		462	288	62.34	174	37.66

*P-value (≤ 0.05)

Table 2. Frequency of AP according to the presence or absence of missed canals in teeth with inadequate endodontic treatments (IET).

Sealing quality	N (%)	Presence of apical periodontitis			
		Yes (n)	%	No (n)	%
IET and missed canal	86	82	39.23	4	8.69
IET without missed canal	169	127	60.76*	42	91.31
Total	255	209	100	46	100

*P-value (≤ 0.05)

Table 3. Association between presence of missed canals and apical periodontitis (AP) in types of teeth.

Types of Teeth	Sample	Missed canal	Missed canal		Without missed canal	Without missed canal	
	Teeth with ET	n (%)	With AP n (%)	Without AP n (%)	n (%)	With AP n (%)	Without AP n (%)
Maxillary							
Central	48	1(2.12)	1(100)	--	47(97.87)	22(46.81)	25(53.19)
Lateral	49		--	--	49(100)	27(55.10)	22(44.90)
Canine	31		--	--	31(100)	13(41.94)	18(58.06)
Premolar	101	10(9.90)	8(80)	2(20)	91(90.10)	54(59.34)	37(40.65)
1 st Molars	71	44(61.9)	42(95.4)	2(4.5)	27(38.1)	20(74)	7(26)
2 nd Molars	34	14(41.1)	14(100)	--	20(58.82)	13(65)	7(35)
Mandibular							
Central	5		--	--	5(100)	2(40)	3(60)
Lateral	5		--	--	5(100)	3(60)	2(40)
Canine	4		--	--	4(100)	--	4(100)
Premolar	32	2(6.25)	2(100)	--	30(93.75)	9(30)	21(70)
1 st Molars	53	10(18.8)	10(100)	--	43(81.13)	29(67.44)	14(32.55)
2 nd Molars	29	5(17.24)	5(100)	--	24(82.75)	14(58.33)	10(41.66)
Total	462	86(18.6)	82(95.3)	4(4.7%)	376(81.39)	206(54.7%)	170(42.3%)

missed canal, with maxillary molars showing the highest percentage (55.23%, 58/105). The presence of AP in these teeth was 96.55% (56/58) (Table 3). In the mesiobuccal (MB) roots of maxillary molars, the MB2 canal was the most frequently missed canal (88.52%, 54/61), with 90.74% being associated with AP (49/54) (Table 4).

Teeth with endodontic treatment but with missed canals had a crude OR: 16.9 (95% CI, 6.07 - 47.10) to be associated with an AP, indicating a greater probability. In addition, according to the statistical significance found in the maxillary and mandibular molar teeth groups in the bivariate analysis, the logistic regression model adjusted for the variable type of tooth found an OR: 10.5 (95% CI 3.61- 30.86). Similarly, the groups of teeth with a statistically significant risk association were maxillary molars, OR :2.5 (95% CI 1.25-5.02), and mandibular molars, OR: 1.96 (95% CI 1.07-3.59), suggesting that a maxillary molar with missed canal had a 2.5-fold risk of having AP, and a mandibular molar with missed canal had a 1.96-fold risk of having AP when a missed canal was present (Table 5 and 6).

DISCUSSION

Several studies have investigated the prevalence of AP in teeth with endodontic treatment and its risk factors^{23,24}; however, they have used 2D images, which have disadvantages compared to 3D images. Due to its high sensitivity, CBCT can more accurately evaluate the presence of AP²⁵, the quality of the endodontic treatment, and the presence of missed canals¹⁴⁻¹⁶. For example, a study conducted in Colombia in 2013 found an AP prevalence of 49% using 2D images for endodontic treatments²¹, whereas the current study using CBCT found a higher prevalence of AP (62.34%), similar to recent CBCT studies that found AP between 51.8% and 88%¹⁴⁻¹⁶.

A noteworthy aspect of the current study is the evaluation of a significant variable, the quality of endodontic treatment, in addition to the presence of missed canals. It was observed that 27.43% of teeth with Adequate Endodontic Treatment (AET) and 72.57% of teeth with Inadequate Endodontic Treatment (IET) presented Apical Periodontitis (AP). This suggests that the presence of missed canals is not the sole factor associated with AP. In fact, 60.76% of teeth with IET and all canals treated had AP, compared to 39.23% of teeth with IET and

Table 4. Association between presence of missed canal and apical periodontitis (AP) in the root and canals of maxillary and mandibular molars.

Root canals	Sample N	With missed canal n (%)	With missed canal		Without missed canal n (%)	Without missed canal	
			AP			AP	
			Yes n (%)	No n (%)		Yes n (%)	No n (%)
Maxillary molars							
1st molar							
Mesiobuccal root							
Mesiobuccal canal	71	1(1.40)	1(100)	--	70(98.59)	53(75.71)	17(24.28)
Mesiobuccal 2 canal	50	43(86)	39(90.69)	4(9.30)	7(14)	5(71.42)	2(28.57)
Distobuccal root							
Distobuccal canal	71	3(4.22)	2(80)	1(20)	68(95.77)	30(44.11)	38(55.88)
Palatal root							
Palatal canal	71	--	--	--	71(100)	32(45.07)	39(54.92)
2nd molar							
Mesiobuccal root							
Mesiobuccal canal	28	2(7.14)	2(100)	--	26(92.85)	19(73.07)	7(26.92)
Mesiobuccal 2 canal	11	11(100)	10(90.9)	1(9.1)	--	--	--
Distobuccal root							
Distobuccal canal	28	2(4.14)	2(100)	--	26(92.85)	13(50)	13(50)
Palatal root							
Palatal canal	28	--	--	--	28(100)	15(53.57)	13(46.42)
Mandibular molars							
1st molar							
Mesial root							
Mesiobuccal canal	51	2(3.92)	2(100)	--	49(96.07)	28(57.14)	21(42.85)
Mesiolingual canal	51	2(3.92)	2(100)	--	49(96.07)	29(59.18)	20(40.81)
Distal root			--				
Distobuccal canal	14	6(42.85)	6(100)	--	8(57.14)	5(62.5)	3(37.5)
Distolingual canal	14	3(21.42)	3(100)	--	11(78.57)	8(72.72)	3(27.27)
2nd molar							
Mesial root							
Mesiobuccal canal	19	2(10.52)	2(100)	--	17(89.47)	10(58.82)	7(41.17)
Mesiolingual canal	19	4(21.05)	4(100)	--	15(78.94)	8(53.33)	7(46.66)
Distal root							
Distobuccal canal	2	--	--	--	2(100)	2(100)	--
Distolingual canal	2	--	--	--	2(100)	2(100)	--

missed canals. This finding could address the letter to the editor by Leprince & Nieuwenhuysen¹⁹, who, after pooling data from the most relevant studies on missed canals¹⁴⁻¹⁶, showed that 81.8% of all evaluated teeth presented AP and had all canals treated. Only 18.2% had presence of AP in teeth with missed canals. This corroborates the idea that multiple variables are associated with the presence of AP.

Furthermore, the current study found 18.61% of missed canals, similar to previous studies, which reported 12% to 23%¹⁴⁻¹⁸. Also, in the present study, 95.3% of missed canals had AP, comparable with previous studies¹⁴⁻¹⁶, which found 82.8%, 82.6%, and 98%, respectively.

Maxillary molars were the teeth with the highest frequency of missed canals, with 55%, similar to prior reports of 40.1%^{14,15}. Another study found

Table 5. Association between presence of apical periodontitis (AP) and quality of endodontic treatment in types of teeth.

Types of Teeth	Sample Teeth with ET	Inadequate n (%)	Inadequate		Adequate n (%)	Adequate	
			With AP n (%)	Without AP n (%)		With AP n (%)	Without AP n (%)
Maxillary							
Central	48	9(18.75)	9(100)	--	39(81.25)	14(35.89)	25(64.10)
Lateral	49	17(34.6)	13(76.47)	4(23.52)	32(65.30)	14(43.75)	18(56.25)
Canine	31	7(22.58)	3(42.85)	4(57.14)	24(77.41)	10(41.66)	14(58.33)
Premolar	101	57(56.4)	43(75.43)	14(24.56)	44(43.6)	19(43.18)	25(56.81)
1 st Molars	71	62(87.3)	58(93.5)	4(6.45)	9(12.6)	4(44.4)	5(55.5)
2 nd Molars	34	26(76.4)	24(92.3)	2(7.69)	8(23.5)	3(37.5)	5(62.5)
Mandibular							
Central	5		--	--	5(100)	2(40)	3(60)
Lateral	5		--	--	5(100)	3(60)	2(40)
Canine	4		--	--	4(100)	--	4(100)
Premolar	32	17(53.1)	10(58.82)	7(41.17)	15(46.9)	1(6.66)	14(93.33)
1 st Molar	53	40(75.4)	32(80)	8(20)	13(24.52)	7(53.84)	6(46.15)
2 nd Molar	29	20(68.9)	17(85)	3(15)	9(31.03)	3(33.33)	6(66.66)
Total	462	255(55)	209(81.96)	46(18.03)	207(45)	80(38.64)	127(61.35)

59.55% and 40% missed canals in maxillary first and second molars, respectively¹⁶, while the present study found similar results for the first and second maxillary molars, with 61.97% and 41.18%, respectively. The reason for the high percentage of missed canals may be the presence of a second mesial buccal (MB) canal in 88.5% of maxillary first molars and 83.4% of second molars²⁵. The MB root has complex anatomy that usually presents Vertucci configurations type II (69.1%)²⁶ and type IV (48.7%)²⁷. MB2 was also the most frequently missed canal in maxillary molars in previous studies, with prevalences of 65%¹⁶, 93%¹⁴, and 89% in the current study. This might be explained by the fact that the MB2 canal is usually narrow, significantly smaller than the MB1 canal, with its opening often covered by secondary dentin or calcifications^{28,29}, and the curvature in the mesial root is pronounced in the apical third of both canals and stronger in the MB2 canal. These anatomical features may make it more difficult to locate and achieve a straight course to the apex²⁹. The presence of an MB2 canal should be considered the rule rather than the exception.

In the current study, mandibular molars had the second-highest frequency of missed canals, with 18%, similar to previous studies, which found 19.7%¹⁵, 26%¹⁴, and 10.42%¹⁶. All mandibular molars with missed canals were associated with AP

in the present study. Other studies have reported similar frequencies, such as 94.1%¹⁶ and 95.5%¹⁴. Another study found 62% of missed canals in the mandibular first molars, with the distolingual canal being the most frequently missed canal, although it did not specify the percentage of AP in mandibular first molars with missed canals¹⁵.

In the current study, the most frequently missed canals were the distobuccal canal in mandibular first molars (42.85%), followed by the mesolingual canal in mandibular second molars (21.05%). This was similar to another study that found in mandibular first molars missed distolingual canals (21%) and mesolingual canals (29%), and in mandibular second molars, missed mesolingual canals (62%)¹⁴. These frequencies of missed canals in mandibular molars may occur due to variations in anatomy and location, which make it difficult to access and treat all root canals¹⁴⁻¹⁶.

In the present study, the probability of a tooth with a missed canal having AP was 16.9 (crude OR) and 10.5 (OR adjusted to the tooth type variable). These findings could be considered higher than those of previous studies, which reported probabilities of 4.38¹⁵, 6.25¹⁴, and 4.4¹⁶. These differences may be related to the higher percentage of teeth with IET in this study.

Good quality endodontic treatment seems to

Table 6. Association between presence of quality of endodontic treatment and apical periodontitis (AP) in the root and canals of maxillary and mandibular molars.

Root canals	Sample N	Inadequate n (%)	Inadequate		Adequate n (%)	Adequate	
			AP			AP	
			Yes n (%)	No n (%)			Yes n (%)
Maxillary molars							
1st molar							
<u>Mesiobuccal root</u>							
Mesiobuccal canal	71	42(59.1)	36(85.71)	6(14.28)	29(40.8)	18(62.06)	11(37.93)
Mesiobuccal 2 canal	50	47(94)	43(91.48)	4(8.52)	3(6)	1(33.33)	2(66.66)
<u>Distobuccal root</u>							
Distobuccal canal	71	34(47.88)	21()	13()	37(52.1)	11(29.72)	26(70.27)
<u>Palatal root</u>							
Palatal canal	71	30(42.25)	19(63.33)	11(36.66)	41(57.74)	13(31.70)	28(68.29)
2nd molar							
<u>Mesiobuccal root</u>							
Mesiobuccal canal	28	18(64.28)	14(77.7)	4(22.2)	10(35.71)	7(70)	3(3)
Mesiobuccal 2 canal	11	11(100)	10(90.9)	1(9.1)	--	--	--
<u>Distobuccal root</u>							
Distobuccal canal	28	15(53.57)	10(66.66)	5(33.33)	13(46.42)	5(38.46)	8(61.53)
<u>Palatal root</u>							
Palatal canal	28	13(46.42)	9(69.23)	4(30.76)	15(53.58)	6(40)	9(60)
Mandibular molars							
1st molar							
<u>Mesial root</u>							
Mesiobuccal canal	51	21(41.17)	15(71.42)	6(28.57)	30(58.83)	15(50)	15(50)
Mesiolingual canal	51	26(50.98)	17(65.38)	9(34.61)	25(49.01)	14(56)	11(44)
<u>Distal root</u>							
Distobuccal canal	14	10(71.42)	9(90)	1(10)	4(28.57)	2(50)	2(50)
Distolingual canal	14	10(71.42)	9(90)	1(10)	4(28.57)	2(50)	2(50)
2nd molar							
<u>Mesial root</u>							
Mesiobuccal canal	19	10(52.63)	7(70)	3(30)	9(47.36)	5(55.55)	4(44.44)
Mesiolingual canal	19	12(63.15)	10(83.33)	2(16.66)	7(36.84)	2(28.51)	5(71.42)
<u>Distal root</u>							
Distobuccal canal	2	2(100)	2(100)	--	--	--	--
Distolingual canal	2	2(100)	2(100)	--	--	--	--

decrease the prevalence of apical periodontitis in teeth with missed canals compared to teeth with IET and missed canals. However, further studies are needed to confirm this hypothesis.

Bivariate analysis of the results obtained in the current study was very similar to that reported in the studies mentioned above. Additionally, a wide confidence interval was observed for crude OR and OR adjusted for the tooth type variable. According to

tooth type, an OR of 2.5 was observed for maxillary molars, similar to the 3.1 previously reported¹⁶.

The major advantage of cross-sectional studies is that, with a large sample size, potential interpretation errors can be diluted¹⁴. However, the nature of cross-sectional studies may also be a limitation owing to the reliability of the observations. Moreover, it is difficult to establish the natural history of the disease, e.g., it is impossible to determine whether

AP is expanding, healing or stable¹. Many of the images classified as AP at the time of analysis could be AP in the process of healing, reducing in size³⁰. Another limitation was the CBCT method, which may show artifacts caused by high-density objects such as gutta-percha, which might generate false positives¹⁴⁻¹⁶.

The diagnosis, histological and microbiological analysis of the small variations seen as areas of low density (0.5 mm radiolucent zone) around the apex in ET teeth when viewed in CBCT images is another crucial factor to consider. However, treatment need not be started immediately based on this finding because when the tooth in question is asymptomatic and functions normally, the clinician should evaluate subtle changes histologically or wait for a

reasonable amount of time³¹. Future studies should also consider factors including lesion size and patient age.

CONCLUSIONS

The frequency of missed canals was 19%, with apical periodontitis being observed in 95,3% of these cases. More than half of the teeth with missed canals were maxillary molars, and the MB2 was the most frequently missed canal, with apical periodontitis being observed in 90.74%. Based on these results we conclude that there was a high frequency of teeth with missed canals and PA and more than half of the teeth with missed canals were maxillary molars, with MB2 being the most common canal, commonly presenting apical periodontitis.

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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Juvenile language disorders and their interaction with dentistry: a bibliometric analysis

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ABSTRACT

Language disorders may interfere with social integration and affect personal development. Because the balance of the stomatognathic system can interfere with language, it is important for speech therapy and dentistry to work together, providing multidisciplinary healthcare. **Aim:** To analyze the 100 most frequently cited articles on language disorders in children and adolescents and assess the interplay with dentistry by means of a bibliometric analysis. **Materials and Method:** A search of the 100 most frequently cited articles up to December 2021 on language disorders was performed in the Web of Science Core Collection database. Four researchers extracted the data on number of citations, title, authors, country, year of publication, journals, study design, prevalent clinical conditions, and area of expertise. The analyses were performed using VOSviewer and Excel. **Results:** The total number of citations ranged from 251 to 1,431. Four articles were cited more than 1,000 times. Bishop DVM (10 articles; 3,653 citations) and Tomblin JD (10 articles; 4,261 citations) were the most frequently cited authors. The institutions with the largest number of publications were the University of Oxford/England (11%) and the University of Kansas/USA (8%). Observational study design was the most frequent (77%). Autism spectrum disorder (18%) and dyslexia (14%) were the most broadly investigated clinical conditions. Speech-language pathology (32%) was the area of expertise with the largest number of articles, and none of the top 100 studies showed interplay with dentistry. **Conclusion:** The 100 most widely cited articles on language disorders are mostly observational, mainly address autism spectrum disorder, and are in the field of speech-language pathology. No study reported interplay with dentistry.

Keywords: bibliometrics - communication disorders - language disorders - interdisciplinary research - dentistry

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Transtornos da linguagem juvenil e sua interação com a odontologia: uma análise bibliométrica

RESUMO

Os distúrbios de linguagem podem interferir na integração social e afetar o desenvolvimento dos indivíduos. O trabalho multidisciplinar entre fonoaudiologia e odontologia é muito importante para a promoção da saúde. O equilíbrio do sistema estomatognático interfere na linguagem. **Objetivo:** Analisar os 100 artigos mais citados sobre distúrbios de linguagem em crianças e adolescentes e verificar a interação com a odontologia por meio de análise bibliométrica. **Material e Método:** Foi realizada uma busca pelos 100 artigos mais citados sobre distúrbios de linguagem na base de dados Web of Science Core Collection até dezembro de 2021. Quatro pesquisadores extraíram os dados referentes a número de citações, título, autores, país, ano de publicação, periódicos, desenho de estudo, condições clínicas prevalentes e área de especialização. As análises foram realizadas utilizando VOSviewer e Excel. **Resultados:** O número total de citações variou de 251 a 1.431. Quatro artigos foram citados mais de 1.000 vezes. Bishop DVM (10 artigos; 3.653 citações) e Tomblin JD (10 artigos; 4.261 citações) foram os autores mais frequentemente citados. As instituições com maior número de publicações foram a Universidade de Oxford/Inglaterra (11%) e a Universidade de Kansas/EUA (8%). O desenho de estudo observacional foi o mais frequente (77%). O transtorno do espectro autista (18%) e a dislexia (14%) foram as condições clínicas mais amplamente investigadas. A Fonoaudiologia (32%) foi a área de atuação com maior número de artigos e nenhum estudo, entre os top 100, mostrou interação com a Odontologia. **Conclusão:** Os 100 artigos mais citados sobre distúrbios de linguagem são em sua maioria observacionais, abordam principalmente transtornos do espectro do autismo e estão na área de fonoaudiologia. Nenhum estudo apresentou interação com a odontologia.

Palavras-chave: bibliometria - distúrbios de comunicação - distúrbios de linguagem - pesquisa interdisciplinar - odontologia.

INTRODUCTION

Language involves exchanging information effectively. Language disorders may interfere with social integration and affect personal development in several domains¹. Chronologically, language acquisition in normotypical children follows an unwavering order, though its pace varies considerably². According to the American Speech, Language, and Hearing Association³, alterations of speech, language and hearing are regarded as language disorders, and they cause negative impact on children's lives and may be considered a public health problem⁴.

The different health professionals who monitor child development should be properly trained to identify the landmarks expected at each age. In addition to speech-language pathologists, who can diagnose and treat language disorders, other health professionals such as pediatricians, pediatric dentists, psychologists and education professionals are part of the supporting network for children's language development⁵. These professionals should pay special attention to children's global development and resort to a multidisciplinary approach when any speech delay or disorder is suspected.

Some studies report that language disorders are the most prevalent conditions among children^{4,6}. Dentistry plays a crucial role in identifying children with speech disorders, and its integration with speech therapy is of the utmost importance. Disharmonies in dental arches, malpositioned teeth, or missing teeth can cause articulatory speech disorders, because the balance of the stomatognathic system, which involves the teeth, lips, and tongue, plays an important role in the articulation of consonants through obstruction and modification of airflow⁷.

Language development disorders may interfere in health promotion as a whole. A multidisciplinary approach can help improve quality of life, especially of children and adolescents, and contribute to providing more precise guidelines and public health promotion⁸.

The use of bibliometric studies has been suggested to help the academic community identify the history and tendencies in a given area or topic, by analyzing the quantitative aspects of the science and scientific production that provides critical input to research on the subject⁹⁻¹¹. The number of times a publication is cited in other articles is a potential marker that represents its influence¹². Widely cited articles

are expected to have high quality and data that significantly contribute to knowledge¹³. Although numerous bibliometric studies have been conducted in areas or topics related to dentistry^{10,14,15}, analyses of children's speech have not been performed to date.

The aim of this study was to assess the 100 most frequently cited articles on language disorders in children and adolescents by means of a bibliometric analysis. Given that a multidisciplinary approach is crucial for a comprehensive and effective treatment, the present study also aimed to ascertain which of these articles reported some interaction with dentistry during their production. Based on the analysis, this report intends to provide an overview of existing research on this topic, identifying areas of interest as well as poorly researched areas, and encouraging future research in the field.

MATERIALS AND METHOD

Search strategy

A literature search was conducted on 17 December 2021. No data or language restrictions were imposed. The search was conducted in the Web of Science Core Collection (WoS-CC) database. The search strategy began with the evaluation of the most widely investigated topics in the field of speech-language pathology. Language was found to be a recurrent topic in the databases. A search using relevant MeSH terms, synonyms, and free terms associated with language disorders in children and adolescents was performed. Boolean operators (AND, OR, and NOT) were used for combinations of terms. The search key is described in Table 1.

Table 1. Search key for the Web of Science Core Collection (WoS-CC) database.

```
TS=(speech OR language* OR linguistic OR communicat*)
AND TS=(disorder* OR impair* OR problem* OR
dysfunction* OR difficult* OR therap* OR train* OR
rehabilitat* OR treat* OR remediat* OR intervention* OR
pathol*) AND TS=(child* OR infant* OR toddler* OR
adolescen* OR teen* OR young* OR newborn* OR
offspring* OR kid OR kids OR pediatric* OR paediatric* OR
neonat* OR juvenile*) NOT TS=(adult* OR elder*)
```

Eligibility criteria and study selection

Articles that addressed language disorders in different fields of health and education and dealt

with the pediatric population were included. Articles that included adults or older people, clinical trial protocols, books or book chapters, and conference/symposium proceedings were excluded.

All the articles retrieved from the database were organized according to the number of citations in decreasing order. Four researchers selected the articles by applying the eligibility criteria, and any disagreements were resolved through consensus. The selection was concluded when the hundredth most frequently cited article was retrieved.

Data extraction

All included studies were transferred to Microsoft Excel® 2010 (Microsoft, Redmond, WA, USA) for classification. The following data were obtained from each article: ranking, number of citations, title, year of publication, country (based on the corresponding author's affiliation), authors, journal, study design (observational, intervention, review, and case report/case series), area of expertise (dentistry, speech-language pathology, psychology, medicine, education, and combinations of these areas), and clinical conditions addressed (autism spectrum disorder, dyslexia, specific language impairment, hearing loss, attention deficit/hyperactivity disorder, auditory processing disorder, dyscalculia, and cerebral palsy).

Bibliometric analysis

The VOS viewer (version 1.6.11, Center for Science and Technology, Leiden University, the Netherlands) was used to create density plots for network maps of authorship and co-authorship¹⁶. On the maps, the items were linked by considering the number of co-authored articles. Each point on the density plot has a color that indicates the density of the items at that point. The colors range from blue to yellow to red. The larger the number of items in the vicinity of a point and the higher the weight of the neighboring items, the closer the point is to red. Conversely, the smaller the number of items in the vicinity of a point and the lower the weights of the neighboring items, the closer the point is to blue¹⁶.

The clinical conditions assessed by the articles were organized into a word cloud, where the larger the size of the word, the higher the frequency of research into that condition¹⁷⁻²⁰.

RESULTS

The searches on the WoS-CC retrieved 88,804 articles. The 100 most frequently cited articles were organized in decreasing order according to the number of citations and are listed in Table 2.

Altogether, the articles were cited 41,108 times (mean of 411.09 citations per article), and only 224 (0.54%) were self-citations. The most widely cited article (1,431 citations) was "Specific reading disability (dyslexia): What have we learned in the past four decades?" by Frank R. Vellutino, published in 2004²¹. The articles were published between 1986 and 2017 and most of them were published in the first decade of the 21st century.

Global publication

Most of the 100 most frequently cited articles were published by North American countries (61 articles; 27,101 citations), followed by European countries (37 articles; 13,695 citations). Of these continents, 58 articles, with 24,171 citations, and 31 articles, with 11,253 citations, were published by the USA and England, respectively. No article was published by South American, Middle Eastern, or African countries.

Authorship metrics

Bishop DVN and Tomblin JB were the authors with the largest number of publications. The co-authorship map shows four major clusters, highlighting authors such as Bishop DVN, Tomblin JB, Catts HW, Zhang XY, and Tall P. Collaborations among authors occurred at national and international levels (Fig. 1).

Journal metrics

The most frequently cited articles were published mainly in the *Journal of Speech Language and Hearing Research* (24%), followed by the *Journal of Child Psychology and Psychiatry* (13%), which, after 2004, was the journal in which the largest number of the most frequently cited articles was published.

Study type analysis

Most studies had an observational design (77%), while 40% were cross-sectional, 25% longitudinal, 8% cohort studies, and 4% case-controls, followed by review articles (11%) and meta-analyses (4%).

Table 1. The 100 most frequently cited articles on language disorders among children and adolescents, in decreasing order.

Rank	Article	Number of citations
1	VELLUTINO, Frank R. et al. Specific reading disability (dyslexia): What have we learned in the past four decades?. <i>Journal of child psychology and psychiatry</i> , v. 45, n. 1, p. 2-40, 2004.	1431
2	TOMBLIN, J. B. et al. E., & O'Brien, M.(1997). Prevalence of specific language impairment in kindergarten children. <i>Journal of Speech, Language & Hearing Research</i> , v. 40, n. 6, p. 1245.	1194
3	YOSHINAGA-ITANO, Christine et al. Language of early-and later-identified children with hearing loss. <i>Pediatrics</i> , v. 102, n. 5, p. 1161-1171, 1998.	1100
4	GATHERCOLE, S. E.; BADDELEY, A. D. The role of phonological memory in vocabulary acquisition. Is there a causal connection. <i>Journal of Memory and Language</i> , v. 29, p. 336-360, 1990.	1021
5	TALLAL, Paula et al. Language comprehension in language-learning impaired children improved with acoustically modified speech. <i>Science</i> , v. 271, n. 5245, p. 81-84, 1996.	821
6	MARTINUSSEN, R. M., Hayden, J., & Tannock, R.(2005). A Meta-Analysis of Working Memory Impairments in Children With Attention-Deficit/Hyperactivity Disorder. <i>J. Am. Acad. Child Adolesc. Psychiatry</i> , v. 8.	801
7	STAVONICH, K. E.; SIEGEL, L. S. The phenotypic performance profile of reading-disabled children: A regression-based test of the phonological-core variable-difference model. <i>Journal of Educational Psychology</i> , v. 86, p. 24-53, 1994.	795
8	CATTS, H. W. et al. Dyslexia and specific language impairment: Same or different developmental disorder. <i>Journal of Speech, Language, and Hearing Research</i> , v. 48, p. 1378-1396, 2005.	730
9	MERZENICH, M. Jenkins W, Johnston P, Schreiner C, Miller S, Tallal P. Temporal processing deficits of language-learning impaired children ameliorated by training. <i>Science</i> , v. 271, p. 77-81, 1996.	687
10	MOELLER, Mary Pat. Early intervention and language development in children who are deaf and hard of hearing. <i>Pediatrics</i> , v. 106, n. 3, p. e43-e43, 2000.	682
11	ZATORRE, Robert J.; BELIN, Pascal. Spectral and temporal processing in human auditory cortex. <i>Cerebral cortex</i> , v. 11, n. 10, p. 946-953, 2001.	638
12	DOLLAGHAN, Chris; CAMPBELL, Thomas F. Nonword repetition and child language impairment. <i>Journal of Speech, Language, and Hearing Research</i> , v. 41, n. 5, p. 1136-1146, 1998.	631
13	CATTS, Hugh W. et al. A longitudinal investigation of reading outcomes in children with language impairments. 2002.	577
14	TAGER-FLUSBERG, Margaret M. Kjelgaard Helen; KJELGAARD, M. M. An investigation of language impairment in autism: Implications for genetic subgroups. <i>Language and Cognitive Processes</i> , v. 16, n. 2-3, p. 287-308, 2001.	561
15	NICOLSON, Roderick I.; FAWCETT, Angela J.; DEAN, Paul. Developmental dyslexia: the cerebellar deficit hypothesis. <i>Trends in neurosciences</i> , v. 24, n. 9, p. 508-511, 2001.	533
16	BOTTING, Nicola; CONTI-RAMSDEN, Gina. Non-word repetition and language development in children with specific language impairment (SLI). <i>International Journal of Language & Communication Disorders</i> , v. 36, n. 4, p. 421-432, 2001.	522
17	LEFEVRE, Jo-Anne et al. What counts as knowing? The development of conceptual and procedural knowledge of counting from kindergarten through Grade 2. <i>Journal of experimental child psychology</i> , v. 93, n. 4, p. 285-303, 2006.	516
18	CATTS, Hugh W.; ADLOF, Suzanne M.; WEISMER, Susan Ellis. Language deficits in poor comprehenders: A case for the simple view of reading. 2006.	496
19	RICE, Mabel L.; WEXLER, Kenneth; HERSHBERGER, Scott. Tense over time: The longitudinal course of tense acquisition in children with specific language impairment. <i>Journal of Speech, Language, and Hearing Research</i> , v. 41, n. 6, p. 1412-1431, 1998.	487
20	BELLINI, Scott; AKULLIAN, Jennifer. A meta-analysis of video modeling and video self-modeling interventions for children and adolescents with autism spectrum disorders. <i>Exceptional children</i> , v. 73, n. 3, p. 264-287, 2007.	483
21	BISHOP, Dorothy VM; NORTH, Tony; DONLAN, CHRIS. Nonword repetition as a behavioural marker for inherited language impairment: Evidence from a twin study. <i>Journal of child Psychology and Psychiatry</i> , v. 37, n. 4, p. 391-403, 1996.	470
22	BESS, Fred H.; DODD-MURPHY, Jeanne; PARKER, Robert A. Children with minimal sensorineural hearing loss: prevalence, educational performance, and functional status. <i>Ear and hearing</i> , v. 19, n. 5, p. 339-354, 1998.	462
23	KASARI, Connie; FREEMAN, Stephanny FN; PAPARELLA, Tanya. Early intervention in autism: Joint attention and symbolic play. In: <i>International review of research in mental retardation</i> . Academic Press, 2000. p. 207-237.	458
24	GEURTS, Hilde M. et al. How specific are executive functioning deficits in attention deficit hyperactivity disorder and autism?. <i>Journal of child psychology and psychiatry</i> , v. 45, n. 4, p. 836-854, 2004.	446
25	HERBERT, M. R. et al. Dissociations of cerebral cortex, subcortical and cerebral white matter volumes in autistic boys. <i>Brain</i> , v. 126, n. 5, p. 1182-1192, 2003.	434
26	HUDRY, Kristelle et al. Preschoolers with autism show greater impairment in receptive compared with expressive language abilities. <i>International journal of language & communication disorders</i> , v. 45, n. 6, p. 681-690, 2010.	412
27	LANDA, Rebecca; GARRETT-MAYER, Elizabeth. Development in infants with autism spectrum disorders: a prospective study. <i>Journal of child psychology and psychiatry</i> , v. 47, n. 6, p. 629-638, 2006.	412
28	BISHOP, D. V. et al. 2017, Phase 2 of CATALISE: A multinational and multidisciplinary Delphi consensus study of problems with language development: Terminology. <i>Journal of Child Psychology and Psychiatry</i> , v. 58, n. 10, p. 1068-1080.	404
29	STEIN, John. The magnocellular theory of developmental dyslexia. <i>Dyslexia</i> , v. 7, n. 1, p. 12-36, 2001.	403
30	CHAVARRIGA, Javier et al. Local analytic integrability for nilpotent centers. <i>Ergodic Theory and Dynamical Systems</i> , v. 23, n. 2, p. 417-428, 2003.	402

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Table 1. The 100 most frequently cited articles on language disorders among children and adolescents, in decreasing order.

Rank	Article	Number of citations
31	WILLCUTT, Erik G. et al. Neuropsychological analyses of comorbidity between reading disability and attention deficit hyperactivity disorder: In search of the common deficit. <i>Developmental neuropsychology</i> , v. 27, n. 1, p. 35-78, 2005.	399
32	WING, Leekam. Leekam S. R. Libby S. J., Gould J. and Larcombe M, p. 307-325, 2002.	396
33	MCCLELLAND, Megan M.; MORRISON, Frederick J.; HOLMES, Deborah L. Children at risk for early academic problems: The role of learning-related social skills. <i>Early childhood research quarterly</i> , v. 15, n. 3, p. 307-329, 2000.	395
34	HIRST, Jessica C. Hodgson Rebecca J.; HUDSON, John M. Hemispheric speech lateralisation in the developing. <i>Language</i> , v. 89, p. 362-369, 2016.	393
35	MODY, Maria; STUDDERT-KENNEDY, Michael; BRADY, Susan. Speech perception deficits in poor readers: Auditory processing or phonological coding?. <i>Journal of experimental child psychology</i> , v. 64, n. 2, p. 199-231, 1997.	389
36	CATTS, Hugh W. The relationship between speech-language impairments and reading disabilities. <i>Journal of Speech, Language, and Hearing Research</i> , v. 36, n. 5, p. 948-958, 1993.	388
37	RIQUE, Luciana Degrade et al. Leitura após formação de classes de equivalência em crianças com implante coclear: Precisão e fluência em palavras e textos. <i>Acta Comportamental: Revista Latina de Análisis de Comportamiento</i> , v. 25, n. 3, p. 307-327, 2017.	387
38	LANDERL, Karin; WIMMER, Heinz; FRITH, Uta. The impact of orthographic consistency on dyslexia: A German-English comparison. <i>Cognition</i> , v. 63, n. 3, p. 315-334, 1997.	386
39	TIMLER, Geralyn R. Use of the Children's Communication Checklist—2 for classification of language impairment risk in young school-age children with attention-deficit/hyperactivity disorder. 2014.	383
40	WRIGHT, Beverly A. et al. Deficits in auditory temporal and spectral resolution in language-impaired children. <i>Nature</i> , v. 387, n. 6629, p. 176-178, 1997.	381
41	KRAUS, Nina et al. Auditory neurophysiologic responses and discrimination deficits in children with learning problems. <i>Science</i> , v. 273, n. 5277, p. 971-973, 1996.	379
42	HAPPÉ, Francesca et al. Executive function deficits in autism spectrum disorders and attention-deficit/hyperactivity disorder: examining profiles across domains and ages. <i>Brain and cognition</i> , v. 61, n. 1, p. 25-39, 2006.	377
43	BIRBAUMER, Niels. Breaking the silence: brain-computer interfaces (BCI) for communication and motor control. <i>Psychophysiology</i> , v. 43, n. 6, p. 517-532, 2006.	376
44	EHRI, Linnea C. et al. Phonemic awareness instruction helps children learn to read: Evidence from the National Reading Panel's meta-analysis. <i>Reading research quarterly</i> , v. 36, n. 3, p. 250-287, 2001.	375
45	LAW, James et al. Prevalence and natural history of primary speech and language delay: findings from a systematic review of the literature. <i>International journal of language and communication disorders</i> , v. 35, p. 165-188, 2000.	370
46	SUSAN, Gathercole; SUSAN, Pickering. Working memory deficits in children with low achievements in the national curriculum at seven years of age. <i>British Journal of Educational Psychology</i> , v. 70, n. 2, p. 177-194, 2000.	368
47	CATTS, Hugh W. et al. Are specific language impairment and dyslexia distinct disorders?. 2005.	361
48	RICE, Mabel L.; WEXLER, Kenneth; HERSHBERGER, Scott. Tense over time: The longitudinal course of tense acquisition in children with specific language impairment. <i>Journal of Speech, Language, and Hearing Research</i> , v. 41, n. 6, p. 1412-1431, 1998.	361
49	BURCHINAL, Margaret et al. Threshold analysis of association between child care quality and child outcomes for low-income children in pre-kindergarten programs. <i>Early childhood research quarterly</i> , v. 25, n. 2, p. 166-176, 2010.	358
50	GOSWAMI, U. et al. Proceedings of the National Academy of Sciences. 2002.	344
51	O'BRIEN, Louise M. et al. Neurobehavioral implications of habitual snoring in children. <i>Pediatrics</i> , v. 114, n. 1, p. 44-49, 2004.	343
52	TROUTON, Alexandra; SPINATH, Frank M.; PLOMIN, Robert. Twins early development study (TEDS): a multivariate, longitudinal genetic investigation of language, cognition and behavior problems in childhood. <i>Twin Research and Human Genetics</i> , v. 5, n. 5, p. 444-448, 2002.	343
53	FUCHS, L. S.; FUCHS, D. Compton, DL, Powell, SR, Seethaler, PM, Capizzi, AM, ... Fletcher, JM (2006). The cognitive correlates of third-grade skill in arithmetic, algorithmic, computation, and arithmetic word problems. <i>Journal of Educational Psychology</i> , v. 98, p. 29-43.	341
54	WEISMER, Susan Ellis et al. Nonword repetition performance in school-age children with and without language impairment. <i>Journal of Speech, Language, and Hearing Research</i> , v. 43, n. 4, p. 865-878, 2000.	334
55	REICHOW, Brian; VOLKMAR, Fred R. Social skills interventions for individuals with autism: Evaluation for evidence-based practices within a best evidence synthesis framework. <i>Journal of autism and developmental disorders</i> , v. 40, n. 2, p. 149-166, 2010.	330
56	HILL, Elisabeth L. GOLDSMITHS Research Online. <i>Brain and Cognition</i> , v. 71, p. 99-107, 2009.	325
57	SHRIBERG, Lawrence D.; TOMBLIN, J. Bruce; MCSWEENEY, Jane L. Prevalence of speech delay in 6-year-old children and comorbidity with language impairment. <i>Journal of speech, language, and hearing research</i> , v. 42, n. 6, p. 1461-1481, 1999.	323
58	GEERS, Ann Elizabeth et al. <i>Ear and Hearing</i> . Lippincott Williams & Wilkins, 2003.	318
59	SNOWLING, Margaret J.; GALLAGHER, Alison; FRITH, Uta. Family risk of dyslexia is continuous: Individual differences in the precursors of reading skill. <i>Child development</i> , v. 74, n. 2, p. 358-373, 2003.	317

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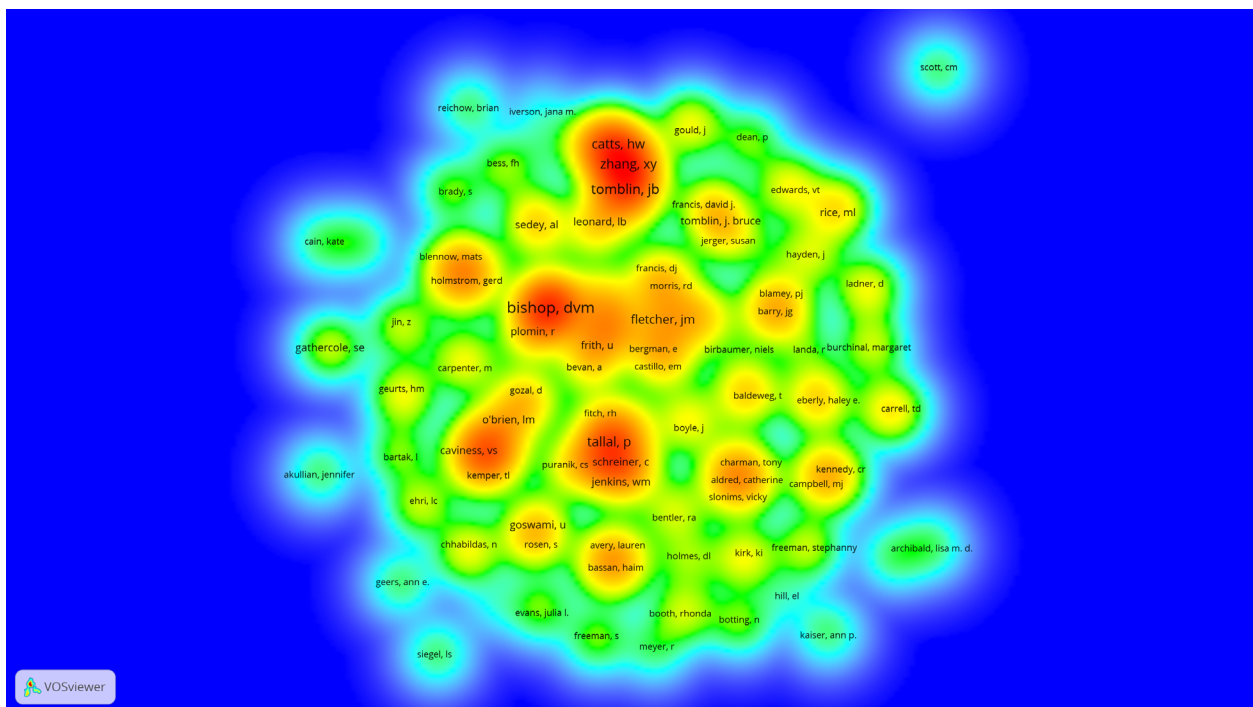
Table 1 The 100 most frequently cited articles on language disorders among children and adolescents, in decreasing order.

Rank	Article	Number of citations
60	LIMPEROPOULOS, Catherine et al. Does cerebellar injury in premature infants contribute to the high prevalence of long-term cognitive, learning, and behavioral disability in survivors?. <i>Pediatrics</i> , v. 120, n. 3, p. 584-593, 2007.	315
61	LEONARD, Laurence B. et al. Speed of processing, working memory, and language impairment in children. 2007.	315
62	NATION, K. és Snowling, MJ (1998): Semantic Processing and the Development of Word-Recognition Skills: Evidence from Children with Reading Comprehension Difficulties. <i>Journal of Memory and Language</i> , v. 39, n. 1, p. 85-101.	314
63	WOLKE, Dieter; MEYER, Renate. Cognitive status, language attainment, and prereading skills of 6-year-old very preterm children and their peers: the Bavarian Longitudinal Study. <i>Developmental medicine and child neurology</i> , v. 41, n. 2, p. 94-109, 1999.	313
64	SCOTT, Cheryl M.; WINDSOR, Jennifer. General language performance measures in spoken and written narrative and expository discourse of school-age children with language learning disabilities. <i>Journal of Speech, Language, and Hearing Research</i> , v. 43, n. 2, p. 324-339, 2000.	311
65	SERENIUS, F. Kållén K, Blennow M, Ewald U, Fellman V, Holmström G, et al. Neurodevelopmental outcome in extremely preterm infants at 2.5 years after active perinatal care in Sweden. <i>Jama</i> , v. 309, n. 17, p. 1810-1820, 2013.	310
66	SIOK, Wai Ting et al. Biological abnormality of impaired reading is constrained by culture. <i>Nature</i> , v. 431, n. 7004, p. 71-76, 2004.	308
67	KENNEDY, Colin R. et al. Language ability after early detection of permanent childhood hearing impairment. <i>New England Journal of Medicine</i> , v. 354, n. 20, p. 2131-2141, 2006.	306
68	GABRIELI, John DE. pufferfish Offline. <i>Science</i> , v. 325, n. 5938, p. 280-283, 2009.	303
69	ARCHIBALD, L. Gathercole S.(2006a). Short-term and working memory in specific language impairment. <i>Int. J. Lang. Commun. Disord</i> , v. 41, p. 675-693.	301
70	MCARTHUR, Genevieve M. et al. On the "specifics" of specific reading disability and specific language impairment. <i>The Journal of Child Psychology and Psychiatry and Allied Disciplines</i> , v. 41, n. 7, p. 869-874, 2000.	299
71	MORRIS, R. et al. Subtypes of reading disability: A phonological core with cognitive variability. <i>Journal of Educational Psychology</i> , v. 90, p. 1-27, 1998.	299
72	MILLER, Carol A. et al. Speed of processing in children with specific language impairment. 2001.	298
73	CATTS, Hugh W. et al. Estimating the risk of future reading difficulties in kindergarten children. 2001.	297
74	BISHOP, Dorothy V. The underlying nature of specific language impairment. <i>Child Psychology & Psychiatry & Allied Disciplines</i> , 1992.	294
75	WEISMER, Susan Ellis; EVANS, Julia; HESKETH, Linda J. An examination of verbal working memory capacity in children with specific language impairment. <i>Journal of Speech, Language, and Hearing Research</i> , v. 42, n. 5, p. 1249-1260, 1999.	289
76	BISHOP, Dorothy VM; NORBURY, Courtenay Frazier. Exploring the borderlands of autistic disorder and specific language impairment: a study using standardised diagnostic instruments. <i>Journal of child Psychology and Psychiatry</i> , v. 43, n. 7, p. 917-929, 2002.	286
77	ROBERTS, Megan Y.; KAISER, Ann P. The effectiveness of parent-implemented language interventions: A meta-analysis. 2011.	285
78	DALE, Philip S. et al. Outcomes of early language delay. 2003.	280
79	BENASICH, April A.; TALLAL, Paula. Infant discrimination of rapid auditory cues predicts later language impairment. <i>Behavioural brain research</i> , v. 136, n. 1, p. 31-49, 2002.	278
80	HERBERT, Martha R. et al. Brain asymmetries in autism and developmental language disorder: a nested whole-brain analysis. <i>Brain</i> , v. 128, n. 1, p. 213-226, 2005.	276
81	SIMOS, Panaqiotis G. et al. Dyslexia-specific brain activation profile becomes normal following successful remedial training. <i>Neurology</i> , v. 58, n. 8, p. 1203-1213, 2002.	276
82	EVANS, Julia L.; SAFFRAN, Jenny R.; ROBE-TORRES, Kathryn. Statistical learning in children with specific language impairment. 2009.	271
83	NICHOLAS, Johanna Grant; GEERS, Ann E. Will they catch up? The role of age at cochlear implantation in the spoken language development of children with severe to profound hearing loss. 2007.	271
84	BISHOP, Dorothy VM; NORTH, Tony; DONLAN, Chris. Genetic basis of specific language impairment: Evidence from a twin study. <i>Developmental Medicine & Child Neurology</i> , v. 37, n. 1, p. 56-71, 1995.	271
85	NATION, Kate et al. Hidden language impairments in children. 2004.	270
86	BIRD, Judith; BISHOP, Dorothy VM; FREEMAN, N. H. Phonological awareness and literacy development in children with expressive phonological impairments. <i>Journal of speech, language, and hearing research</i> , v. 38, n. 2, p. 446-462, 1995.	270
87	DAVIS, Julia M. et al. Effects of mild and moderate hearing impairments on language, educational, and psychosocial behavior of children. <i>Journal of speech and hearing disorders</i> , v. 51, n. 1, p. 53-62, 1986.	269
88	ZIEGLER, Johannes C. et al. Developmental dyslexia in different languages: Language-specific or universal?. <i>Journal of experimental child psychology</i> , v. 86, n. 3, p. 169-193, 2003.	268
89	NORBURY, Courtenay Frazier; BISHOP, Dorothy VM. Narrative skills of children with communication impairments. <i>International journal of language & communication disorders</i> , v. 38, n. 3, p. 287-313, 2003.	268

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Table 1. The 100 most frequently cited articles on language disorders among children and adolescents, in decreasing order.

Rank	Article	Number of citations
90	KASARI, Connie et al. Language outcome in autism: randomized comparison of joint attention and play interventions. <i>Journal of consulting and clinical psychology</i> , v. 76, n. 1, p. 125, 2008.	267
91	LE, Charlop-Christy M. Carpenter M. L LeBlanc LA Kellet K Using the picture exchange communication system (PECS) with children with autism assessment of PECS acquisition, speech, social-communicative behavior, and problem behavior <i>Appl. Behav. Anal.</i> , v. 35, n. 3, p. 213, 2002.	267
92	BLAMEY, Peter J. et al. Relationships among speech perception, production, language, hearing loss, and age in children with impaired hearing. 2001.	267
93	TOMBLIN, J. Bruce; RECORDS, Nancy L.; ZHANG, Xuyang. A system for the diagnosis of specific language impairment in kindergarten children. <i>Journal of Speech, Language, and Hearing Research</i> , v. 39, n. 6, p. 1284-1294, 1996.	261
94	MOELLER, Mary Pat et al. Current state of knowledge: Language and literacy of children with hearing impairment. <i>Ear and hearing</i> , v. 28, n. 6, p. 740-753, 2007.	258
95	SWAN, Denise; GOSWAMI, Usha. Phonological awareness deficits in developmental dyslexia and the phonological representations hypothesis. <i>Journal of experimental child psychology</i> , v. 66, n. 1, p. 18-41, 1997.	258
96	STEVENSON, Ryan A. et al. Multisensory temporal integration in autism spectrum disorders. <i>Journal of Neuroscience</i> , v. 34, n. 3, p. 691-697, 2014.	257
97	TEMPLE, Elise et al. Disrupted neural responses to phonological and orthographic processing in dyslexic children: an fMRI study. <i>Neuroreport</i> , v. 12, n. 2, p. 299-307, 2001.	256
98	CAIN, Kate; OAKHILL, Jane; BRYANT, Peter. Investigating the causes of reading comprehension failure: The comprehension-age match design. <i>Reading and Writing</i> , v. 12, n. 1, p. 31-40, 2000.	254
99	LEONARD, Laurence B. et al. Three accounts of the grammatical morpheme difficulties of English-speaking children with specific language impairment. <i>Journal of Speech, Language, and Hearing Research</i> , v. 40, n. 4, p. 741-753, 1997.	252
100	LIÉGEOIS, Frederique et al. Language reorganization in children with early-onset lesions of the left hemisphere: an fMRI study. <i>Brain</i> , v. 127, n. 6, p. 1229-1236, 2004.	251

**Fig. 1: Co-authorship map.**

There was an editorial (1%) among experimental studies (1%). No case reports or case series were retrieved.

Clinical conditions

The most frequent clinical conditions were autism spectrum disorder (ASD) (18%), followed by dyslexia (14%), specific language impairment (9%), hearing loss (9%), and attention-deficit/hyperactivity disorder (3%).

Area of expertise

Speech-language pathology was the most frequent area of expertise (32%), followed by psychology (27%) and neurosciences (9%). Some articles covered two or more areas, such as neuropsychology and linguistics (3%). No article on dentistry was found (Fig. 2).

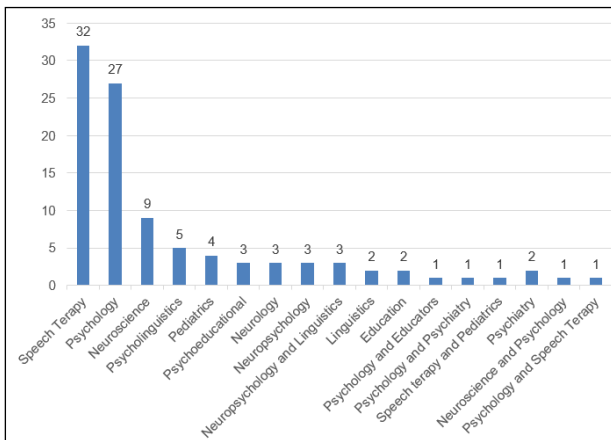


Fig. 2: Area of expertise.

DISCUSSION

Both speech-language pathologists and dentists deal with the functions of the stomatognathic system, so their interdisciplinary work is of the utmost importance to providing successful treatment¹⁷. Interaction between them has increased substantially. The number of citations of scientific articles depends on the year in which the study was submitted for publication, given the profusion of articles over time. Scientific papers are not usually cited until 1–2 years after publication, generally reaching a peak after 3–10 years, after which they continue to be cited at a lower rate. In general, a widely cited article is regarded as a milestone and may therefore have considerable influence on research and practice¹⁸. To become a classic, an article must be cited at least 400 times¹⁹. In the present bibliometric study, 30 articles were cited 400 or more times and four were cited more than 1,000 times.

Self-citations, albeit in a low number, were observed in this study. They can affect bibliometric data because they may be considered inappropriate²⁰. Nevertheless, they are sometimes necessary, as most authors have been researching a topic for a very long time and can provide deeper insights into it. In such cases, self-citations are useful and contribute to the body of knowledge^{10,20}.

The most frequently cited article had 1,431 citations. Its main goal was to provide an updated review of the previous four decades, drawing upon historically influential and contemporary concepts regarding the etiologies of “specific reading disorder” or “dyslexia”²¹. This large number of citations over the years shows that this article has had considerable impact on the understanding of dyslexia, a language disorder that mainly affects the ability to read and write. The fact that it was so often cited was probably because of the influence it can have on people, especially during the literacy development phase²¹.

Europe and North America were the continents with the largest number of articles in the list of the top 100 citations. The United States had the largest number of articles. Some of the world’s leading research centers are in the USA, where large-scale funding is made available to the scientific community²². No publications were retrieved from South America, Africa, or the Middle East. Other bibliometric studies have shown that these continents have linguistic and financial barriers, as well as few partnerships with other institutions and researchers^{23,24}. This scenario shows the need for and importance of collaborative networks among authors from different countries for scientific construction in developing countries, as well as in those with striking differences in culture, history, capacity, and productivity.

The *Journal of Speech Language and Hearing Research* and the *Journal of Child Psychology and Psychiatry* were the journals with the largest number of published articles. The former was founded in 1936 and addresses normal and disordered speech, language, and hearing processes and related areas such as cognition, oral motor function, and swallowing, with significant contributions to the advancement of knowledge (<https://pubs.asha.org/journal/jslhr>). The latter was founded in 1960, with the main objective of selecting research articles on children in different areas of expertise such as psychiatry, psychology, pediatrics, psychoanalysis, social case work, and sociology. It is concerned with developmental psychopathology and child development and has gained additional importance for research and clinical practice, having made numerous contributions, especially to emotional factors with an impact on language disorders (<https://acamh.onlinelibrary.wiley.com/journal/14697610>). Bishop DVN and Tomblin JB were the authors with

the largest number of publications in the field. The studies by Dorothy Bishop, a psychologist from the University of Oxford in the UK, have had an extremely relevant impact on neuropsychology, speech-language pathology, and developmental psychology. Around 1990, she presented numerous findings on topics such as autism spectrum disorder, dyslexia, and specific language impairment, which had been poorly investigated until then. She devised many methods for evaluating children's language, including the test for reception of grammar and the children's communication checklist. Psychology has increasingly contributed to scientific breakthroughs, providing broader understanding of human relationships, and enabling us to rethink and analyze some issues^{25,26}. The second most frequently cited author, James Bruce Tomblin, a psychologist from the University of Iowa, USA, has made remarkable contributions to the epidemiology, etiology, assessment, and treatment of childhood language disorders. He and his colleagues developed a valid and reliable system for diagnosing specific language impairment.

In the bibliometric analysis, the prevalence of observational studies was higher than that of intervention studies. Observational studies are less expensive, more convenient, and easier to perform, especially on children and adolescents²⁷. Most observational studies dealt with etiology and risk or prevalence and incidence, which is expected, given that the investigator in these studies does not interact with the participants, but examines the natural relationships between factors and outcomes²⁸. Etiology and prevalence are still highly investigated, considering that the etiopathogenesis of language disorders is not yet fully understood and that it may involve organic, intellectual/cognitive, and emotional factors (family context), and in most cases, all possible factors are related²⁹.

Autism spectrum disorder (ASD) was the most frequently addressed clinical condition. It is characterized by various alterations in language development (alterations of speech, echolalia, and mutism, among others)^{1,30}. The fact that this was the most frequently addressed topic in this bibliometric study shows how concerned global research is with its elucidation. Studies have reported high worldwide prevalence of ASD, which affects 62 out of every 10,000 individuals, with higher prevalence among male children³¹. Autism is still heatedly

debated worldwide and has come under the spotlight because of its different clinical manifestations, high incidence, and the concern regarding its impact on child development¹. The multidisciplinary approach is of the utmost importance in cases of ASD¹.

Speech-language pathology (32%) and psychology (27%) were the areas under which most studies were published. Both fields seek to understand the behavior of language. Human behavior, emotional factors, stress, anxiety, and depression interfere with language development, and here, the role of both psychologists and speech-language pathologists is important^{25,31}. Clinical manifestations of behavior and human development in different age groups were the target of these two main fields in this bibliometric analysis. Psychology seeks to understand human behavior and its complexities, whereas speech-language pathology seeks to prevent and treat disorders that affect human communication skills^{25,31,32}.

The current bibliometric study did not retrieve any publications in the field of dentistry. A child's first contact with healthcare professionals often involves pediatricians and pediatric dentists³³. Pediatric dentists address different clinical conditions and should endeavor to understand the patients' language development in addition to their initial complaint.

The need for a multidisciplinary approach to healthcare means that the roles of all professionals are crucial to providing comprehensive, humane care³⁴. This is especially true regarding children and adolescents with language disorders, because they do not easily understand instructions, which could compromise their general health. Although language disorders have been widely studied in the past few decades, and despite the large number of pediatric dentistry articles published on oral health comorbidities^{33,35,36}, the paucity of studies on language disorders shows the need for further research.

The present bibliometric study has some limitations such as the fact that it included only research papers, case reports, case series, and review articles, leaving out a sizable number of important books or book chapters and conference proceedings. As this was a bibliometric analysis rather than a systematic review, it did not evaluate the methodology, quality, or risk of bias of the studies. The fact that the 100 most frequently cited articles on language disorders did not address dental problems does not indicate

a lack of integration between the areas, but only that they were not among the most frequently cited articles. Nevertheless, it is essential to encourage dental researchers to investigate language disorders.

CONCLUSION

To conclude, the analysis of the 100 most widely cited articles on language disorders provides better understanding of children's language development and shows the major characteristics of important articles published in this field. Most of the studies were performed in North America. Bishop DVN (UK) and Tomblin JB (USA) were the most

frequently cited authors, and the *Journal of Speech Language and Hearing Research* presented the highest number of publications. Most studies on ASD had an observational design. Speech-language pathology was the most prevalent area of expertise, and there was no collaboration with the dental field that involved children and adolescents. Therefore, our findings demonstrate that dental researchers should be encouraged to investigate language disorders, and the interplay between speech-language pathology and dentistry should be strengthened and worked on further.

ACKNOWLEDGMENT

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Relationship between periapical lesions and sinus changes on multi-slice computed tomography scan

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ABSTRACT

Differentiating orofacial odontogenic pain/disorders from pain/disorders associated with maxillary sinusitis is important to avoid unnecessary dental procedures and to properly refer patients to colleagues/dentists and vice versa. Aim: To analyze the association between apical lesions and sinus changes and to evaluate the agreement between the diagnoses of an endodontist, a radiologist, an oral and maxillofacial surgeon, and an otolaryngologist. Materials and Method: 385 axial, coronal, and sagittal MSCCT scans were selected using an image archiving and communication system (PACS). The examinations had been performed between 2018 and 2022. Results: Apical lesions were observed in 36.10% of sinusitis cases, 73.8% of unilateral sinusitis cases, 48.7% of sinus floor discontinuity cases, and 67.2% of cases in which endodontic treatment had been performed. Agreement between the diagnoses made by the endodontist and those made by the other investigators was high for most study variables ($k > 0.60$). The exceptions were mucosal thickening, for which agreement between the endodontist and the other investigators was intermediate ($k=0.397$), and the presence of periapical lesions ($k=0.010$), previous endodontic treatment ($k=0.013$), and mucosal thickness ($k=0.024$), for which agreement between endodontists and radiologists was low. Conclusions: There was an association between sinus changes and apical lesions.

Keywords: differential diagnosis - maxillary sinus - periapical periodontitis - sinusitis - X ray computed tomography

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Relação entre lesões periapicais e alterações sinusais na tomografia computadorizada multislice

RESUMO

Diferenciar a dor/desordens odontogênicas orofaciais da dor/desordens associadas à sinusite maxilar é importante para evitar procedimentos odontológicos desnecessários e para encaminhar adequadamente os pacientes aos colegas/dentistas e vice-versa. Objetivo: Analisar a associação entre lesões apicais e alterações sinusais e avaliar a concordância entre os diagnósticos de um endodontista, um radiologista, um cirurgião bucomaxilofacial e um otorrinolaringologista. Material e Método: foram avaliadas 385 imagens. Resultados: As lesões apicais foram observadas em 36,10% dos casos de sinusite, em 73,8% dos casos de sinusite unilateral, em 48,7% dos casos de descontinuidade do assoalho do seio e em 67,2% dos casos em que o tratamento endodôntico havia sido realizado. A concordância entre os diagnósticos feitos pelo endodontista e os feitos pelos outros pesquisadores foi alta para a maioria das variáveis do estudo ($k > 0,60$). As exceções foram o espessamento da mucosa, para o qual a concordância entre o endodontista e os outros pesquisadores foi intermediária ($k=0,397$) e a presença de lesões periapicais ($k=0,010$), tratamento endodôntico prévio ($k=0,013$) e espessura da mucosa ($k=0,024$), para os quais a concordância entre endodontistas e radiologistas foi baixa. Conclusões: Houve uma associação entre as alterações sinusais e as lesões apicais.

Palavras-chave: diagnóstico diferencial -seio maxilar - periodontite periapical - sinusite - tomografia computadorizada por raios X



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INTRODUCTION

Diffuse posterior maxillary pain and certain abnormalities can be difficult to diagnose given the close anatomic relationship between the maxillary sinus and the posterior maxillary teeth¹. It is important to distinguish orofacial odontogenic pain/disorders from pain/disorders associated with maxillary sinusitis, to avoid unnecessary dental procedures and properly refer patients to colleagues/dentists and vice versa.

Periapical infection is one of the most important etiologic factors responsible for sinus abnormalities of odontogenic origin². The development of periapical lesions on teeth that are close to or even in contact with the maxillary sinus can cause inflammatory changes in the sinus mucosa and lead to the onset of sinusitis³. After the onset of pulpal necrosis, bacterial virulence factors such as enzymes and lysosomal toxins promote bacterial invasion and tissue breakdown in the periapical bone⁴. The resulting inflammation can lead to rupture of Schneiderian membrane⁵, causing dental infection and the spread of its byproducts into the maxillary sinuses. This process impairs mucociliary function in the maxillary sinuses and irritates the maxillary sinus mucosa⁶. As the bacteria reach the maxillary sinus and sinusitis sets in⁷, symptoms progressively worsen.

The prevalence of odontogenic maxillary sinusitis is often underestimated by medical radiologists and otolaryngologists because they are unaware of the association between apical disease and odontogenic sinusitis. Consequently, this association is rarely investigated in routine clinical practice¹.

Studies based on cone beam computed tomography (CBCT) images have demonstrated positive association between odontogenic sinusitis and the presence of periapical or periodontal lesions. The prevalence rates of this association range from 10% to 86% of sinusitis cases^{8,9}. Accurate diagnosis of the odontogenic origin of maxillary sinusitis by all professionals involved is crucial for effective management of this condition. It is important to note that the recommended treatment of odontogenic sinusitis differs significantly from those for other forms of maxillary sinusitis⁷. A comprehensive approach therefore needs to be used, including detailed anamnesis, complete physical examination, and imaging studies such as intraoral (periapical and occlusal) radiographs, extraoral (panoramic and Waters incidence) radiographs, and CBCT¹⁰.

CBCT provides a three-dimensional view of the affected anatomical structures in the axial, sagittal, and coronal planes, enabling more accurate assessment of the maxillary sinus, posterior teeth, and surrounding structures than do other imaging modalities¹. CBCT offers high-resolution imaging in multiple planes and eliminates overlap of maxillary molars, enabling detailed examination of the patient's maxillary sinus anatomy and detection of sinus inflammation¹¹.

While other tests such as magnetic resonance imaging, ultrasound, endoscopy, and scintigraphy may also be indicated, CBCT is the most commonly used and preferred method due to its high quality and ability to provide an accurate assessment of sinus anatomy, sinus mucosa lesions, bone structures, presence of air in the sinuses, and anatomic changes of the sinuses¹².

During the past decade, CBCT has gained popularity as a valuable technique for dentomaxillofacial images. However, multi-slice computed tomography images (MSCT) remain more familiar to radiologists and the medical profession. MSCT replaced conventional radiography as the gold standard for facial sinus examination many years ago and continues to be the preferred imaging modality in cases with suspected complicated sinusitis. This is due to the need to evaluate the soft tissues surrounding the sinuses to rule out orbital or intracranial complications, regardless of whether the examination is conducted with or without intravenous contrast material¹³.

Nevertheless, there are few studies in the literature that have explored the relationship between apical lesions and paranasal sinus disease using MSCT¹³. Therefore, the aim of this study was to investigate the relationship between periapical lesions and paranasal sinus changes on multi-slice computed tomography scans, as well as the concordance between diagnoses made by an endodontist, a radiologist, an oral and maxillofacial surgeon, and an otolaryngologist.

MATERIALS AND METHOD

This retrospective observational study was approved by the local research ethics committee (number 4.601.417) and conducted in accordance with the requirements of resolution 196/96 of the National Health Council. Examination reports and images were retrieved from a private medical diagnostic

radiology service and analyzed after obtaining written permission from the technical director of the service. The selected images were analyzed after careful anonymization of the patients.

Selection of images

A total 385 axial, coronal, and sagittal MSCT scans were selected using an image archiving and communication system (PACS). The examinations had been performed between 2018 and 2022. Inclusion criteria were MSCT examinations of patients aged 20 to 65 years, of either sex, with all first and second premolars, at least one of the maxillary first and second molars, and fully erupted teeth with fully developed roots. Images of edentulous patients, images with questionable tips of posterior teeth, images of patients with an orthodontic retainer, bone abnormalities, or suspected tumors around the zone of interest were excluded.

Calculation of the sample size required to determine the association between the presence of periapical lesions and sinus disease was based on previous studies^{4,7,14}. Sample size was estimated using the SurveyMonkey Audience program (<https://pt.surveymonkey.com/mp/sample-size-calculator/>; Momentive.ai, San Mateo, CA, USA), considering a 95% confidence level and a 5% margin of error, resulting in a minimum size of 385 images.

Image assessment

Images were analyzed independently by four previously trained and calibrated examiners: an endodontist, a radiologist, an oral and maxillofacial surgeon, and an otolaryngologist, all of whom had extensive experience in radiology. OsiriX Lite software (Pixmeo, Bernex, Switzerland) was used to evaluate images in DICOM (Digital Imaging and Communications in Medicine) format. The variables evaluated were the presence of an apical lesion or bone thickening (no/yes), the location of the sinus change (bilateral/unilateral), contact of the lesion with the sinus floor (absent/present), discontinuity of the sinus floor (absent/present), mucosal thickening (absent/present), thickness of mucosa (1, 2, or 3 cm), opacity (absent/present), tooth involvement (both, molars, premolars, or neither), and previously performed endodontic treatment (no/yes). Data were recorded individually in a spreadsheet and the degree of agreement between investigators was assessed.

Statistical analysis

The mean age of patients with and without periapical lesion was compared using Student's t-test. The association between the presence of an apical lesion and the other variables of interest was evaluated using the Pearson chi-square test with Bonferroni correction, the Pearson chi-square test, and Fisher's exact test. Agreement between the diagnoses made by the endodontist and those made by the other investigators was assessed using the Kappa agreement test. Statistical analyses were performed using the software IBM SPSS (version 26.0, IBM Corporation, Armonk, NY, USA) with a significance level of 5%.

RESULTS

The mean age of patients with an apical lesion was higher than that of patients without an apical lesion (41.81 ± 10.92 versus 38.58 ± 8.74 ; $p = 0.003$). Apical lesions were observed in 36.10% of all cases of maxillary sinusitis, 73.8% of cases of unilateral maxillary sinusitis, 48.7% of cases of sinus floor discontinuity, and 67.2% of cases with previous endodontic treatment (Fig. 1, Table 1).

Agreement between diagnoses made by the endodontist and those made by the radiologist, oral and maxillofacial surgeon, and otolaryngologist was substantial for most study variables ($k > 0.60$), except for mucosal thickening, for which agreement between endodontists and the other investigators was intermediate ($k = 0.397$); and presence of a periapical lesion, previous endodontic treatment, and mucosal thickness, for which agreement between endodontists and radiologists was low ($0 < k < 0.20$; Table 2).

DISCUSSION

The purpose of this study was to determine the presence of apical disease and its association with sinus disease on MSCT scans. This finding of this study confirms the observations made by numerous authors^{9,15,16} regarding the direct impact of periapical lesions on the maxillary sinuses. Factors such as the size of the lesion its proximity to the maxillary sinus^{9,17}, the presence or absence of previous endodontic treatment^{14,18}, and the anatomic relationship between the maxillary sinus and the maxillary posterior teeth⁷ play a role in this association.

One study¹⁷ analyzed the frequency of anatomic changes and pathologic findings in the maxillary

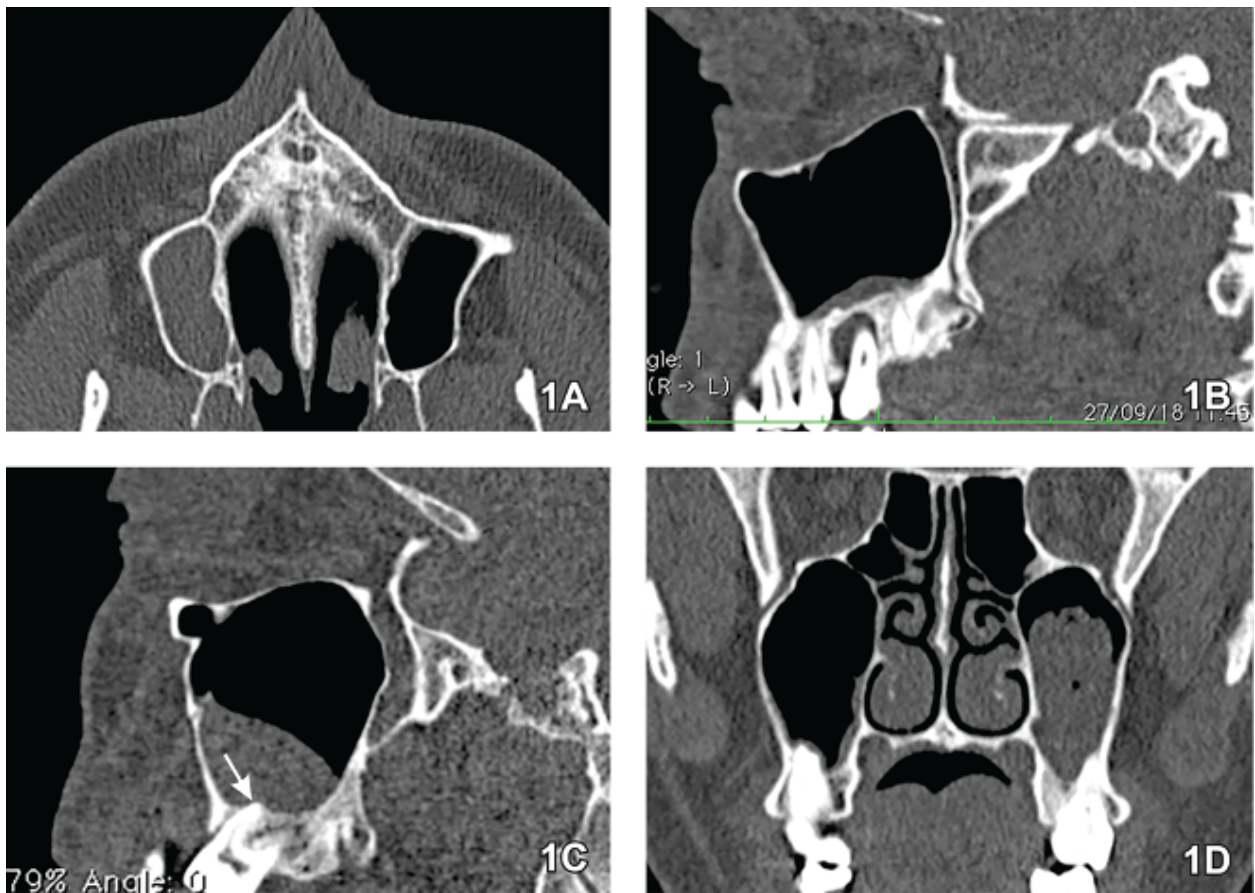


Fig. 1: Representative images of the MSCT evaluated in the study. A) Axial section. Unilateral maxillary sinusitis with total opacification of the right maxillary sinus. B) Sagittal section. Alveolar bone resorption surrounding the apices of the maxillary second molar, with involvement of the maxillary sinus. C) Sagittal section. Discontinuity of the maxillary sinus floor related to the apex of the buccal root of the maxillary molar, with resorption of the cortical bone (arrow). D) Coronal section. Intense opacification of the left maxillary sinus. Note the close contact of the maxillary molar roots with the sinus floor.

sinuses of patients undergoing CBCT. The prevalence of maxillary sinus disease ranged from 7.5% to 66%, with mucosal thickening and opacification, or sinusitis, being the most common conditions observed. These broad findings reflect the wide variation in the scientific literature regarding the definition of maxillary sinusitis. Another study¹⁹ defined sinusitis as any obvious thickening of the maxillary sinus mucosa. However, this definition does not distinguish between sinogenic and odontogenic causes of sinusitis, and clinical findings alone are generally insufficient to make this distinction. Therefore, imaging examination of the maxillary sinuses under various conditions is recommended for an accurate diagnosis².

Different radiographic techniques are used to diagnose maxillary sinus mucosal thickening and apical periodontitis, including conventional radiography of the face sinuses, magnetic resonance

imaging, CBCT, and conventional periapical radiography⁹. Among these options, CBCT provides more detailed information about changes in the maxillary sinus^{20,21}, and high-resolution images that enable accurate assessment of the maxillary sinuses, teeth, and adjacent tissues in all planes, as well as the relationships between these structures^{4,14}. However, in the present study, MSCT images were chosen for evaluation by different healthcare professionals because these images are considered the gold standard for assessing the soft tissues surrounding the sinuses¹³.

According to one study²¹, odontogenic infection was unilateral in approximately 70% of cases of patients treated for sinusitis, which is consistent with the findings of the present study (73.8%). Furthermore, the present study revealed that patients with an apical lesion were on average older (41.81 ± 10.92) than those without apical lesion (38.58 ± 8.74 ; p

Table 1. Analysis of the association between the presence of apical lesion and the other variables of interest.

Variable		Apical lesion		p-value
		No	Yes	
Location of sinus change	Bilateral	209(85.7%)	35 (14.3%)	< 0.001**
	Unilateral	37 (26.2%)	104(73.8%)	
Lesion contact with the sinus floor	Absent	40 (52.6%)	36 (47.4%)	0.001*
	Present	206(66.6%)	103(33.4%)	
Discontinuity of the sinus floor	Absent	125(83.9%)	24 (16.1%)	< 0.001*
	Present	121(51.3%)	115(48.7%)	
Mucosal thickening	Absent	2 (50.0%)	2 (50.0%)	0.278*
	Present	244(63.9%)	13(36.1%)	
Thickness of the mucosa	1 cm	78 (67.2%)	38 (32.8%)	0.465**
	2 cm	107(64.5%)	59 (35.5%)	
	3 cm	61 (59.2%)	42 (40.8%)	
Opacification	Absent	3 (60.0%)	2 (40.0%)	0.081*
	Present	243(63.9%)	137(36.1%)	
Tooth involved	Both	18 (50.0%)	18 (50.0%)	0.001*
	Molar	148(60.7%)	96 (39.3%)	
	Premolar	80 (77.7%)	23 (22.3%)	
	None	0 (0.0%)	2 (100.0%)	
Endodontic treatment	No	226(69.7%)	98 (30.3%)	< 0.001**
	Yes	20 (32.8%)	41 (67.2%)	

* Pearson's chi-square test with Bonferroni correction; ** Pearson's chi-square test; *** Fisher's exact test. Level of significance = 5%

= 0.003), confirming the results of other studies^{5,9}. This can be attributed to the fact that increasing age increases the likelihood of dental diseases, particularly periodontal diseases, apical abscesses, missing teeth, or other pathologic conditions, which also increases the risk of maxillary sinusitis²². It is estimated that dental origin accounts for 10% to 12% of all cases of maxillary sinusitis¹⁷. The present study demonstrated a positive correlation between apical lesion and thickening of the maxillary sinus mucosa, which is consistent with previous studies^{8,9}. Earlier studies reported prevalence rates of sinus mucosal thickening ranging from 37% to 62%^{9,23,24}. In the present study, mucosal thickening was observed in 36.1% of patients with periapical lesions, in agreement with Block and Dastoury²⁵ and Souza-Nunes³, who reported rates of 36.8% and 38.19%, respectively.

This discrepancy in prevalence rates can be attributed to differences in inclusion criteria and diagnostic techniques. In the present study, the thickness of the

maxillary sinus mucosa was recorded at three levels, although there is still no consensus on what mucosal thickness should be considered pathological. Various authors have defined pathologic thickening as ≥ 1 mm³¹, ≥ 2 mm¹³ or > 3 mm²³. Mucosal thickening greater than 2 mm (grades 2 and 3), with or without a periapical lesion, was found in 269 (69.8%) of patients in the present study. Additionally, the prevalence of mucosal thickening greater than 2 mm with a periapical lesion was 26.2% of patients, which can be considered relatively low compared to the 42.1% rate found by another study⁷.

The association between mucosal thickening and periapical lesions in the maxillary posterior region is attributed to their anatomic proximity. Another study reported that odontogenic sinusitis most commonly involves the maxillary first molars, followed by maxillary second molars and maxillary first premolars⁷. Therefore, both maxillary premolars and maxillary molars were examined in the present study. Maxillary first molars, being the first permanent teeth to erupt, are more prone to caries, pulp disease and surgical procedures. This, along with their anatomy, may explain their higher prevalence as an etiologic factor of maxillary sinusitis¹⁵.

The anatomical proximity between the maxillary sinus and the apices of maxillary teeth can result in inflammatory changes in the mucosa that may remain asymptomatic and persist for months or even years if the affected tooth is left untreated^{2,5}. Another study²⁶ found that the risk of odontogenic sinusitis decreased by up to 2.5-fold when the location of the endodontically infected tooth moved away from the maxillary sinus ($p < 0.05$).

Subsequently, the affected mucosa becomes more susceptible to infection, becoming a risk factor for the development of sinusitis. Sinus inflammation may be limited to the floor of the maxillary sinus, such as in osteoperiostitis or mucositis, or may also progress and cause partial or complete obstruction of the maxillary sinus due to mucous secretions and inflammatory exudate, exhibiting clinical and radiographic features resembling those of sinogenic sinusitis². The presence of vital pulp excludes the possibility of sinusitis of endodontic origin since the tooth must have necrotic pulp or an unsuccessful root canal treatment².

After pulp necrosis occurs, potent bacterial virulence factors promote bacterial invasion and tissue

Table 2. Analysis of inter-examiner agreement regarding the diagnoses performed by the endodontist versus those performed by the radiologist, oral and maxillofacial surgeon and otorhinolaryngologist based on the assessment of MSTC.

Variable		Endodontist			Kappa
		No	Yes		
Apical lesion		No	Yes		
Radiologist	No	130	75		0.010
	Yes	116	64		
OMF surgeon	No	246	4		0.977
	Yes	0	135		
OL physician	No	246	4		0.977
	Yes	0	135		
Location of sinus change		Bilateral	None	Unilateral	
Radiologist	Bilateral	242	0	1	0.983
	None	0	0	0	
	Unilateral	2	0	140	
OMF surgeon	Bilateral	244	0	0	0.983
	None	0	0	3	
	Unilateral	0	0	138	
OL physician	Bilateral	244	0	0	0.983
	None	0	0	3	
	Unilateral	0	0	138	
Lesion contact with the sinus floor		Absent	Present		
Radiologist	Absent	68	3		0.176
	Present	7	305		
OMF surgeon	Absent	76	0		1.000
	Present	0	309		
OL physician	Absent	76	0		1.000
	Present	0	309		
Discontinuity of the sinus floor		Absent	Present		
Radiologist	Absent	149	235		0.747
	Present	1	0		
OMF surgeon	Absent	143	5		0.940
	Present	6	231		
OL physician	Absent	143	5		0.940
	Present	6	231		
Mucosal thickening		Absent	Present		
Radiologist	Absent	2	0		0.397
	Present	2	381		
OMF surgeon	Absent	2	0		0.397
	Present	2	381		
OL physician	Absent	2	0		0.397
	Present	2	381		
Thickness of the mucosa		1 cm	2 cm	3 cm	
Radiologist	1 cm	36	51	38	0.024
	2 cm	30	72	37	
	3 cm	50	43	28	

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Table 2. Analysis of inter-examiner agreement regarding the diagnoses performed by the endodontist versus those performed by the radiologist, oral and maxillofacial surgeon and otorhinolaryngologist based on the assessment of MSTC.

Variable		Endodontist				Kappa
OMF surgeon	1 cm	116	0	0		0.996
	2 cm	0	165	0		
	3 cm	0	1	103		
OL physician	1 cm	116	0	0		0.996
	2 cm	0	165	0		
	3 cm	0	1	103		
Opacification		Absent	Present			
Radiologist	Absent	0	5			0.855
	Present	15	365			
OMF surgeon	Absent	5	0			1.000
	Present	15	365			
OL physician	Absent	5	0			1.000
	Present	15	365			
Tooth involved		Both	Molar	Premolar	None	
Radiologist	Both	36	1	0	0	0.990
	Molar	0	242	0	0	
	Premolar	0	1	103	0	
	None	0	0	0	2	
OMF surgeon	Both	36	0	0	0	1.000
	Molar	0	244	0	0	
	Premolar	0	0	103	0	
	None	0	0	0	2	
OL physician	Both	36	0	0	0	1.00
	Molar	0	244	0	0	
	Premolar	0	0	103	0	
	None	0	0	0	2	
Previous endodontic treatment		No	Yes			
Radiologist	No	273	51			0.013
	Yes	51	11			
OMF surgeon	No	324	0			1.000
	Yes	0	61			
OL physician	No	324	0			1.000
	Yes	0	61			

Inter-examiner agreement test (Kappa test). Level of significance = 5%.

breakdown in the periapical bone^{4,9}. Consequently, dental infections and their byproducts may spread to the maxillary sinuses and irritate the sinus mucosa. Odontogenic sinusitis is more likely to involve anaerobic bacteria²⁷, making any antibiotics used to treat normal sinusitis less effective in odontogenic cases. It is therefore crucial to diagnose the cause of sinusitis accurately before starting treatment⁹. In

addition, imaging findings should be correlated with clinical information to ensure an accurate diagnosis and appropriate treatment of sinusitis¹⁴. Patients with periapical lesions and possibly associated mucosal thickening in the sinus floor should be referred to an endodontist for evaluation, even if they are asymptomatic².

All sinusitis cases investigated in the present study

were reported as non-odontogenic, neglecting the possibility of endodontic involvement. This observation highlights the importance of incorporating the endodontics discipline into treatment planning for maxillary sinus disease, not only to preserve teeth and promote oral cavity health, but also to address the health of other areas, including the maxillary sinuses. Further research is needed to investigate both imaging and clinical aspects to provide more comprehensive information for dental and medical professionals.

In conclusion, this study, within its limitations, demonstrates a clear association between maxillary

sinus changes and apical lesions in the maxillary premolars and molars as observed through MSCT. There was also overall agreement among the diagnoses made by the endodontist and other specialists for most sinus and tooth changes. However, there were disagreements between the endodontist and the radiologist regarding the presence of a periapical lesion, previous endodontic treatment, and mucosa thickness. These results underscore the importance of interdisciplinary collaboration among healthcare professionals to ensure precise diagnosis and effective treatment of maxillary sinusitis.

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Effect of different design and surface treatment on the load-to-failure of ceramic repaired with composite

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ABSTRACT

Glass ceramics are widely used to manufacture esthetic veneers, inlays, onlays, and crowns. Although the clinical survival rates of glass-ceramic restorations are favorable, fractures or chips are common. Certain cases can be repaired with direct composite. **Aim:** The aim of this study was to investigate the interaction effect of different designs and surface treatments on the load-to-failure of lithium disilicate glass-ceramic repaired with nanofilled composite. **Materials and Method:** Lithium-disilicate glass-ceramic slabs (IPS e.max Press, Ivoclar Vivadent) with three different designs of the top surface (flat, single plateau, or double plateau) (n=11) received 'no treatment', '5% HF etching', or "Al₂O₃ sandblasting". HF-etched and sandblasted slabs also received silane and universal one-step adhesive application. All slabs were incrementally repaired with nanofilled composite (Filtek Z350, 3M ESPE) up to 6 mm above the highest ceramic top plateau. Specimens were stored in artificial saliva at 37 °C for 21 days and then subjected to 1,000 thermocycles between 5 and 55 °C. The interface composite-ceramic of each specimen was tensile tested until failure in a universal testing machine and the mode of failure was determined under a stereomicroscope. The ceramic surface morphology of one representative tested specimen from each subgroup (design/surface treatment) was observed through scanning electron microscopy (SEM). **Results:** Regardless of ceramic design, the absence of surface treatment resulted in significantly lower load-to-failure values. No significant differences in load-to-failure values were observed between HF-etched and sandblasted specimens for the flat design; however, HF etching resulted in significantly higher load-to-failure values than sandblasting for both single plateau and double plateau designs. The majority (60%) of HF-etched specimens with single plateau or double plateau presented mixed failures. SEM photomicrographs showed that HF-etched specimens had smoother surfaces than sandblasted specimens. **Conclusion:** The surface treatment of a defective lithium disilicate glass-ceramic restoration has more influence than its macroscopic design on the retention of the composite repair. HF etching seems to provide higher bond strength to the composite repair.

Keywords: dental restoration repair - composite repair - ceramic repair - hydrofluoric acid - sandblasting

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RESUMO

Embora fraturas e lascamento de restaurações vitrocerâmicas sejam comuns, alguns casos podem ser reparados com compósito direto. **Objetivo:** investigar o efeito da interação de diferentes formas e tratamentos de superfície na carga de ruptura de uma vitrocerâmica reforçada com dissilicato de lítio reparada com compósito nanoparticulado. **Materiais e Método:** A superfície superior de espécimes de vitrocerâmica (IPS e.max Press, Ivoclar Vivadent) foi preparada com três formas (plana, platô único, ou duplo) e recebeu (n=11): 'nenhum tratamento', 'condicionamento com ácido hidrófluorídrico 5%', ou 'jateamento com Al₂O₃'. Ambos espécimes condicionados e jateados receberam silano e adesivo universal. Todos os espécimes foram reparados incrementalmente com compósito (Filtek Z350, 3M ESPE) até 6 mm acima do platô cerâmico mais alto, armazenados em saliva artificial à 37 °C por 21 dias, e submetidos à 1.000 termociclos (5 e 55 °C). A interface compósito-cerâmica de cada amostra foi testada à tração até sua falha em máquina universal e o modo de falha foi determinado com estereomicroscópio. A morfologia da superfície de uma amostra representativa de forma/tratamento de superfície foi observada através de microscopia eletrônica de varredura (MEV). **Resultados:** Independentemente da forma cerâmica, a ausência de tratamento superficial resultou em valores de carga de ruptura significativamente menores. Não foi observada diferença significativa entre os espécimes planos condicionados ou jateados; no entanto, o condicionamento resultou em valores significativamente maiores que o jateamento para espécimes com platô único e duplo. A maioria (60%) dos espécimes condicionados e com platô único ou duplo apresentou falhas mistas. Imagens SEM demonstraram rugosidade superficial mais regular dos espécimes condicionados que os jateados. **Conclusões:** O tratamento superficial de uma restauração defeituosa de vitrocerâmica reforçada por dissilicato de lítio tem maior influência na retenção do reparo de compósito do que sua forma macroscópica; ainda, o condicionamento com ácido hidrófluorídrico parece proporcionar maior resistência de união ao reparo com compósito.

Palavras-chave: reparo de restauração dental; reparo com compósito; reparo cerâmico; ácido hidrófluorídrico; jateamento.

INTRODUCTION

Glass ceramics are widely used to manufacture esthetic veneers, inlays, onlays, and crowns¹. However, they are brittle and often become cracked or chipped due to secondary caries, trauma, parafunctional habits, manufacturing flaws, or stress concentration induced by occlusal adjustments²⁻⁵. Several factors such as cost, time, wear of sound tooth structure, and risk to pulp vitality must be considered before replacing a defective ceramic restoration⁶. Removing restorations luted with adhesive inevitably enlarges the new preparation and weakens the tooth^{7,8}. However, certain cases can be repaired with direct composite, which is a minimally invasive, low-cost, less time-consuming procedure^{2,5}.

The advantages of restorative repair have been routinely included for more than 10 years in most European and North American dental school syllabuses⁹⁻¹¹. Some longitudinal clinical trials indicate that repaired composite restorations can remain clinically acceptable for up to 12 years¹²⁻¹⁵. Despite the lack of long-term evidence, the repair of ceramic restorations has shown a success rate of 89% and a survival rate of 3 years, which makes the approach feasible in certain cases¹⁶.

Several factors, including ceramic type, composite type, aging condition, and surface treatment protocol can influence the composite repair bond strength to ceramic restorations⁶. The success of adhesion depends on the roughness of the surface to which the composite is bonded²¹. Different protocols for intraoral repair of chipped and/or fractured ceramic restorations have been suggested to increase the bond strength to the composite: roughening by diamond burs²², etching with hydrofluoric acid (HF)^{23,24} sandblasting with aluminum oxide (Al₂O₃) microparticles¹⁵, laser irradiation, and tribochemical silica coating²⁵. HF etching has been widely reported as a reliable extraoral surface treatment for glass-ceramic restorations prior to adhesive luting²⁶. However, in an intraoral repair scenario, HF is highly toxic and may cause severe damage to oral tissues, and its use is forbidden in dental clinics in several countries²⁷. Although lower bond strength values have been reported, sandblasting with Al₂O₃ microparticles at adequate pressure does not harm soft tissues nor decrease the flexural strength of lithium disilicate glass-ceramics²⁸.

Though most studies investigate the effect of

microscopic changes promoted by different surface treatments on the ceramic surface to be repaired, it would be also relevant to address the question of whether the macroscopic design of the ceramic restoration could affect adhesive bonding to the composite repair. The aim of this study was therefore to investigate the interaction effect of different designs and surface treatments on the load to failure of lithium disilicate glass-ceramic repaired with nanofilled composite. The null hypothesis was that different macroscopic ceramic designs did not influence the load-to-failure of composite repairs, regardless of different previous surface treatments.

MATERIALS AND METHOD

Ceramic slabs 4 mm thick (10 x 10 mm) with three different designs of the top surface (flat, single 2-mm-deep plateau, or double 2-mm-deep plateau) were prototyped virtually using computer aided-design software, milled in wax using a computer aided-manufacturing unit, invested, and then heat-pressed with lithium disilicate glass-ceramic ingots (IPS e.max Press, Ivoclar Vivadent, Liechtenstein). The injection sprues were removed with diamond burs (#881 and #881F, Jota do Brasil, Florianópolis, SC, Brazil) mounted on a high-speed water-cooled air turbine, and the ceramic slabs were cleaned ultrasonically in distilled water for 30 sec (Fig. 1).



Fig. 1: Ceramic slabs with different macroscopic designs: flat, single plateau, and double plateau.

The ceramic slabs of each design were assigned to subgroups (n=11) according to the following top surface treatments:

- No treatment.
- Etching with 5% HF (Condac Porcelain, FGM, Joinville, SC, Brazil) for 20 sec, followed by water rinsing and air-drying. One layer of silane (Relyx Ceramic Primer, 3M ESPE, Saint Paul MN, USA) was applied and air-dried after 60 sec. Then, one layer of a universal one-step adhesive

(Single Bond Universal, 3M ESPE, Saint Paul, MN, USA) was applied for 20 sec, air-dried for 5 sec, and light-cured for 10 sec using an LED unit with an output of 1000 mW/cm² (VALO, Ultradent, South Jordan, UT, USA).

- Sandblasting with 50- μ m Al₂O₃ particles for 10 sec from a distance of 5 mm. Then, both silane and adhesive were applied as described above.

Each slab was placed in a polyvinyl chloride mold and incrementally repaired with nanofilled composite (Filtek Z350, 3M ESPE) up to 6 mm above the highest ceramic top plateau. The molds had marks every 2 mm to guide the thickness of each composite layer (Fig. 2), which was light-cured for 20 sec on each side using the abovementioned LED unit. All specimens were stored in artificial saliva at 37 °C for 21 days (ECB 1.3 bacteriological oven, Odontobrás, Ribeirão Preto, SP, Brazil). Then, they were subjected to 1,000 thermocycles between 5 and 55 °C (30 sec dwell time) and stored in distilled water at 37 °C before testing.

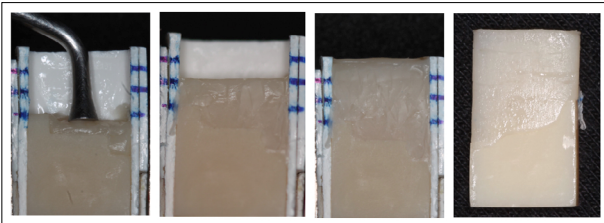


Fig. 2: Ceramic slab placed in a polyvinyl chloride mold and repaired with 2-mm-thick layers of nanofilled composite.

The upper and the lower edges of each specimen were attached to a tensile test setup (Fig. 3) and the interface composite-ceramic was tested until failure in a universal testing machine with a 200-kgf load cell (DL2000, EMIC, São José dos Pinhais, PR, Brazil) at a crosshead speed of 1 mm/min. The load-to-failure of each specimen was recorded in Newtons (N).

The mode of failure was determined under a stereomicroscope with 40x magnification (EK3ST, Eikonol, São Paulo, SP, Brazil) and classified as ‘adhesive’ (at the interface between ceramic and composite), ‘cohesive in composite’, ‘cohesive in ceramic’, or ‘mixed’ (combination of interfacial failure and cohesive in composite). One representative tested specimen of each subgroup (design/surface treatment) was sputter-coated with gold and the ceramic surface morphology was observed through scanning electron microscopy

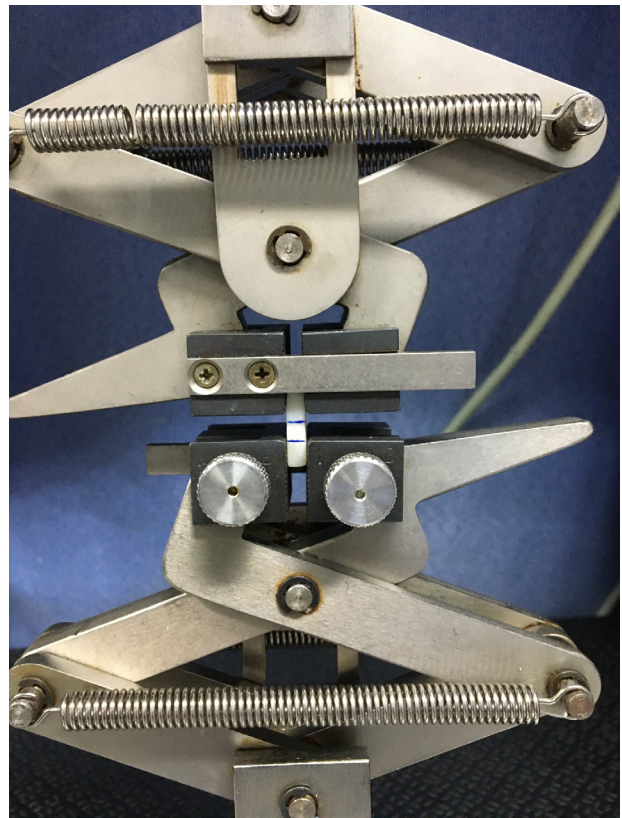


Fig. 3: Composite-repaired ceramic specimen attached to a tensile test setup in a universal testing machine.

(SEM; VEGA3, Tescan, Brno, Czech Republic) at magnifications of 100X, 160X, and 500X.

Since the data did not meet the assumptions of normality and homoscedasticity, the ceramic design and surface treatment effects were analyzed using the Kruskal-Wallis test followed by Dunn’s multiple comparisons test. The failure modes were compared using G-tests. The data were analyzed with statistical software at a significance level of $p < 0.05$ (SPSS 23.0, IBM Corp., Chicago, IL, USA; BioEstat 5.0, Mamirauá Institute, Belém, PA, Brazil).

RESULTS

Considering the flat ceramic design, no significant differences in load-to-failure values were observed between HF-etched and sandblasted specimens (Tables 1 and 2). However, HF etching resulted in significantly higher load-to-failure values than sandblasting for both single plateau and double plateau designs (Tables 3 and 4).

Regardless of ceramic design, the absence of surface treatment resulted in significantly lower load-to-failure values between lithium disilicate glass-ceramic and composite repair (Table 1).

Table 1. Mean (\pm standard deviation) and median load to failure values (N) between lithium disilicate glass-ceramics and composite repair for different ceramic designs and surface treatments.

Ceramic design	No treatment	HF etching	Sandblasting	p-value
Flat	2.19 (\pm 4.62); 0.00 ^{Ba}	148.62 (\pm 66.06); 144.81 ^{Aa}	61.29 (\pm 50.96); 33.7 ^{7Aa}	< 0.001
Single plateau	5.49 (\pm 10.99); 0.00 ^{Ba}	171.02 (\pm 84.40); 208.6 ^{4Aa}	16.84 (\pm 12.58); 16.9 ^{8Bb}	< 0.001
Double plateau	8.90 (\pm 17.78); 0.0 ^{Ba}	127.59 (\pm 64.10); 116.3 ^{9Aa}	34.34 (\pm 35.17); 19.53 ^{Bab}	< 0.001
p-value	0.733	0.550	0.031	—

Values with identical superscript uppercase letters for each ceramic design within the same rows are not significantly different ($p > 0.05$). Values with identical superscript lowercase letters in the same columns for each surface treatment are not significantly different ($p > 0.05$).

Table 2. Dunn's multiple comparison test for flat specimens with different surface treatments.

Comparisons	Rank difference	calculated Z	critical Z	p<0.05
No treatment vs. HF etching	18.0	4.5720	2.394	Yes
No treatment vs. Sandblasting	10.2	2.5908	2.394	Yes
HF vs. Sandblasting	7.8	1.9812	2.394	No

* Rank sum: No treatment 61.0; HF etching 241.0; Sandblasting 163.0.

* Mean rank: No treatment 6.1; HF etching 24.1; Sandblasting 16.3.

Table 3. Dunn's multiple comparison test for single plateau specimens with different surface treatments.

Comparisons	Rank difference	calculated Z	critical Z	p<0.05
No treatment vs. HF etching	17.95	4.5593	2.394	Yes
No treatment vs. Sandblasting	5.90	1.4986	2.394	No
HF vs. Sandblasting	3.0607	2.394	2.394	Yes

* Rank sum: No treatment 75.5; HF etching 255.0; Sandblasting 134.5.

* Mean rank: No treatment 7.55; HF etching 25.5; Sandblasting 13.45.

Table 4. Dunn's multiple comparison test for double plateau specimens with different surface treatments.

Comparisons	Rank difference	calculated Z	critical Z	p<0.05
No treatment vs. HF etching	18.00	4.5720	2.394	Yes
No treatment vs. Sandblasting	8.10	2.0574	2.394	No
HF vs. Sandblasting	9.90	2.5146	2.394	Yes

* Rank sum: No treatment 68.0; HF etching 248.0; Sandblasting 149.0.

* Mean rank: No treatment 6.88; HF etching 24.8; Sandblasting 14.9.

Approximately 80%, 70%, and 60% of the untreated specimens of flat, single plateau, and double plateau ceramic designs, respectively, had pre-testing failures during thermocycling, and their respective load-to-failure values were recorded as zero. Only 20% of sandblasted specimens with flat and single plateau designs presented pre-testing failures. Conversely, HF-etched specimens did not present failures during thermocycling.

The load-to-failure values of HF-etched specimens did not differ significantly according to the ceramic design. For specimens with sandblasted surface treatment, the load-to-failure values of flat specimens were significantly higher than for specimens with a single plateau. The double plateau design resulted in intermediate load-to-failure values, which did not differ significantly from the other two ceramic designs (Table 5).

Table 5. Dunn's multiple comparison test for sandblasted specimens with different ceramic design.

Comparisons	Rank difference	calculated Z	critical Z	p<0.05
Flat vs. Single plateau	10.25	2.6035	2.394	Yes
Flat vs. Double plateau	6.4	1.6256	2.394	No
Single plateau vs. Double plateau	3.85	0.9779	2.394	No

* Rank sum: Flat 210.5; Single plateau 108.0; Double plateau 146.5.

* Mean rank: Flat 21.05; Single plateau 10.8; Double plateau 14.65.

Significant differences were found among failure modes ($p < 0.001$) Regardless of the design, untreated and sandblasted lithium disilicate glass-ceramic specimens, respectively, presented only adhesive failures and cohesive failures in composite. The percentage of cohesive failures in composite was high (80%) for HF-etched flat specimens, while most (60%) of the HF-etched specimens with single plateau or double plateau presented mixed failures. Cohesive failure in ceramic was observed only in HF-etched with single plateau design (10%) (Fig. 4). SEM photomicrographs showed that the surface was smoother in HF-etched specimens than in specimens sandblasted with Al_2O_3 microparticles (Fig. 5).

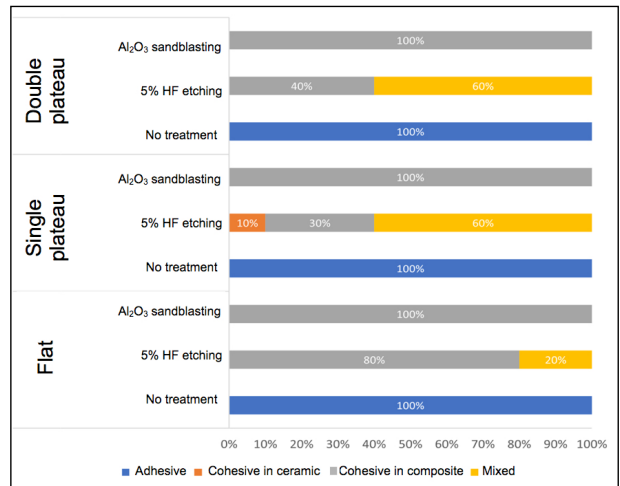


Fig 4: Failure mode percentages of composite-repaired lithium disilicate glass-ceramic specimens after tensile testing for each ceramic design and surface treatment.

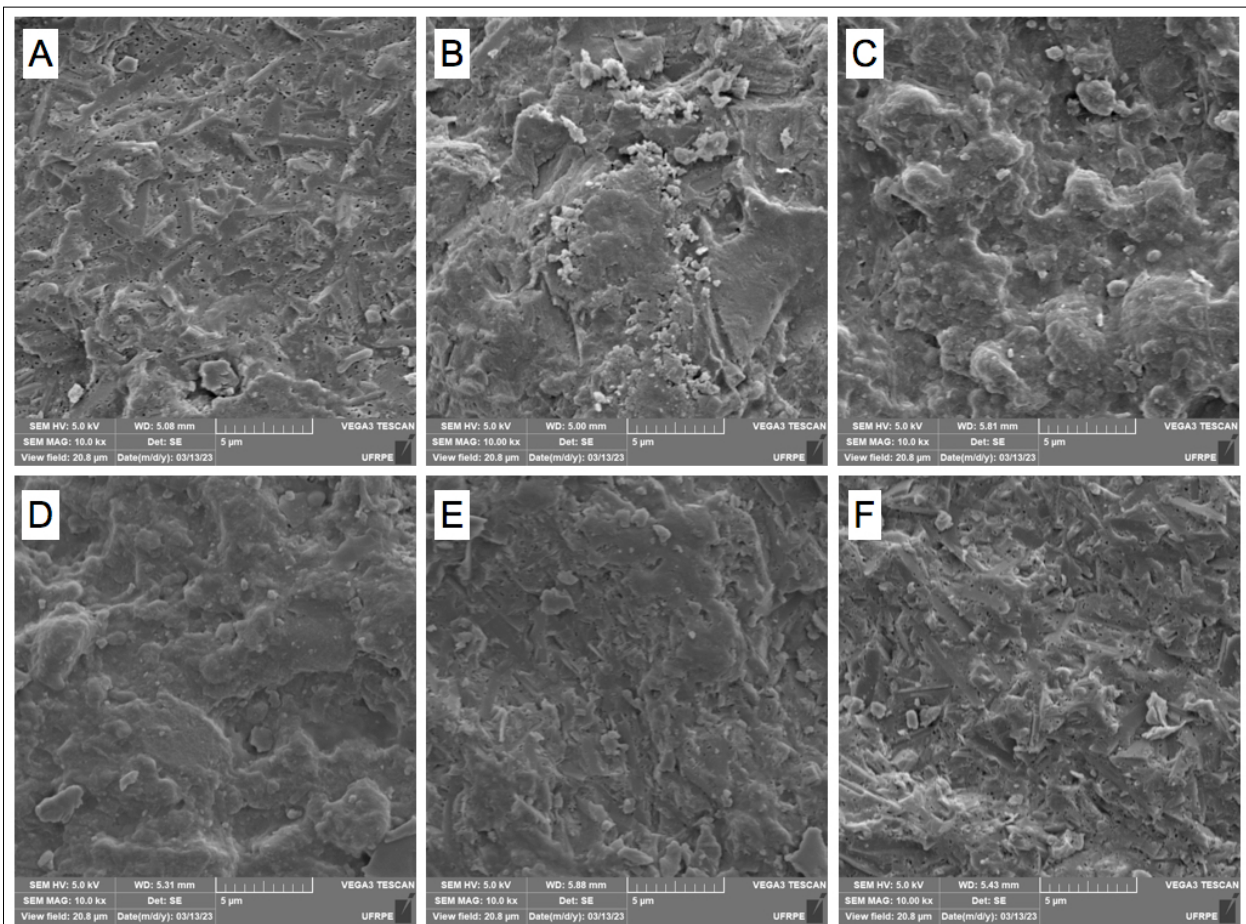


Fig. 5: SEM photomicrographs of tensile tested surfaces: A) Untreated specimen with single plateau design; B) Sandblasted specimen with single plateau design; C) Sandblasted specimen with double plateau design; D) HF-etched specimen with flat design; E) HF-etched specimen with single plateau design and F) HF-etched specimen with double plateau design.

DISCUSSION

Since repairing defective ceramic restorations with direct composite can be a valuable approach due to its reliability, low cost, and conservative characteristics²⁹, this study addressed the effect of macroscopic design and surface treatment on the load-to-failure of lithium disilicate glass-ceramic repaired with nanofilled composite. The null hypothesis was rejected because there was no significant difference between macroscopic ceramic design and load-to-failure values.

The bonding effectiveness of composite to ceramic depends strongly on micromechanical retention⁶, so the lithium disilicate glass-ceramic surface was roughened by HF etching or Al₂O₃ sandblasting. Regardless of the ceramic design, the highest values of load-to-failure were observed for specimens etched with 5% HF before composite repair. Although the microstructure of lithium disilicate glass-ceramic has high crystal content, HF etching dissolves the glassy matrix of ceramic, creating a superficial porous microretentive surface which increases the surface free energy and wettability for adhesive bonding^{30,31}. Both HF concentration and etching time were within the acceptable range that does not jeopardize the bond strength to lithium disilicate glass-ceramic^{15,24}.

The results also demonstrated that Al₂O₃ sandblasting created a certain amount of micromechanical retention on lithium disilicate glass-ceramic. Flat ceramic surfaces resulted in significantly higher load-to-failure values than did single plateau design, suggesting that Al₂O₃ sandblasting is less effective when applied on angulated ceramic walls. Moreover, sandblasting was significantly less effective than HF etching for both single plateau and double plateau ceramic designs; however, the difference was not significant between HF-etched and sandblasted flat ceramic surfaces. Sandblasting increases the surface roughness and surface area of glass-ceramics; however, surface roughness above certain microlevels can form microcracks that reduce micromechanical retention and decrease bond strength; in addition, deep irregular pits on the ceramic surface do not provide retentive features³². The application of a silane coupling agent produces a chemical link between the silicate in the ceramic surface and the polymer-based hydrophobic components in the composite through covalent siloxane bonds^{4,33}. Thus, the combination of

mechanical and chemical retention increases the bond strength of ceramic and repair composite³⁴. The results of the current study corroborate some other studies that recommend HF etching followed by silanization as the gold standard surface treatment for silica-based glass-ceramics^{4,5,35}.

The specimens were stored in artificial saliva for 21 days and thermocycled between 5 and 55 °C (1,000 cycles) because these techniques are widely accepted to simulate aging of the interface between ceramic and composite repair^{6,8}. Regardless of the ceramic design, 80% of the specimens that did not receive surface treatment presented pre-testing failure during thermocycling, which indicates the importance of creating microretention on the ceramic surface before composite repair.

In the HF-etched specimens, the load-to-failure values were higher for single plateau ceramic design than for flat design. Although the double plateau was expected to provide even more retention for the composite repair, it had the lowest load-to-failure values. Since the load-to-failure values of all ceramic designs were relatively high and did not differ significantly, it seems that the failures (mostly cohesive in composite or mixed) occurred due to intrinsic characteristics of the composite. Furthermore, the relatively high standard deviations presented by most of the groups may be related to the macro design of the specimens, in which the occurrence of internal gaps along the ceramic/composite interface as well as stress accumulation, particularly at the internal corners of single and double plateau specimens, may have influenced the overall results³⁶.

All lithium disilicate glass-ceramic specimens that did not receive previous surface treatment failed at the interface between ceramic and composite, which was not observed in any HF-etched or sandblasted specimen. Regardless of the ceramic design, all sandblasted specimens presented cohesive failure in composite, which suggests that the interfacial bond strength provided by sandblasting is higher than the cohesive strength of the nanofilled composite. Moreover, the higher load to failure values of specimens with flat design in comparison to both single plateau and double plateau indicates that a thick, uniform layer of repair composite when sandblasting is used as ceramic surface treatment. In contrast to flat specimens, HF-etched specimens

with single or double plateau presented the most mixed failures, which suggests better stress distribution throughout the composite and the lithium disilicate glass-ceramic. The optical profilometry analysis conducted by Lima et al. (2021) showed that both sandblasting with 50- μm Al_2O_3 particles and silica coating with 30- μm Al_2O_3 particles resulted in the most pronounced alterations on the ceramic surface. The authors reported that although sandblasting created the highest surface roughness, it also promoted surface damage in all evaluated ceramic types. Moreover, 10% HF etching increased flexural strength, particularly when applied for 20 sec.

Strasser et al. (2018) reported that HF provided strong, homogenous etching patterns on lithium disilicate glass-ceramic, in which the glass phase was dissolved and the crystals were found to be relatively protruded. Nevertheless, sandblasting

with 50- μm Al_2O_3 particles resulted in the highest values of surface roughness. Gul & Uygun (2020) reported that sandblasting caused the most remarkable alterations on ceramic surfaces. In the current study, the SEM images of sandblasted specimens also showed deeper, more irregular roughness than HF-etched specimens. In addition, the Al_2O_3 microparticles potentially abraded the lithium disilicate glass matrix and crystals to a certain level that weakened the surface.

The results of this study therefore indicated that the surface treatment of a defective lithium disilicate glass-ceramic restoration has more influence on the retention of the composite repair than its macroscopic design. Moreover, the highest load-to-failure values, the failure mode pattern, and the regular surface roughness observed for HF-etched lithium disilicate glass-ceramic specimens suggest higher bond strength to the composite repair.

CONFLICT INTERESTS

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

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None.

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LVII SAIO 2024 Annual Meeting, City of Buenos Aires - Argentina

We are delighted to announce that the LVII Annual Meeting of the Argentine Society of Dental Research will take place from November 15th to 16th, 2024, in the city of Buenos Aires, Argentina. The event will be hosted by the Faculty of Dentistry at the University of Buenos Aires. Organized by the Organizing Committee of the 2024 Annual Meeting, chaired by Dr. Romina De Lucca, the meeting will include presentations and lectures by prominent experts in contemporary dental research, an awards ceremony, and the presentation of research papers in both oral and poster formats.



LVI Annual Scientific Meeting of the Argentine Society for Dental Research 2023

On November 9th, 10th, and 11th, 2023, the LVI Annual Scientific Meeting of the Argentine Society for Dental Research took place at the Faculty of Health Sciences of the Catholic University of Cordoba and the Faculty of Dentistry of the National University of Cordoba. Dr. Pablo Rodríguez, President of the Society, highlighted during his speech at the opening ceremony the fundamental role of publications and scientific dissemination within the dental community. He also emphasized his interest, along with that of the Board of Directors, in supporting and fostering a new image and wider dissemination of the official publication of SAIO, the journal Acta Odontológica Latinoamericana. Additionally, he underscored the current significance of social media networks and the website in promoting the journal to more researchers, thereby facilitating its expansion and growth.



The Organizing Committee of the Annual Meeting was comprised of the following members:
President: Dr. Gabriela Martín; Secretary: Pablo Fontanetti; Assistant Secretary: Valentín Mendoza; Treasurer:
Dr. Carlos Rozas; General Abstract Coordinator: Dr. Mariana Rocamundi; Commercial Relations: Dr. Jorgelina Ulloque.

LVI Annual Scientific Meeting of the Argentine Society for Dental Research 2023

The LVI Annual Meeting received 214 abstracts, comprising 87 oral presentations, 112 posters, and 15 research projects.

Thirty-four papers were submitted for awards. The Annual Meeting commenced on Thursday, November 9th. The day featured award sessions, lectures, group meetings, the Acta Odontológica Latinoamericana Journal meeting, an Inaugural Ceremony, and a welcome reception.

Subsequent days were dedicated to oral and poster presentations, conferences, concluding on Saturday, November 11th, with the closing dinner and awards ceremony.

Additionally, a photo competition was organized to encourage the dissemination of findings related to dental research in both basic and clinical areas.

The winners of the awards were:

PREMIO DIVISIONAL IADR UNILEVER HATTON DIVISIONAL AWARD CIENCIAS BÁSICAS

"Valoración de alteraciones óreas asociadas al crecimiento orofacial experimental en ratos hipercolesterolémicos"



Autores: María Lorena Cabrita, Pablo Alejandro Rodríguez, Flaminia Luisi NUNO, Eugenio Molisevsky, U. JRG LEE, Eugenio SERRAJO, Leonardo Nori, Gerardo Martínez, Patricia Rodríguez, Domingo Correas DeLuca

PREMIO DIVISIONAL IADR UNILEVER HATTON DIVISIONAL AWARD CIENCIAS CLÍNICAS

"Análisis de la anatomía dentaria interna y externa en una población argentina"



Autores: Eugenia Rivero Corrali Lari, Pablo Alejandro Rodríguez, Adrián Rodríguez

PREMIO RODOLFO ERAUSQUIN

"Perfil profilométrico en placa y neurolíbilas asociado al estado gingivo periodontal de gestantes y no gestantes"



Autores: Luciana Doger, Benicio Lora, María Lourdes, Sofía Navas, Pablo Pobiano, Guillermo Coll, Luciana O'Grady, Laura Blanco, Vanesa Hualde, Adela Silvestri, Claudia Pérez Larrea

PREMIO DRA. MARÍA INÉS EGOZUE

"Frecuencia de hipersensibilidad dentaria en pacientes concurrentes a un servicio de atención odontológica"



Autores: Valerina Alberti Concalor, Julianna O'Grady, María Florencia Escalante Vito, Roberto Solgado, Aldo Scavini

PREMIO SUZEL M. SCOZZARO

"La durcimina modula el remodelado óseo en ratos hipercolesterolémicos con periodontitis"



Autores: María Eugenia Antón, Gastón Roberto Troncoso, Valeria Diaz, Javier Esteban Parera Montero, Silvio María Paderny, Elise Kowalski, Tatiana Shigley

PREMIO COLGATE-PALMOLIVE

"Resorción de todas de octos grasos N-3 de ratos: relación con carcinógenos lingual experimental"



Autores: César Darío Hernández, David Cervera, María Eugenia Percevali, Adrián Benito Lora

PREMIO GSK Cat. Graduado

"Análisis digital de PSI en lesiones reactivas, leucoplásticas y cáncer bucal"



Autores: Gonzalo David Remonier, Gerardo Gilgari, René Luis Paricio, Jerónimo Roberto Lopez, Magdalena Añón, Oriana Peña, Vanesa Pella

PREMIO GSK Cat. Estudiante

"Caracterización de tratamientos endodénticos realizados por estudiantes de grado FOUBA con ENDOSCAN"



Autores: Belén Noéla Benítez, Eugenio Pilar Corrali Lari, Romina Crandau Rojas, Axel Peltz Guether, Pablo Sebastián Rodríguez

PREMIO SALUD BUCAL

"Necesidad de tratamiento de caries en escolares antes y después de la pandemia COVID-19"



Autores: Pablo Ullrich, Florencia Venturi, Gisela Rossi, Aldo Solgado, Roberto Solgado

PREMIO BIOLOGÍA PULPAR Y REGENERACIÓN

"Calificación intracanal asociada a revascularización"



Autores: Rigoberto Gutiérrez, Karina Leyra Martín, Silvio Andrés Álvarez, Marcela Adela Piya

PREMIO ODONTOPEDIATRÍA

"Análisis postural, oclusión, deglución y respiración en niños con SAHDS, Córdoba, Argentina"



Autores: Andrea Fernández Armador, Lucía Lucero, Pablo Cristian Eggera

PREMIO 75 AÑOS FOUBA

"Estudio de la morfología de los conductos radiculares de los primeros molares superiores en TCHC"



Autores: Gilda Julieta Sarraf, Victoria Atalio, Luciana Figueroa, Lucía Vázquez, Ana Socheles, Jorge Pérez, Francisco Soriano, Mario Julio Carlos Astorino, Pablo Solgado, Diego Vázquez

PREMIO 1º puesto CATEDRA ENDODONCIA FOUBA

"Evaluación in vitro del pH de materiales endodénticos bioactivos"



Autores: Eugenia Molisevsky, María Lorena Cabrita, Margarita Chiloja Jordan, Sofía Perceval, Diego Correas, Axel Luis Leonardi, Liliana Gabriela Sierra, Pablo A. Rodríguez

PREMIO 2º puesto CATEDRA ENDODONCIA FOUBA

"Tratamiento con tetraciclina como estrategia de terapia de pulpa vital en ratos"



Autores: Romina Crandau Pérez, Agrinía De Luca, Mariana Lopez, Pablo A. Rodríguez, Mariana Hill, Javier Fernández Sator, Claudio Hain

CONCURSO FOTOGRAFÍA CIENTÍFICA

"Cribado de un emulsionador" Ciencias Básicas

Autores: Marcela Ricci, Romina De Luca, Luciana Urquiza

"Actividad bacteriana de la biopelícula" Ciencias Clínicas

Autor: Jorge Nicolás Juárez

