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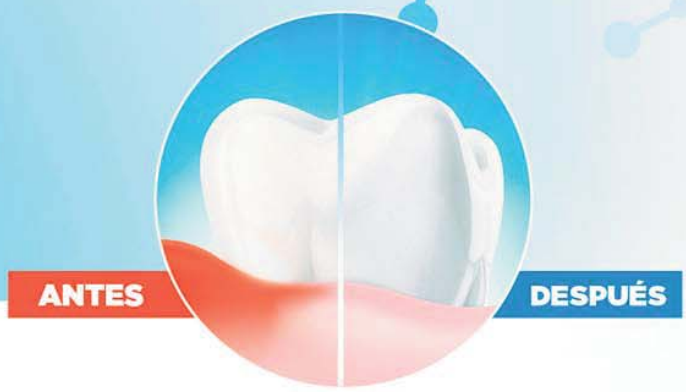

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Epithelial rests of Malassez in experimental animals at different ages

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ABSTRACT

Epithelial rests of Malassez (ERMs) are fragments of Hertwig's sheath in the periodontal ligament. There is extensive knowledge of their role in the etiology of pathological processes and current evidence links them to maintenance of periodontal homeostasis. The aim of this study was to assess the behavior of ERMs with relation to the changes in periodontal tissues during growth in an experimental model with Wistar rats. Mesiodistal sections were made of the first lower molars from Wistar rats aged 1 month (n=7), 3 months (n=7) and 5 months (n=6). Sections were stained with H&E to evaluate number of ERMs/mm, size/area of ERMs (µm2), height of periodontal ligament (PL.h) (µm), area of cement in the furcation zone (C.Ar) (µm2) and bone area related to the ERM zone (BAr/TAr)(%). Post-hoc Bonferroni and ANOVA were applied for statistical analysis of results. Number of ERMs/mm declined significantly with age (1m: 4.34±1.51, 3m: 1.48±0.89, 5m: 0.27± 0.50, p≤0.05), and there was great variability in size. There was significant increase in C.Ar (1m: 3418.96 ± 905.88, 3m: 19365.76 ± 5500.52, 5m: 32182.76 ± 7114.51, p≤ 0.05) and interradicular (BAr/TAr) (1m: 25.26 ± 2.37, 3m: 44.70 ± 3.95, 5m: 46.81 ± 7.80, p≤ 0.05: 1 vs 3, 1 vs 5). There was significant decline in PLh at 5 months (1m: 141.42 ± 29.25, 3m: 162.06 ± 28.21, 5m: 124.72 ± 18.67, p≤ 0.05: 1 vs. 3, 3 vs. 5). The decline in number of ERMs as animal age increases may be related to the significant increase in C.Ar and reduction in PL.h. It remains to ascertain its relationship with the increase observed in BAr/TAr. Further studies are needed to learn more about the role of ERMs and their relationship with periodontal tissues when confronted with different normal and pathological stimuli.

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Keywords: *epithelial rests of Malassez - periodontal ligament - alveolar bone - periodontium - histomorphometry.*

Restos epiteliales de Malassez en animales experimentales a diferentes edades

RESUMEN

Los restos epiteliales de Malassez (ERMs) son fragmentos de la vaina de Hertwig, en el ligamento periodontal. Se conoce extensamente su rol en la etiología de procesos patológicos y actualmente las evidencias los vinculan al mantenimiento de la homeostasis periodontal. El objetivo del presente trabajo fue evaluar el comportamiento de los ERMs en relación a los cambios de los tejidos periodontales durante el crecimiento en un modelo experimental de ratas Wistar. Se obtuvieron cortes mesio-distales del 1er molar inferior de ratas Wistar de 1 mes (n=7), 3 meses (n=7) y 5 meses (n=6) de edad. En cortes coloreados con H&E, se evalúo: Nº de ERMs/mm, tamaño/área de ERMs (µm2), altura del ligamento periodontal (PL.h) (µm), área de cemento en la zona de furcación (C.Ar) (µm2) y área del hueso relacionado con la zona de los ERMs (BAr/TAr) (%). Los resultados se analizaron estadísticamente mediante ANOVA y Bonferroni post-hoc. El Nº de ERMs/mm disminuyó significativamente con la edad (1m: 4.34±1.51, 3m: 1.48±0.89, 5m: 0.27± 0.50, p≤0.05), observándose una gran variabilidad de tamaño. Se observó un aumento significativo del C.Ar (1m: 3418.96 ± 905.88, 3m: 19365.76 ± 5500.52, 5m: 32182.76 ± 7114.51, p≤ 0.05) y del (BAr/TAr). interradicular (1m: 25.26 ± 2.37, 3m: 44.70 ± 3.95, 5m: 46.81 ± 7.80, p≤ 0.05: 1 vs 3, 1 vs 5). Además, se halló una disminución significativa en PLh a los 5 meses (1m: 141.42 ± 29.25, 3m: 162.06 ± 28.21, 5m: 124.72 ± 18.67, p≤ 0.05: 1 vs 3, 3 vs 5). La disminución hallada del número de ERMs conforme aumenta la edad del animal podría vincularse con el aumento significativo del C.Ar y la reducción del PL.h. Resta por dilucidar su relación con el aumento de BAr/TAr. observado. Se requieren más estudios para profundizar sobre el rol de los ERMs y su relación con los tejidos periodontales ante distintos estímulos normales y patológicos.

Palabras clave: *restos epiteliales de Malassez - ligamento periodontal - hueso alveolar - periodontium - histomorfometría.*

INTRODUCTION

The insertion periodontium components that make up the topographic and functional unit are root cementum, periodontal ligament and alveolar bone. These structures evolve in an interrelated, coordinated manner over the life of the tooth, adapting continuously to changes that take place in the mouth. The periodontal ligament is located between the cementum and the alveolar bone. It is a fibrous connective tissue comprising a wide variety of cells: fibroblasts, osteoblasts, cementoblasts, osteoclasts, macrophages, mastocytes, undifferentiated ectomesenchymal cells and epithelial rests of Malassez (ERMs). Cell activity is mediated by several signaling factors that regulate the complex growth and regeneration machinery of periodontal structures[1]. After root formation, remnants of Hertwig's sheath persist as ERMs throughout the life of the tooth[2,3]. Although their etiological role in the formation of odontogenic tumors and cysts is widely known, ERMs are currently a subject of interest as structures that take part in the histophysiology of the periodontal ligament. Histologically, ERMs are identified as small groups of epithelial cells within the periodontal ligament, in close contact with the cementum surface, becoming increasingly distant from the cementum surface towards the coronal third of the root[4]. Oblique sections of the periodontal ligament have shown ERMs to form part of mesh around the root[5]. ERMs have also been shown to have an irregular nucleus with dense heterochromatin and a small, scarcely distinguishable peripheral halo of cytoplasm[6]. ERM ultrastructure in sections of rat periodontal ligament is similar to that in humans and other animals[7,8]. A basal lamina separates the ERM cell islands from the connective tissue[9], and there are tight junctions between them and hemidesmosomes, and tonofilaments within the cytoplasm. ERMs can produce different proteins and macromolecules, including cytokeratins[10,11] and neuropeptides[12]. The expression of CK 17 could be a marker used for identification[13]. Other studies[14,15] also report the expression of cell surface proteins such as epidermal growth factor receptors. It has been observed that proteins typical of the enamel matrix such as amelogenin and ameloblastin are expressed from samples of human periodontal ligament[16,17]. It has been suggested that these two proteins may act as growth factors and participate in adhesiveness, proliferation, migration and differentiation of periodontal ligament fibroblasts[18,19]. Moreover, it has been found that disruption of periodontal integrity induced early expression of APINA (another protein of the enamel matrix), forming part of a cascade of events possibly leading to the activation of the ERMs during periodontal healing and regeneration[20].

Despite the ectodermal origin and epithelial nature of these cell groups, they can synthesize components that are frequently associated to cells of mesenchymal origin, such as glycosaminoglycans, hyaluronic acid, dermatan sulfate and chondroitin sulfate[21], as well as osteopontin (OPN), bone sialoprotein (BSP) and osteoprotegerin (OPG)[4,22]. They can also degrade collagen by synthesis of collagenases and proteinases[23,24]. It has therefore been suggested that they may contribute to periodontal regeneration by synthesizing a series of proteins related to the cementum and the bone tissues[25]. Current scientific evidence suggests that the possible role of ERMs in adult periodontal ligament is related to maintaining homeostasis of the periodontal space, thereby preventing anchylosis, inhibiting root resorption and contributing to cementum repair[26,27].

In previous papers, our group has reported that in an experimental periodontitis model, ERMs exhibited cell hypertrophy when there was root resorption[28]. Other authors have evaluated ERM cell response to *in vivo* mechanical stimuli, observing cell proliferation and hypertrophy[29]. Regarding this point, our group studied ERMs in an experimental model of root resorption mediated by orthodontic forces in rats, finding no significant morphological change that would highlight their role in root resorption induced by orthodontic forces[30]. Other studies have described the expression of several proteins[27,31] that may contribute to the maintenance of cementogenesis and osteogenesis. Among these proteins, the expression of HSP 70 (Heat Shock Protein) by ERMs may provide protection against different forms of attack, including oxidizing agents, inflammation, hypoxia, hyperthermia and mechanical stimuli such as orthodontic forces[32].

The **aim** of this study was to assess the behavior of periodontal tissues with relation to ERMs in healthy Wistar rats at different ages.

MATERIALS AND METHODS

We used 20 healthy male Wistar rats aged 1 month (n=7), 3 months (n=7) and 5 months (n=6). All

animals had "ad libitum" access to balanced feed and water. They were housed in galvanized wire cages containing not more than 5 animals each, with temperature 21 ºC to 24 ºC; Humidity 52% to 56%; light/dark cycles: 12 hours/12 hours.

The experimental protocol was approved by the Ethics Committee of the School of Dentistry 012/2016 CICUAL-ODONTO-FOUBA of Buenos Aires, Argentina, and is in keeping with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals.

Histology and Histomorphometry

Following euthanasia at the relevant experimental times, lower jaws were extracted and fixed in 10% buffered formalin (pH 7.4). Samples were desiccated and decalcified in 10% EDTA solution, pH 7.0, for 25 days.

The section of each hemimandible corresponding to the three lower molars was embedded in paraffin to prepare mesiodistal histological sections of the lower first molar. Sections were stained with hematoxylin-eosin. The section with best orientation from each hemimandible, showing mesial and distal roots up to the apices, was selected.

The following parameters were evaluated at the furcation zone:

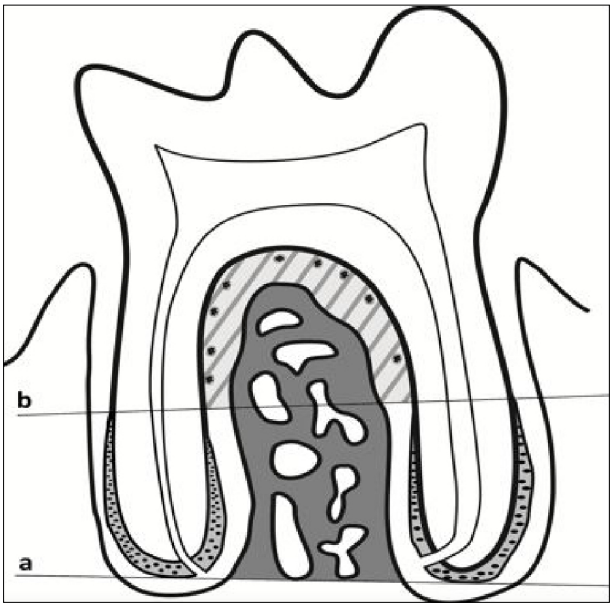

Fig. 1: Diagram of rat molar in situ. Line "a" joins the two apices and limits the total interradicular space in which B.Ar/T.Ar (%) was evaluated. Line "b", drawn at the beginning of the cellular cementum, limits the periodontal area (shaded and lined) where ERMs were evaluated.

- ***Number of ERMs/mm**:* number of ERMs in the periodontal ligament in the perimeter limited by the beginning of cellular cementum, expressed with relation to root surface (Fig. 1).
- ***Size/Area of ERMs (µm²):*** Area of ERMs
- ***Height of periodontal ligament (PL.h) (µm):*** average of five equidistant linear measurements between the cementum surface of the furcation zone and the beginning of the bone tissue (Fig. 2).
- ***Area of cementum in the furcation zone: C.Ar (µm²):*** measurement of the area of cementum in the zone limited by the projection of two lines, on the mesial and distal root surfaces (Fig. 3).

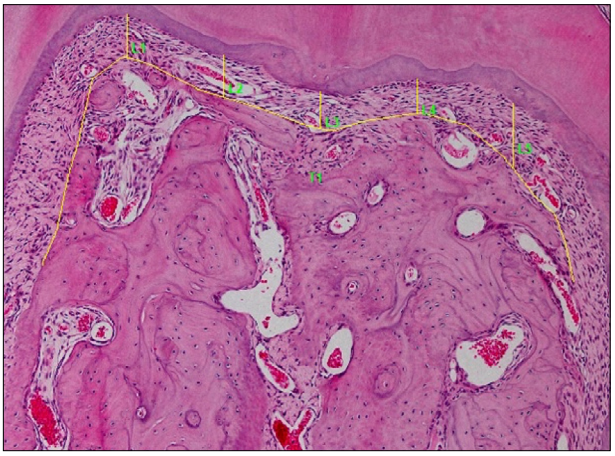

Fig. 2: Microphotograph of a mesiodistal section of lower molar showing the 5 lines used for measuring height of the periodontal ligament. H&E. 100X.

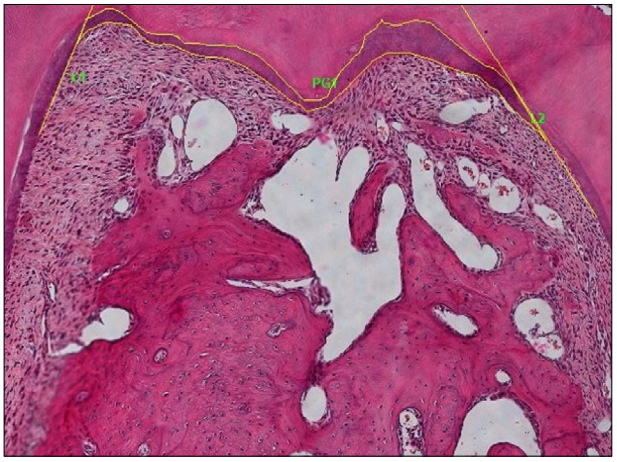

Fig. 3: Microphotograph of a mesiodistal section of lower molar. The two lines (L1 and L2) projected on the cementum surface demarcate the furcation zone where cementum area (C.Ar) was evaluated. H&E. 100X.

- ***Bone area B.Ar/T.Ar:*** Area of trabecular bone tissue in the zone selected for measuring ERMs, expressed as percentage of total area of interradicular bone (Fig. 1).

Number of ERMs was counted directly under 100x magnification with a Bausch & Lomb microscope. The rest of the histomorphometric measurements were taken using 100X digital microphotographs taken with a Nikon Eclipse photomicroscope and *Image Pro-Plus* Software.

Statistical analysis was performed with post-hoc Bonferroni and ANOVA, considering significance at $p < 0.05$.

RESULTS

The results show that number of ERMs/mm declined significantly with age of animals, with major variability and high standard deviations in size of ERMs found in each group. This variability is reflected by the high standard deviations obtained for this parameter. In the 5-month-old group, ERMs were only observed in 3 of the 6 animals. Moreover, there was a significant increase in cementum area and bone area in the interradicular zone and a significant decrease in the height of the periodontal ligament in the 5-month-old group in relation to the other groups (Table 1 and Figs 4 and 5). Interestingly, we observed nuclei compatible with ERMs associated with and even embedded in the cementum matrix in the furcation zone (Fig. 6).

Table 1: Values of the different parameters expressed as mean ± SD

	1 month	3 months	5 months
Number of ERMs/mm	4.34 ± 1.51 **a**	1.48 ± 0.89 **b**	0.27 ± 0.50 **c**
Size/Area of ERMs (μm²)	213.61 ± 61.84 **a**	431.91 ± 180.21 **b**	203.43 ± 82.23 **a**
Height of periodontal ligament (PL.h) (μm)	141.42 ± 29.25 **a b**	162.06 ± 28.21 **b**	124.72 ± 18.67 **a**
Area of cementum at furcation (C.Ar) (μm²)	3418.96 ± 905.88 **a**	19365.76 ± 5500.52 **b**	32182.76 ± 7114.51 **c**
Bone area (B.Ar/T.Ar) (%)	25.26 ± 2.37 **a**	44.70 ± 3.95 **b**	46.81 ± 7.80 **b**

Means with same letter do not differ significantly ($p \geq 0.05$).

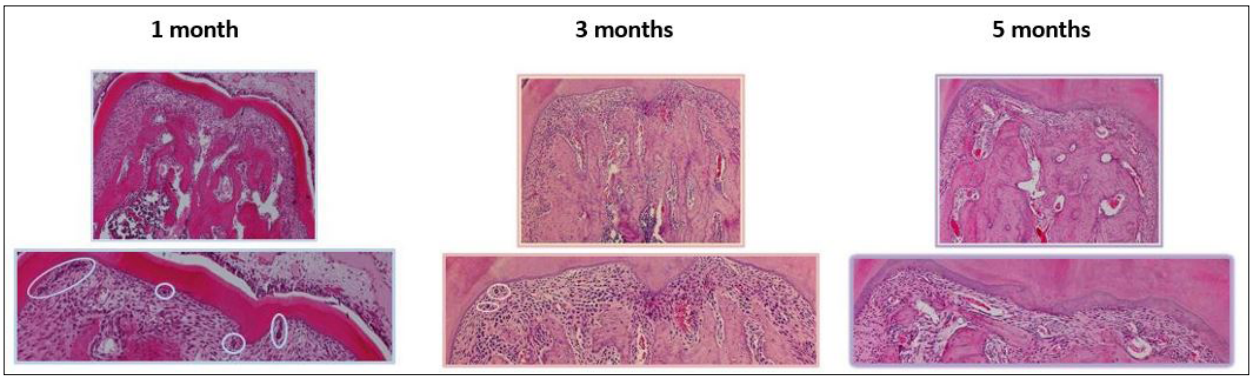

Fig. 4: Microphotographs of mesiodistal sections of lower molar of Wistar rats aged 1, 3 and 5 months, showing the decline in number of ERMs, the increase in cementum area at the level of the furcation and the reduction of the periodontal space as animal chronological age increases. H&E. 40 and 100X.

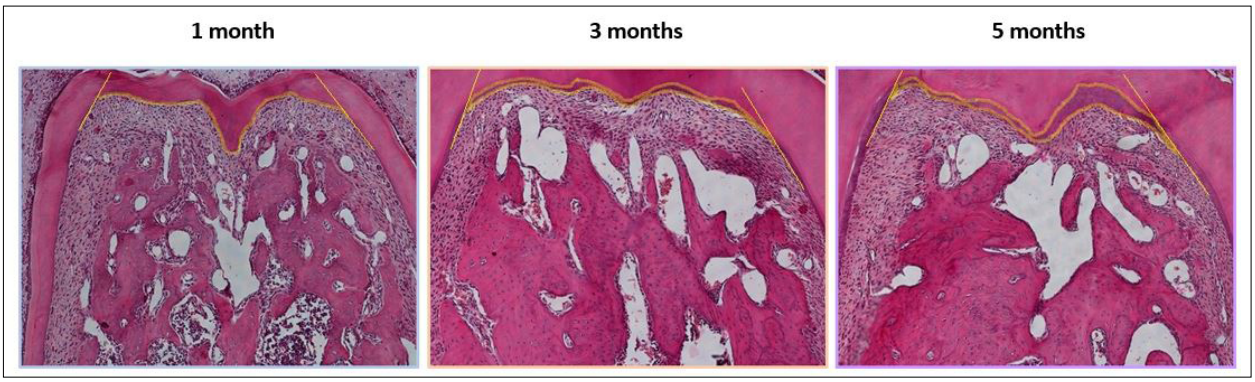

Fig. 5: Microphotographs of mesiodistal sections of lower molar in Wistar rats aged 1, 3 and 5 months, showing delimitation established for evaluating the cementum area in the furcation zone in the 3 age groups. H&E.100X.

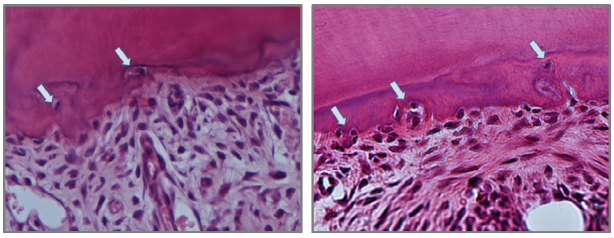

Fig. 6: Microphotographs of mesiodistal sections of lower molar in Wistar rat aged 5 months, showing cell nuclei compatible with ERMs embedded in the cementum (arrows). H&E. 40X.

DISCUSSION

The results of this study show that the number of ERMs of lower molars in Wistar rats declines significantly as animal chronological age increases, and that there is major variability in size. The decline in number of ERMs was accompanied by a significant increase in interradicular cementum area at the level of the furcation and the interradicular bone area, entailing a reduction in height of the periodontal ligament when the animals were 5 months old.

The decline observed in number of ERMs with increase in age agrees with different papers published since 1950. Wentz et al.[33] suggests that the decline in number of ERMs with age may be because they degenerate and then become calcified. Studies on human ERMs have also reported that they decline as age increases, and studies on older people report the onset of ERM calcification, which persist as cementum-like structures/cementicles[34]. A study by Gonçalves et al.[35] suggests that such decline may be partly due to cell apoptosis. In our study, the decline with age was accompanied by, and probably related to, an increase in the cementum area and interradicular bone volume and the consequent reduction in the height of the periodontal ligament. Sims et al.[36] also reports a reduction in the thickness of the periodontal space as age increased in mice.

It is important to note that although our study did not find isolated partial calcifications in the periodontal ligament, it did find nuclei of cells compatible with possible ERMs embedded in the cementum associated to the interradicular space. The hypothesis that the nuclei observed in the radicular cementum matrix may have originated from nearby ERMs is also supported by the well-known fact that the topographic zone of the furcation and radicular cervical third is not characterized by presenting cellular cementum.

Hasegawa et al.[37] showed that during cementum repair, ERMs express osteopontin and ameloblastin, suggesting that they may be involved in cementum repair. More recent findings suggest that within the ERMs, there is a cell population with properties similar to those of undifferentiated mesenchymal cells, which, under the right stimulus, can differentiate into cells capable of synthesizing mineralized matrices in a particular microenvironment[38].

It has been more than twenty years since enamel matrix derivatives were introduced in clinical periodontics for use in regenerative therapies. Nevertheless, further research is still needed for better characterization of their effects on the behavior of different tissues and on the different cells[39]. In this context, it is worth noting that ERMs can express proteins typical of the enamel matrix, such as amelogenin and ameloblastin[16]. These proteins may have a strong effect on cell activity of the insertion periodontum[18,19]. Considering that ERMs may be a natural source of these proteins within the periodontal ligament itself, it is essential to continue learning about the behavior of ERMs in different contexts and under different stimuli.

The current study also found an increase in interradicular bone volume as chronological age increases, in agreement with other studies conducted by our group[40]. This finding poses a question that is yet unanswered, regarding whether there is any association between the increase in bone volume found and the behavior and/or presence of ERMs in the periodontal ligament. In further studies we will endeavor to ascertain whether there are mechanisms that operate in said association and how they work.

CONCLUSIONS

Further studies are needed to learn more about the role of ERMs and their association with the cementum, periodontal ligament and bone tissue, both in health and in situations requiring periodontal repair and/or regeneration mechanisms. In addition to providing insight regarding the biology of ERMs, this study contributes to a better characterization of an animal model that is extensively used in research on different aspects of oral pathology.

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DECLARATION OF CONFLICTING INTERESTS

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Assessment of permeability of eroded dentin after the use of universal, self-etch, and conventional systems

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ABSTRACT

Dentin hypersensitivity is caused by increased dentinal permeability due to total or partial exposure of dentinal tubules, which in turn can be produced by alterations of dental structures or failure of restorative procedures. The purpose of this in vitro study was to evaluate the efficacy of the application of different kinds of adhesive systems to prevent dentin permeability before and after an erosive challenge. Fifty bovine dentin discs (6x1 mm) were prepared and the specimens were divided into 5 groups (n=10): (SB2) Single Bond 2, (SBU) Universal Single Bond, (CSB) Clearfil SE Bond, (SM) Scotchbond Multipurpose and (C) Control. Hydraulic conductance of dentin was recorded after adhesive application (HC-1) and after erosive challenge (HC-2). Dentin surface images of post-treatment and post-erosive challenge were obtained by scanning electron microscopy (SEM). Data were analyzed using Kruskal Wallis, Mann-Whitney with Bonferroni correction and Wilcoxon tests ($p<0.05$). Reduction in dentin permeability was observed with the application of adhesive systems ($p<0.05$). After the erosive challenge, dentin permeability increased for SBU and CSB ($p<0.05$), while SB2 and SM did not differ in HC-1 or HC-2 ($p>0.05$). The conventional, self-etching and universal adhesive systems reduce dentinal permeability by more than 80%, and dentin demineralization may contribute to the increased permeability of universal and self-etching systems.

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Keywords: *dentin - dentin bonding agents - dentin permeability - tooth erosion.*

Avaliação da permeabilidade da dentina com erosão, após o uso de sistemas universais, autocondicionantes e convencionais

RESUMO

Diversos pacientes apresentam hipersensibilidade dentária ou falha nos procedimentos restauradores, devido à exposição total ou parcial de túbulos dentinários. O objetivo deste estudo in vitro foi o de avaliar a influência da aplicação de diferentes tipos de sistemas adesivos na permeabilidade da dentina e após o desafio erosivo. Cinquenta discos de dentina bovina (6x1 mm) foram confeccionados e os espécimes foram divididos em 5 grupos (n = 10): (SB2) Single Bond 2, (SBU) Universal Single Bond, (CSB) Clearfil SE Bond, (SM) Scotchbond Multiuso e (C) Controle. A condutância hidráulica da dentina foi registrada após a aplicação do adesivo (HC-1) e após o desafio erosivo (HC-2). Imagens da superfície da dentina de pós-tratamento e pós-desafio erosivo foram obtidas por microscopia eletrônica de varredura (MEV). Os dados foram analisados pelos testes de Kruskal Wallis, Mann-Whitney com correção de Bonferroni e Wilcoxon ($p <0,05$). Redução da permeabilidade dentinária foi observada com a aplicação dos sistemas adesivos ($p <0,05$). Após o desafio erosivo, a permeabilidade dentinária aumentou para SBU e CSB ($p <0,05$), enquanto SB2 e SM não diferiram em HC-1 e HC-2 ($p> 0,05$). Os sistemas adesivos convencionais, autocondicionantes e universais reduzem a permeabilidade dentinária em mais de 80%, e a dentina desmineralizada pode gerar um aumento da permeabilidade dos sistemas adesivos universais e autocondicionantes.

Palavras-chave: *dentina - adesivos dentinários - permeabilidade da dentina - erosão dentária.*

INTRODUCTION

Numerous clinical conditions may affect dentin and the permeability of the dentinal complex. Dentin permeability involves the passage of fluids, ions, molecules, particles and bacteria through dentinal tubules and can be modified by different oral conditions. Physiologically, this is a very evident mechanism that enables the transport of nutrients and pulp impulses through dentinal tubes via odontoblasts, influencing the maintenance of vitality of the dental tissues[1].

Clinically, dentinal tubules may be exposed due to pathological conditions such as loss of enamel, inducing the presence of erosive or abrasive dentin exposure and gingival recession, presence of caries, cracked tooth,[2] or even due to cavity preparation for direct/indirect restorations[3]. Adhesive systems are presented as a relatively effective material for sealing exposed dentinal tubules, acting through the formation of the hybrid layer [4]. Resin materials may thus be indicated both for protecting pulp against exposure to bacterial products between the period of dental preparation and cementation of the final indirect restoration (prehybridization)[3], and for dentin hypersensitivity[2] in non-carious cervical lesions (NCCL). Hybrid coatings can be alternative for the treatment of clinical dentin hypersensitivity because they form a thin, colorless film on the dentin,[5] reducing the communication between the external environment and the pulp.

However, the formation and maintenance of the hybrid layer are objects of investigation,[4, 6] because the technique is challenging due to the complexity of the dentin tissue. Moreover, the hybrid layer is subject to protein and hydrolytic degradation [4, 6].

Single-bottle or multi-bottle adhesives, either self-etching or total-etch, are employed for the dentin treatment. The behavior of these materials varies according to the type of adhesive system used. The single-step self-etching adhesives appear to allow fluid conductance *in vitro* similarly or slightly more than dentin covered by the smear layer[7]. Transmission electron microscopy reveals the formation of water trees in single-step self-etching adhesives, which facilitate water movement through the polymerized adhesives, making them highly permeable[7]. In contrast, better dentin surface sealing[3] has been observed in 3-step total-etch or 2-step self-etching adhesive systems, because the final seal is achieved by a resinous layer of hydrophobic monomers.

"Universal", "multimode" or "multipurpose" adhesive systems are increasingly used due to the improvement in their composition by having the 10-MDP (methacryloyloxydecyl dihydrogen phosphate) molecule, in addition to other monomers. The functional phosphate ester monomer (10-MDP) was already part of the composition of self-etching adhesive systems (Clearfil SE-2 steps). Its phosphate group has the potential for interaction with hydroxyapatite and is capable of forming strong ionic bonds with calcium due to the relatively low rate of calcium dissolution contributing significantly to the durability of the restorations.

In addition, the 10-MDP monomer enables polar behavior which is favorable to adhesion, and protects the collagen fibers through the formation of MDP-calcium salts[8]. Therefore, studies demonstrating dentin permeability after the use of these simplified "universal" materials compared to the already established 3-step etch-and-rinse and 2-step self-etch systems are needed. It is interesting to note that this analysis is important mainly in conditions of demineralized dentin that has mineral depletion with loss of Ca and P [9].

With regard to this concept, the literature has shown that even after the photopolymerization, fluid transudation through the polymerized adhesives is observed[4,10] and that these dentin adhesives are susceptible to surface degradation by the erosive challenge. The presence in the oral cavity of acids of intrinsic or extrinsic origin[11] may result in dental erosion, affecting dental tissues (enamel/dentin) and degrading restorative materials[12].

Knowing that the dentinal fluid rate changes when hybrid coatings are applied after erosive challenges[5], we became interested in learning about the behavior regarding hydraulic conductance of simplified universal systems. It is important to evaluate the intrinsic water permeability in adhesive systems because it is known to have a significant effect on the quality of bond strength and adhesive interface [13].

The aim of this *in vitro* study was therefore to use the hydraulic conductance test to evaluate the influence of the use of different adhesive systems on dentin permeability immediately after application and after being submitted to the erosive challenge. The null hypotheses tested are: there is no statistically significant difference in dentin permeability 1) immediately after the application of different adhesive systems, and 2) after being submitted to erosive challenge.

MATERIALS AND METHODS

Specimen preparation

Fifty bovine incisors were cleaned and stored in distilled water, which was changed weekly until use, for a period not exceeding 6 months.

The teeth were sectioned, under constant water cooling, below the cementoenamel junction with a diamond disc (Dremel, Campinas, SP, Brazil) coupled to a high-rotation lathe (Nevoni, São Paulo, Brazil) for root removal.

A trephine drill (6mm internal Ø) adapted to a cutting machine (Micro Mill - Washington, USA) under abundant irrigation, was used to prepare circular samples of enamel and dentin obtained from the flatter central portion of the buccal surface. For enamel removal and standardization of dentin thickness at 1 mm, the samples were worn in a circular polishing machine (DP-10, Panambra, São Paulo, SP, Brazil) with P600, P800, P1200 granular silicon carbide sandpaper (Fepa P, Extec, Enfield, CT, USA) and sanded to P2400 granularity (Fepa P, Extec, Enfield, CT, USA) under constant water cooling.

Opening of the dentinal tubules

To remove the smear layer and expose dentinal tubules, the samples were immersed in 37% phosphoric acid solution for 30 s, washed with deionized water for 30 s and stored in 0.1% thymol.

Dentin permeability

Dentin permeability was determined using the apparatus, split chamber model, THD 03 (ODEME Equipamentos médicos e odontológicos Ltda, Joaçaba, SC, Brazil) (Fig. 1). Dentin permeability was established by hydraulic conductance (HC, Lp) using the following formula: Lp=Q/(SA.P), where Lp=hydraulic conductance expressed in $\mu l.cm^{-2}.min^{-1}.cmH_2O^{-1}$, Q=infiltration rate in $\mu L.min^{-1}$, SA=surface area exposed to filtration in cm², P=hydrostatic pressure across dentin in cmH_2O [5,14]. Hydraulic conductance was measured at three times: after removal of the smear layer - initial (HC-0) (PI), after treatments (application of adhesive systems) (HC-1) and after erosive challenge (HC-2).

To determine hydraulic conductance, the samples were placed between two rings that enabled the standardization of the available dentin area for deionized water filtration (0.03801 cm²) and adequate sealing. The pulp face remained in contact with the fluid (deionized water) under a pressure of 703 cm H_2O [15] and the external side faced the environment, according to the dental structure. Fluid filtration through the dentin was followed for 2 min by linear displacement of an air bubble

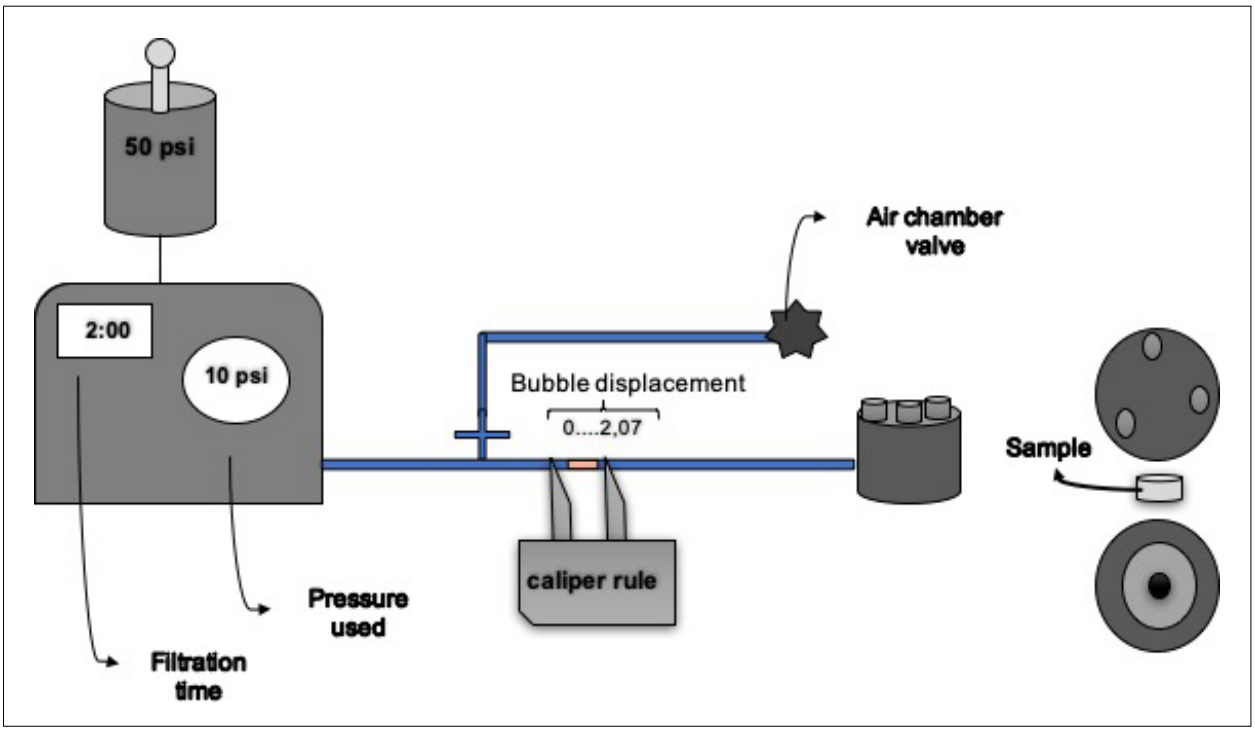

Fig. 1: Schematic drawing of the permeability device

inserted into the glass capillary using a digital caliper. This glass capillary (internal volume 75 µl and length 101 mm) is responsible for connecting the water reservoir and the perfusion chamber. Three consecutive measurements of the linear displacement of the bubble were recorded for each sample and the average of these measurements was used to determine dentin permeability (Fig. 1).

Distribution of experimental groups

After reading the (HC-0), the samples were divided into 5 groups (n=10 each) so that they had similar average values of initial hydraulic conductance. Adhesive systems were applied according to the manufacturers' instructions (Table 1). After the treatments, the specimens were stored in deionized water at 37±1°C for 24 h.

Erosive challenge

The erosive challenge was performed on the external face (vestibular) of the specimens by four daily cycles of erosion for five days. Each cycle consisted of immersing the samples in 0.3% citric acid solution at pH 2.3 for 2 minutes, followed by washing in ultrapure water and immersion in artificial saliva (pH=7) for 1 hour, [16] a formulation proposed by Gohring et al.[17] Before starting a new cycle, the samples were washed with ultrapure water and new solutions of citric acid and artificial saliva were used. The sequence of 4 cycles of erosive challenge and 20 hours of immersion in artificial saliva was repeated for 5 consecutive days. After the erosive challenge, the samples were kept in ultrapure water at 37±1°C for 24 h.

Table 1. Materials used in the experimental groups.

GROUPS	MATERIAL/LOT	COMPOSITION	APPLICATION METHOD
CSB	Clearfil SE Bond Primer -L: 01245A Adhesive -L: 01882A	Primer: 10 –Methacryloxydecyl dihydrogen-phosphate (MDP), 2-hydroxyethyl methacrylate (HEMA), camphorquinone hydrophilic dimethacrylate, N, N - diethanol-P-toluidine, water Adhesive: MDP, bisphenol A glycidyl dimethacrylate (Bis-GMA), HEMA, hydrophobic dimethacrylate, camphorquinone, N, N - diethanol-P-toluidine, silanized colloidal silica	• Drying with absorbent paper. • Primer application for 20 s. • Light air blast for 5 s. • Adhesive application for 20 s. • Light air blast for 5 s. • Light cure for 10 s.
SBU	Single Bond Universal – 3M -L: 1432500600	Bisphenol A diglycidyl ether dimethacrylate (Bis-GMA), 2-hydroxyethyl methacrylate, silica treated with silicon, ethyl alcohol, decamethylene dimethacrylate, water, 1,10-decanediol methacrylate phosphate, acrylic copolymer and itaconic acid, camphoroquinone, N, N - dimethylbenzocaine, 2-dimethylamonoethyl methacrylate, methyl ethyl ketone	• Surface drying with absorbent paper. • Active application of the adhesive with a disposable applicator for 20 s. • Light air blast for 5 s. • Light cure for 10 s.
SB2	Single Bond 2 – 3M L: N587475	Ethanol, Bis-GMA, Silane Treated with Silica Particle, 2-Hydrosethylmethacrylate, Glycerol 1,3 Dimethacrylate, Acrylic Acid Copolymer, and Itaconic Acid and Diurethane Dimethacrylate	• Surface drying with absorbent paper. • Application of 37% phosphoric acid for 15 s. • Washing with water for 15 s and drying with absorbent paper. • Two consecutive Single Bond 2 layers actively applied for 15 s. • Light air blast for 5 s. • Light cure for 10 s
SM	Scotchbond Multipurpose – 3M Primer- L: 1502200616 Adhe-sive-L:1411401017	Primer: 2-hydroxyethyl methacrylate (HEMA) and polyalkene acid Adhesive: bismethacrylate (1-methylethylidene) bis[4,1-fenilenooxi (2-hydroxy-3,1-propanediol)] and 2-hydroxyethyl methacrylate	• Surface drying with absorbent paper. • Application of 37% phosphoric acid gel for 15 s. • Washing for 15 s and drying with absorbent paper. • Primer application for 15 s. • Light air blast for 5 s. • Adhesive application. • Light cure for 10 s.
C	NO TREATMENT	Control	• Storage in distilled water during all the studied periods.

Permeability percentages

The initial permeability calculated from HC-0 was considered to be 100% for each sample analyzed. The dentin percentage permeability for each sample was calculated after the treatment (%PPT) and after erosive challenge (%PPEC), and each sample was its control. To obtain these permeability values, the following formula was applied: %P = (Lp.100)/ Lpinitial, where %P = percentage of permeability regarding the initial permeability, Lp = hydraulic conductance at each moment, Lpinitial = initial hydraulic conductance (CH-0) considered after removal of the smear layer and tubular opening.

Scanning electron microscope (SEM) micrographs

Micrographs (3000x and 5000x) were obtained after the application of the adhesive systems and after the erosive challenge, to observe the behavior of different types of treatment on dentin. To do so, the samples were dried in a graded series of alcohol and desiccator for 24 h. Subsequently, the samples were placed on an aluminum stub with the aid of a conductive carbon tape and metal-coated in a SC7620 Sputter Coater (Emitech, FEI, Czech Republic) employing 25 KV. Samples were analyzed by capturing the images through software coupled to the SEM (Inspect 550, Fei).

Statistical analysis

The average values (standard deviations) and medians of %PPT and %PPEC were calculated.

The Kruskal-Wallis test and Mann-Whitney post hoc test with Bonferroni correction were used to test the hypothesis that the groups were different in %PPT and %PPEC. The Wilcoxon test was employed to test the hypothesis that there was no significant difference in the dentin permeability change of each material between %PPT and %PPEC. The adopted significance level was 5%. The statistical program used was SPSS 24.0 (IBM, Armonk, NY, USA).

RESULTS

Dentin permeability analysis

Table 2 shows the average values of %PPT and %PPEC for the different groups. A significant reduction in permeability is observed after the use of adhesives ($p<0.05$). From the initial values of hydraulic conductance, it is verified that permeability was reduced in all the systems by more than 80%.

In demineralized dentin, both the self-etch and total-etch systems exhibited similar immediate behaviors, differing only from the untreated demineralized group. However, on the same substrate, after the erosive challenge, a significant increase in permeability was found for self-etch adhesives (CSB and SBU) (Table 2).

Scanning Electron Microscopy (SEM) Analysis

Micrographs (3000 and 5000 X) of the different experimental groups showed the presence of porosity and irregularities, with the presence of valleys and depressions in the dentin surface, even after the application of adhesive systems. The degradation of the adhesives after erosive challenge demonstrated greater uniformity of the surface layer (Fig. 2). The demineralized dentin had evidently open, exposed collagen fibers, and after erosion, the presence of smear layer.

DISCUSSION

Several adhesive systems are available, but their influence on dentinal tubule sealing, and consequently

Table 2. Mean values (standard deviation) of the percentage of median dentin permeability after treatment and post-erosive challenge.

Groups	%P Post-treatment		% Permeability Reduction	%P Post-erosive challenge		% Permeability Reduction
	Means (SD)	Median (IC 5-95%)		Means (SD)	Median (IC 5-95%)	
CSB	7.97 (6.31) ^{aA}	5.92 (3.45-12.49)	92.0	10.65 (8.43) ^{bA}	7.30 (4.61-16.68)	89.3
SBU	7.98 (5.26) ^{aA}	6.95 (4.21-11.75)	92.0	12.12 (9.16) ^{bA}	10.52 (5.56-18.68)	87.8
SB2	10.38 (10.62)^{aA}	6.54 (2.78-17.99)	89.6	11.94 (9.90) ^{aA}	7.22 (4.85-19.02)	88.0
SM	16.10 (10.19)^{aA}	14.52 (8.80-23.39)	83.9	15.61 (9.84) ^{aA}	12.05 (8.57-22.66)	84.3
C	104.29 (13.50)^{aB}	104.15(4.61-16.68)	-4.29	157.93 (77.49) ^{aB}	143.38(102.50-213.37)	-57,9

*Different lowercase letters, statistical difference between columns and different uppercase letters, statistical difference between lines ($p<0.05$)

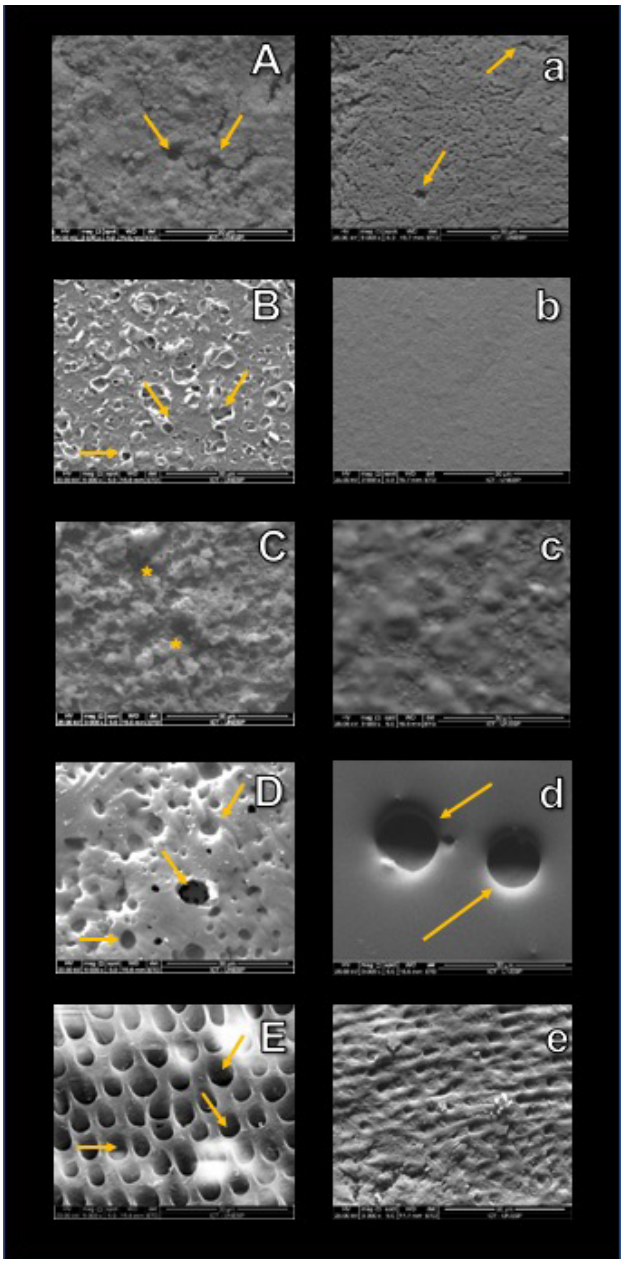

Fig. 2: Micrographs of the surfaces treated with the different adhesive systems before (uppercase letters) and after (lowercase letters) the erosive cycle: CSB – A. Irregular layer, porosity. a. Eroded layer presenting microporosity (arrows). SBU – B. Numerous pores b. More regular eroded surface layer. SB2 – C. Irregular layer with valleys and depressions (asterisk). c. More regular eroded layer. SM – D. Layer with numerous pores (arrow). d. Layer with dilated pores (arrow). C – E. Tubule opening and exposure of collagen fibers (arrow). e. Eroded dentin.

on dentinal permeability, is still a relevant object of study. In the present study, the first hypothesis was rejected, because after being applied on dentin, all the adhesive systems promoted a significant reduction in dentin permeability compared to the control group, without statistical differences among them. The second null hypothesis was also rejected, because after the erosive challenge, there was an increase in dentin permeability of the self-etching (CSB) and universal (SBU) systems.

Most studies have evaluated dentinal permeability by imaging techniques such as scanning electron microscopy (SEM),[13,18] transmission electron microscopy (TEM)[7,13], and confocal laser microscopy[13]. The evaluation of hydraulic conductance has been suggested as an adequate way to quantify dentin permeability. The evaluation of hydraulic conductance, even when associated with the intra-pulp pressure simulation [13], provides significant information regarding the behavior of materials on dentin. However, few studies have evaluated hydraulic conductance after dentin treatment with different adhesive systems.

As observed in the literature, regardless of the composition of the materials used, the adhesive systems [conventional, self-etching (multiple bottles) and universal (single bottle)] did not differ significantly and did not completely seal dentinal tubule fluid percolation[18], corroborating the study by Carvalho et al.[15] *In vitro* and *in vivo* evaluations demonstrate that adhesive systems applied on the dentin allow the dentin fluid to pass through the polymerized resinous materials[3], but their application significantly reduces dentin permeability[15,16].

Changes in the chemical and molecular structure of the dentin interface are observed with the use of 3-step adhesives such as Scotchbond Multi-Purpose or OptiBond FL and 2-step self-etching adhesives[3]. It has been reported that Clearfil SE, whose acidic primer is covered with a solvent-free, dimethacrylate-rich adhesive, could present a longer life than simplified adhesives that allow greater dentin fluid passage through the resin[3]. Application of the simplified adhesive system SB2 was expected to present higher permeability than the other systems studied. Being a simplified system, it contains solvents and hydrophilic components such as the polyalkene acid polymer, which has multiple pendant carboxylic acids along with a linear skeleton and tends to bind water to the adhesive as well as to prevent its penetration into the interfibrillar spaces, due to its high molecular weight[14]. However, a factor that contributed to the good performance of the simplified conventional adhesive was the fact that 2 layers of the adhesive were actively applied, waiting for solvent

evaporation, as recommended by the manufacturer. It is known that more than one layer of adhesive is recommended because a single layer promotes lower μTBS and higher permeability values[18].

Self-etching systems are widely indicated for pre-hybridization and sealing of dentin before indirect adhesive procedures [19] because they decrease sensitivity and protect the pulp by partially sealing the dentin[3]. Due to their wide acceptance and good clinical and laboratory results [19], two-bottle (CSE) and single-bottle (SBU) self-etching systems were expected to present better dentinal sealing values. Kamazu et al.[20] observed that two-step etch-and-rinse adhesive (Single Bond Plus) showed relatively stable dentin bond performance under all degradation conditions, and the three-step etch-and-rinse adhesive showed decreased dentin shear bond strength with prolonged degradation. The universal adhesive [Scotchbond Universal] used in normal dentin did not show any significant decrease in shear bond strength from the baseline under any degradation condition.

However, in our study, the smear layer was completely removed and the dentinal tubules were fully opened in order to evaluate hydraulic conductance, according to the studies that evaluate dentinal permeability[5]. In agreement with the methodology employed, in cases of dental erosion, it has been observed that dentin has a certain degree of demineralization, and microscopic evaluations show that teeth with dentin hypersensitivity present eight times more dentinal tubules per area unit and tubular diameter twice the size compared to non-sensitive teeth[21]. Therefore, the self-etching adhesives with 10-MDP could not react chemically with the mineral component of dentin [22], not establishing a strong chemical adhesion of the phosphate group with hydroxyapatite. Simplified adhesives used in etch-and-rinse mode are mainly characterized by hydrolysis and collagen degradation, while in self-etch mode, mainly hydrolysis of the polymeric matrix is observed[23]. The degradation of the hybrid layer occurs through the enzymatic degradation of its collagen fibrils by endogenous dentinal enzymes, such as the matrix metalloproteinases (MMPs) and cysteine cathepsins, and the leaching of the resin from the hybrid layer[4].

After the erosive challenge, chemical stress became more evident. The mechanical barrier failure for the CSB and SBU systems applied to demineralized dentin occurred due to the formation of a weak ionic bond of functional monomers with calcium. In contrast, the micro-tags formed by acid etching/ adhesive and the performance of a judicious technique promoted greater stability of dentinal tubule sealing for the SM and SB2 groups. After being submitted to the erosive challenge, restorative materials can show superficial degradation with reduction of surface roughness and microhardness[24]. The micrographs of the present study illustrated that the adhesive systems presented porosities, demonstrated by the presence of bubbles and cracks, becoming semi-permeable membranes when compared to the control sample (total opening of the tubules). And after the erosive challenge, the samples presented greater surface degradation.

Although single-bottle self-etching adhesive systems are more susceptible to hybrid layer degradation[23] and consequently to increased dentin permeability than are other types of adhesive systems, the 24-hour 5-day periods of the erosive challenge may not have been enough for hydrolytic degradation to occur and to result in a significant increase in permeability. Some studies suggest hydrolytic activity during the 6 months of water storage[25] , which means that from that time on, water must have been able to travel freely through the hybrid layer.

The results of this study enabled us to observe that, for immediate use, all materials when applied to demineralized dentin appeared to work as a mechanical barrier, presenting similar behavior. However, in the long-term, one must carefully analyze not only the monomeric composition of the materials, but also the quality of the substrate. Mainly when facing eroded dentin, the permeability of the substrate treated with self-etching systems may be compromised.

In conclusion, the use of conventional, self-etching, and universal adhesive systems reduces immediate dentin permeability by more than 80%[16]. The high degree of dentin demineralization may contribute to the increase in the permeability of universal and self-etching systems subjected to erosion challenge.

DECLARATION OF CONFLICTING INTERESTS
The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Resistance to fracture due to cyclic fatigue of stainless steel manual files and its association to surface roughness

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ABSTRACT
The aim of this study was to evaluate the possible association between the roughness of 5 brands of stainless steel endodontic files and their resistance to fracture due to cyclic fatigue. The study included five different brands of stainless steel endodontic files: SybroEndo Triple-Flex Files (Kerr, Glendora, USA), Ready Steel K-Flexofile (Dentsply Sirona, Ballaigues, Switzerland), Mani Flexile Files (Mani, Tochigi- Ken, Japan), FKG K-Files (FKG, La Chaux-de-Fonds, Switzerland) and Zipperer Flexicut Files (VDW, Munich, Germany). Twelve files per brand (total 60 files) were evaluated. File surface roughness over an area (Sa) was quantified using a focus variation microscope. Then the files were subject to a cyclical fatigue test to determine the number cycles to fracture due to fatigue and length of fractured fragment. Finally, fractographic analysis was performed using a scanning electron microscope. The electropolished Ready Steel K-Flexofile® files had the highest roughness according to Sa parameters, though they also had the highest resistance to fracture due to cyclic fatigue and the longest fractured fragment. Moderate positive correlation was found between fractured fragment length and roughness. The fractured surface showed characteristics of ductile fracture with cracks and plastic deformation. The electropolished stainless steel Ready Steel K-Flexofile® files were the most resistant to fracture due to cyclic fatigue even though they had highest surface roughness.
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Keywords: *dental instruments - endodontics - flexural strength - root canal preparation - stainless steel.*

Resistencia a la fractura por fatiga cíclica de las limas manuales de acero inoxidable y su relación con la rugosidad superficial

RESUMEN
El objetivo del presente estudio fue evaluar la posible asociación entre la rugosidad de 5 marcas de limas endodónticas de acero inoxidable y su resistencia a la fractura por fatiga cíclica. Se incluyeron cinco grupos diferentes de limas endodónticas de acero inoxidable: SybroEndo Triple-Flex Files (Kerr, Glendora, EE. UU.), Ready Steel K-Flexofile (Dentsply Sirona, Ballaigues, Suiza), Mani Flexile Files (Mani, Tochigi-Ken, Japón), FKG K-Files (FKG, La Chaux-de-Fonds, Suiza) y Zipperer Flexicut Files (VDW, Munich, Alemania); se evaluaron doce instrumentos por grupo para un total de 60 limas. Mediante un microscopio de variación focal se cuantificó la rugosidad superficial por área (Sa) de los instrumentos, posteriormente fueron sometidos a una prueba de fatiga cíclica donde se determinó el número de ciclos de fractura por fatiga y la longitud del fragmento fracturado. Finalmente, se realizó un análisis fractográfico mediante microscopía electrónica de barrido. Las limas Ready Steel K-Flexofile® con electropulido mostraron el mayor valor de rugosidad en los parámetros Sa; sin embargo, también tenían la mayor resistencia a la fractura por fatiga cíclica y la mayor longitud del fragmento fracturado del instrumento. Además, se encontró una correlación moderada positiva entre la longitud del fragmento fracturado y la rugosidad. La superficie fracturada mostró características de fractura dúctil con grietas y deformación plástica. Las limas Ready Steel K-Flexofile® fabricadas en acero inoxidable con electropulido, fueron más resistentes a la fractura por fatiga cíclica a pesar de tener la mayor rugosidad superficial..

Palabras clave: *acero inoxidable - endodoncia - fatiga cíclica - instrumentos dentales - preparación del conducto radicular.*

INTRODUCTION

Endodontic files have surface features, resulting from their manufacturing process, which affect their resistance to fracture due to cyclic fatigue. The presence of surface roughness on endodontic files has been reported to concentrate stress, giving rise to areas where cracks initiate during clinical use[1], leading to fracture due to cyclic fatigue[2].

To date, mechanical, physical and heat surface treatments have been reported for stainless steel alloys –including sanding, grinding, abrading, electropolishing, nitriding, and combined plasma carburizing, among others– to alter surface texture and improve properties such as microhardness and resistance to fracture due to fatigue of the alloy[3-5].

However, in contrast to nickel-titanium files, stainless steel alloy files have not often been subjected to procedures to improve their properties, even though they are so frequently used in endodontic treatment. In general, there are reports of prevalence of fractures in stainless steel endodontic files of up to 7.4%[6], which justifies the development of manufacturing techniques which could increase their useful life.

For nickel-titanium instruments, it has been reported that low surface roughness is associated to higher resistance to fracture due to cyclic fatigue[7]. However, for stainless steel alloys, electropolishing may not be associated to an increase or decrease in surface roughness, because it may produce a regular surface with micro-deformation that is not necessarily detrimental to resistance[8].

Although it has been reported that surface treatment of stainless steel medical devices has a significant effect on roughness, it is not clear whether such roughness causes higher or lower resistance to cyclic fatigue[9].

There is no report in the literature on evaluation of surface roughness of stainless steel endodontic files, and therefore no report analyzing the association between their roughness and fracture due to cyclic fatigue. The aim of this study was thus to assess the possible association between surface roughness and resistance to fracture due to cyclic fatigue in 5 brands of stainless steel endodontic files. The null hypothesis is that there is no difference in roughness and resistance to fracture due to cyclic fatigue in the stainless steel endodontic files evaluated.

MATERIAL AND METHODS

This was an experimental in vitro study. Probabilistic sample size was calculated with a power of 80% and significance level 5%. Sample size per group was thus 12 manual stainless steel endodontic files No. 25, with length 25 mm long and taper 0.02, which had not been used previously. Total sample consisted of 60 files of 5 different brands: A: SybroEndo Triple-Flex Files (Kerr, Glendora, USA); B: Ready Steel K-Flexofile (Dentsply Sirona, Ballaigues, Switzerland); C: Mani Flexile Files (Mani, Tochigi- Ken, Japan); D: FKG K-Files (FKG, La Chaux-de-Fonds, Switzerland); E: Zipperer Flexicut Files (VDW, Munich, Germany).

Surface finish/roughness

Three-dimensional measurements were considered to analyze the topography of the stainless steel using *Sa* area parameters. This analysis was performed using an Alicona Infinite Focus G5 focus variation microscope (FVM) (IFM, Alicona Imaging, Grambach, Graz, Austria). The handle of each file was attached to a mini chuck (Alicona Imaging, Grambach, Graz, Austria) adapted to the microscope (Fig. 1). Each file was adjusted in the 3D rotation unit. The instrument was positioned and focused, taking a value of 0 for the coordinates of axes X, Y and Z. It was moved automatically towards the lens at a distance of -3.5 mm on axis X to locate it on the respective point measured at 20x, after which the 160 µm * 160 µm measurement window was defined. The *Sa* parameters were measured using the *Surface Texture Measurement* module of the MeX 6.1 software (Alicona Imaging, Grambach, Graz,

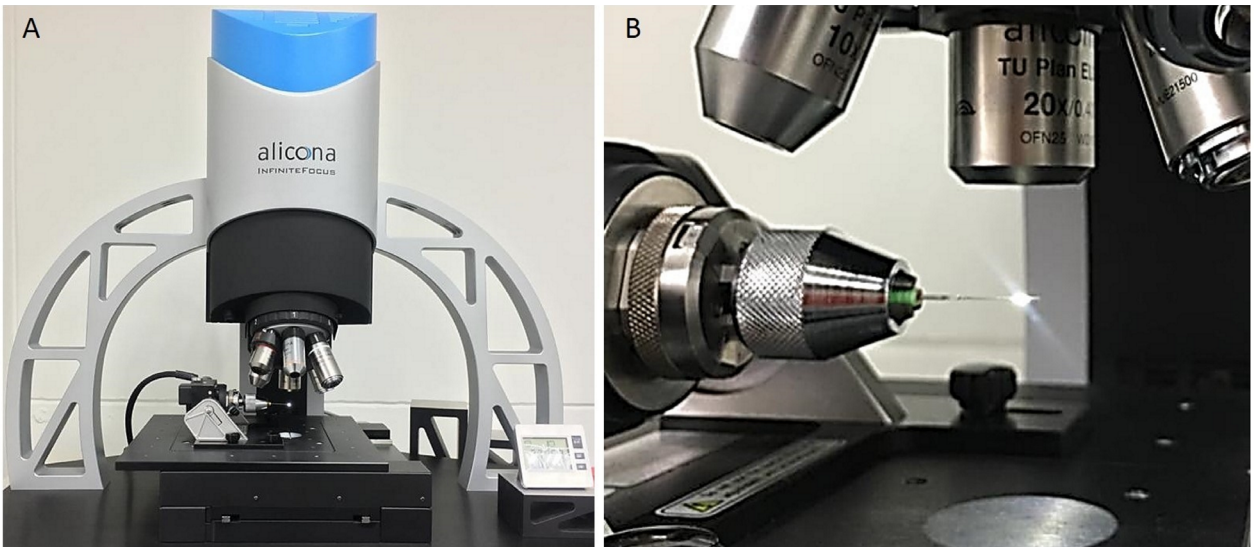

Fig. 1: A. Focus variation microscope (FVM). B. Endodontic file attached to the 3D rotation unit of the FVM by means of a chuck.

Austria). Surface roughness over an area (*Sa*) was evaluated for each file at four sites: 3.5 mm, 4.5 mm, 5.5 mm and 6.5 mm from the tip of the file.

Cyclic fatigue

Cyclic fatigue was tested using an ad hoc device (Fig. 2A) which included a simulated root canal made of stainless steel, designed and built as described by Lopes et al.[7] and Rodrigues et al.[10], with curvature 86°, diameter 1.5 mm, radius of curvature 6 mm and total length 21 mm. The curved segment of the canal measured 9 mm, the longest straight segment 8 mm and the shortest straight segment 4 mm.

Each file was placed in a mini chuck (Electric Drill Bit Collet, Mini Twist Drill Tool Chuck Set Pretty RF, Koyot, Shangai, China) adapted to a shaft and powered by an endodontic motor (Fig. 2B). The active parts of the manual files were placed and secured in this device. Once in position, a dynamic cyclic fatigue test was conducted using the TriAuto ZX rotary motor (J. Morita Corp., Kyoto, Japan) at 280 rpm in continuous rotation with axial in-and-out movement of the file, with stroke 4mm and frequency 1/3 Hz, until the file fractured. Synthetic lubricant (WD-40 Company, Milton Keynes, England) was used in all groups to reduce friction.

Cyclic fatigue time to fracture was measured with a digital chronometer. Number of cycles to fatigue (NCF) for each file was calculated by multiplying time to fracture (s) by motor rotations per minute (rpm), divided by 60[11]. The fractured

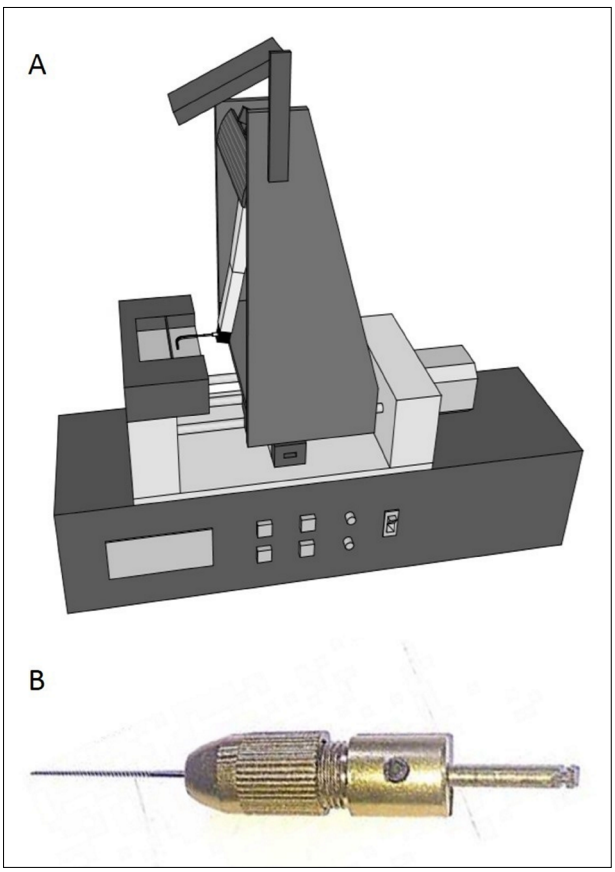

Fig. 2: Devices used to evaluate cyclic fatigue. A. Diagram of the cyclic fatigue device used. B. Mini Twist Drill Tool with machined adjustment for use in endodontic rotary motor.

fragment of each file was measured with a digital caliper (Digimess, Sao Paulo, SP, Brazil).

Scanning Electron Microscopy

To observe the features of the fractured surface, one file per group was selected randomly and cleaned ultrasonically to eliminate debris, and examined with a TESCAN VEGA 3 scanning electron microscope (Libusina tr, Brno-Kohoutovice, Czech Republic) at 500x magnification.

Statistical analysis

Data were initially analyzed with Shapiro-Wilk's test, and showed non-normal distribution *(p= 0.001)*. Then, the Kruskal-Wallis test was applied to analyze roughness, file time to fracture and NCF. Finally, Spearman's correlation coefficient test was applied between file roughness and fracture level to determine whether they were associated. Statistical significance level was 5%. Software was Stata 12 (StataCorp, Texas, USA).

RESULTS

Table 1 shows the results for roughness by evaluation with FVM, cyclic fatigue, length of fractured fragment and number of fractured cases per level (distance from tip). The Ready Steel K-Flexofile® files had the highest roughness (*Sa*) (p= 0.0001), highest resistance to fracture due to cyclic fatigue (p= 0.0001) and longest fractured fragments (p= 0.000).

A 54% association was found between file roughness and fracture level according to Spearman's test, indicating moderate positive correlation (rho= 0.5440, p= 0.000). This means that the fractured fragments of the least rough files were closer to the tip of the instrument, whereas the fractured fragments of the roughest files were farther from the tip of the instrument.

Figure 3 shows the surface roughness evaluated by FVM for the different groups of stainless steel files. After fractographic analysis of the surface, SEM can be used to observe the characteristics of ductile fractures with cracks, plastic deformation and fibrous areas with dimples (Fig. 4).

Table 1. Results for roughness (Sa), NCF, Length of fractured fragment and Number of fractured cases per level for the groups analyzed

Groups		A Sybro Endo Triple-Flex Files	B Ready Steel K-Flexo-file	C Mani Flexile	D FKG K-Files	E Zipperer Flexi-cut Files
Roughness (µm)	Mean ± SD*	0.383 ± 0.01	0.709 ± 0.120	0.441 ± 0.030	0.289 ± 0.010	0.456 ± 0.030
	P value	*<0.001*	*<0.001*	*0.003*	*<0.001*	*0.004*
NCF**	Mean ± SD*	2,091 ± 563	3,369 ± 788	1,587 ± 449	354 ± 212	953 ± 295
	P value	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*
Length of fracture from the tip (mm)	Mean ± SD*	3.14 ± 0.92	4.97 ± 0.77	4.89 ± 1.00	3.2 ± 0.33	4.57 ± 0.66
	P value	*<0.001*	*<0.001*	*1.000*	*<0.001*	*1.000*
Number of cases fractured per level	*a*	9	0	2	10	1
	b	2	5	1	2	4
	c	1	2	4	0	7
	d	0	5	5	0	0

Measurement level (distance from tip)
a: 3.5 mm
b: 4.5 mm
c: 5.5 mm
d: 6.5 mm

* Arithmetic mean ± Standard deviation; ** Number of cycles to fracture.
Significant association at 5% *(P<0.05)*

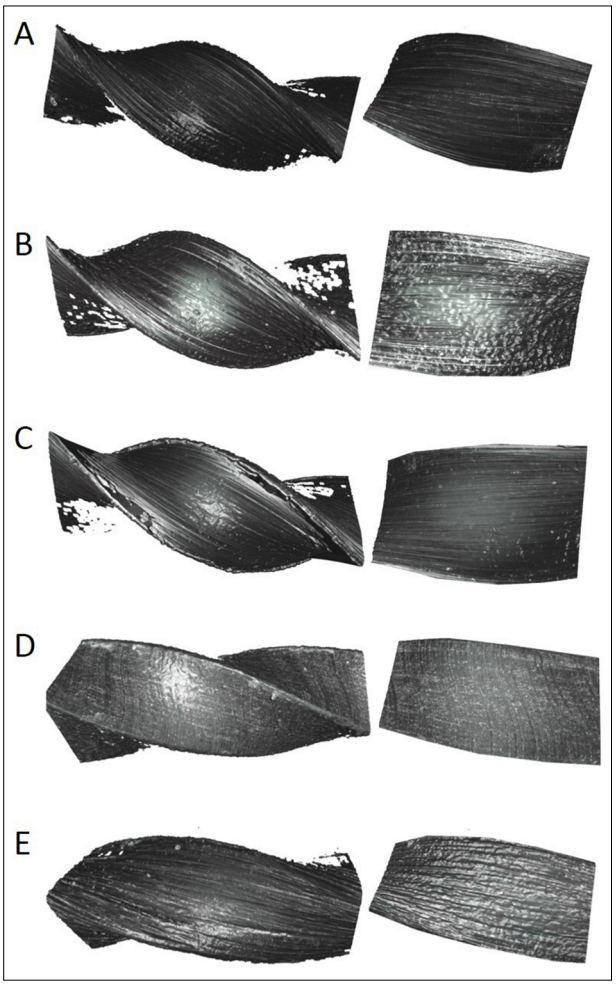

Fig. 3: Topographic images taken with FVM at 20x magnification (left) and their respective 160 x 160 µm fields of measurement (right) for the 5 groups evaluated. A. SybroEndo Triple-Flex Files®; B. Ready Steel K-Flexofile®, which had the greatest roughness; C. Mani Flexile Files®; D. FKG K-Files®, which had the lowest roughness; and E. Zipperer Flexicut Files®.

DISCUSSION

Stainless steel endodontic files are useful for endodontic treatment because they provide greater tactile sensitivity than nickel-titanium files, which is helpful for determining the internal anatomy of the root canal[12]. Moreover, the use of stainless steel is needed as a complement to the handling protocols of various nickel-titanium rotary systems[13-15].

The concept of cyclic fatigue has been adopted in endodontics regarding the application of repeated alternate tension and compression forces[16]. Based on this idea, purely mechanical tests have been proposed to evaluate whether parameters such as heat treatments[17], speed of rotation[18] or roughness[7] can affect file resistance to fracture due to cyclic fatigue in endodontics. Thus, even though the current study evaluated stainless steel files, tests like the ones used in the current study for evaluating cyclic fatigue in endodontic files are valid and have been widely reported in the literature on endodontics[7,10,19].

The fatigue model used in the current study at ambient temperature provides a valid, reproducible simulation of the clinical setting, since, according to recently reported information[20], body temperature does not interfere with the result of the cyclical fatigue test. Body temperature is counteracted by factors such as the low thermal conductivity of dentin[21] and the cooling action of the irrigant within the root canal[22], which allow the fatigue test to be performed at ambient temperature without altering its clinical relevance[20].

Haikel et al.[23] evaluated the resistance to cyclic fatigue of six types of stainless steel manual files in a simulated root canal at 60° in dynamic fatigue, finding that Flexofile® instruments had the greatest resistance to cyclic fatigue. This is

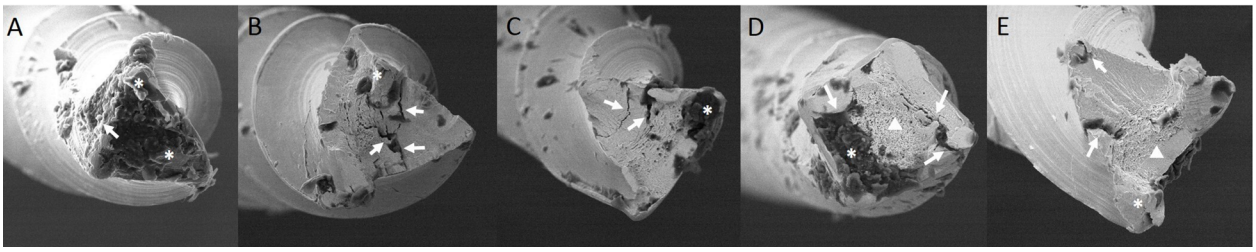

Fig. 4: Scanning electron microphotographs of the fractured surface of the 5 groups analyzed at 500x magnification. A. SybroEndo Triple-Flex Files®; B. Ready Steel K-Flexofile®; C. Mani Flexile®; D. FKG K-Files®; and E. Zipperer Flexicut Files®. The images show ductile fractures with cracks (white arrows), plastic deformation (asterisks) and fibrous areas with presence of dimples (arrowheads).

similar to our results, which also found that the group Ready Steel K-Flexofile® had the greatest resistance to fracture due to cyclic fatigue.

Gambarini et al.[24] compared the resistance to cyclic fatigue between nickel-titanium Pathfile® files and stainless steel k-type files for use with the reciprocating handpiece M4®, also in a simulated 60° stainless steel root canal. They found that M4 stainless steel files were more resistant to fracture due to cyclic fatigue. Although it is clear that their reciprocating motion had definitive impact on the results, they present stainless steel files as a possible alternative for rotatory mechanical instrumentation. These findings agree with our results, as we too found that stainless steel endodontic files had good resistance to fracture due to cyclic fatigue.

Piasecki et al.[25] reported an experiment with stainless steel and carbon steel Pathfinder® instruments in double curved root canals (middle third 60° and apical 70°), also using the M4® handpiece with reciprocating motion, finding no significant difference between the two groups evaluated. Our results showed a longer time to fracture due to cyclic fatigue, comparable to the time reported by Piasecki et al.[25] for the group of stainless steel files. However, it is important to consider that we performed the evaluation in a continuous rotary motion rather than the reciprocating motion used by Piasecki et al[25].

Roughness has generally been evaluated in profile or area parameters. In the literature, the most frequently used profile parameter is *Ra*, and the most frequently used area parameter is *Sa*[26]. The following advantages have been reported for the *Sa* parameter over the *Ra* parameter: better topographic evaluation of three-dimensional surface; *Ra* roughness parameter depends on the direction in which the profile is drawn, while *Sa* roughness parameter takes the average of the spikes in a given area, so its results have been considered more reliable[26,27].

In contrast to nickel-titanium files, which are manufactured by machining, stainless steel files are manufactured by torsion. In nickel-titanium files, the electropolishing procedure is intended to smooth or eliminate cracks or defects that may remain as a result of machining[7]. The results of electropolishing of the nickel-titanium alloy has been evaluated by SEM and surface roughness analysis[7,28].

Although the Ready Steel K-Flexofile® manufacturers say they electropolish these stainless steel files (Bolle A, 2018, communication from the manufacturer, unpublished data), we found that these were the files with the greatest surface roughness. Nevertheless, the same group –Ready Steel K-Flexofile®– had the greatest resistance to fracture due to cyclic fatigue. One possible explanation is the shape and appearance of the roughness on these files, which was found by FVM to have no cracks, angles or edges that might concentrate tensions, thereby contributing to initiation of crack formation. On the contrary, this roughness presents regularly distributed, rounded plastic surface micro-deformations, probably as a result of some type of electropolishing, which may induce greater resistance to cyclic fatigue by introducing residual compression stress. It has been shown that the aforementioned procedures for stainless steel alloy, such as electropolishing, are not necessarily associated to less roughness[8].

With regard to the presence of residual compression stress, Yibo et al.[29] reported the use of ultrasonic cavitation in stainless steel alloys, which increased surface roughness while at the same time generating residual compression stress that increases microhardness and resistance to cyclic fatigue. Moreover, Kadarno et al.[30] reported a mechanical micro-punching process on stainless steel sheets that resulted in a regular plastic deformation surface that increased resistance to cyclic fatigue. The same authors mentioned that residual compression forces are responsible for this greater resistance to cyclic fatigue.

Abbott et al.[31] observed that stainless steel surfaces present regular undulations and variations

after manufacturing and electropolishing processes, which is consistent with our findings, in which FVM shows regularly distributed undulating surface deformations, which may be attributed to an electropolishing process in stainless steel files.

We suggest that, in contrast to residual tensile stresses, which are detrimental to material resistance and promote crack formation[32], the presence of residual compressive stresses in the surface of a metal such as endodontic file stainless steel may prevent the propagation of cracks occurring as a result of repeated stress, thereby reducing the likelihood of fracture due to cyclic fatigue.

In contrast to our results, lower roughness has been reported to be associated to resistance to cyclic fatigue. Silva et al.[33] reported that heat treatments reduce roughness and increase resistance to cyclic fatigue of rotary nickel-titanium files. Likewise, Vaz-García et al.[34] reported that the smooth surface of nickel-titanium endodontic files is associated to a lower likelihood of cracks initiating due to fatigue and leading to instrument fracture[34]. Lopes et al.[7] found that instruments treated with surface polishing, which are therefore smoother, are more resistant to cyclic fatigue than instruments not subjected to polishing. The difference from our results may be explained by two factors: (1) the manufacturing process, since stainless steel files are manufactured by torsion, while nickel-titanium files are machined, and (2) the difference may lie in the material itself, i.e., the mechanical properties of stainless steel are different from those of nickel-titanium, which may affect behavior under cyclic fatigue.

One limitation of this study was not being able to determine the type of electropolishing procedure performed by the manufacturer for the Ready Steel K-Flexofile® group, because it is industry confidential information and therefore unavailable to the public. Thus, all the findings described herein were deduced based on observations of the surface of the material and on the cyclic fatigue resistance assay.

The fracture surface characteristics observed were similar to those reported by Haikel et al.[23], who also observed stainless steel file fractures by SEM. Our study observed presence of ductile fracture and fibrous areas with presence of dimples characteristic of overload and plastic deformation.

Evaluation of resistance to fracture due to cyclic fatigue of different nickel-titanium rotary files has found that the instrument with the longest fractured fragment was the one with highest resistance to fracture due to cyclic fatigue[35,36]. These results agree with ours, since we too found that the file with the longest fragment was the one with greatest resistance to fracture due to cyclic fatigue.

Based on our search in the literature, this is the first article to relate surface roughness to cyclic fatigue resistance in stainless steel files. It also uses focus variation microscopy to evaluate the topography of surface roughness of files, of which the main use is representing roughness by means of *Sa* area parameters.

Further studies are needed to evaluate resistance to dynamic and torsional cyclic fatigue in rotary and manual endodontic files in reciprocating motion or continuous rotation.

To conclude, files in the Ready Steel K-Flexofile® group have significantly higher resistance to fracture due to cyclic fatigue than the other instruments evaluated, as well as significantly higher roughness. As moderate positive correlation was found between length of the fractured fragment and roughness.

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Structural resistance of orthodontic mini-screws inserted for extra-alveolar anchorage

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ABSTRACT

The risk of fracture or strain in mini-screws is higher if diameter, length, type of alloy or insertion angle is selected inappropriately. The aims of this study were to test the structural resistance of two types of orthodontic mini-screws –one made of stainless steel and another of titanium– from an international brand and to evaluate the efficacy of two other titanium mini-screws of Brazilian origin, during an extra-alveolar anchorage procedure. The mini-screws analyzed were: Bomei stainless steel and Bomei titanium / Taiwan, Morelli titanium and Neodent titanium/ Brazil. Experiments were conducted on pig mandibles to simulate the process of extra-alveolar anchorage. Two insertion processes were used: Direct at 30º, and Indirect, starting at 60º and ending at 30º with gradual continuous movement. Strain was evaluated using Optical and Scanning Electron Microscopy. Data were evaluated using Kruskal-Wallis non-parametric statistical analysis and post hoc Tamhane test. Significant statistical differences in strain were observed among the mini-screws used in the extra-alveolar insertions, both for the direct and indirect procedures. In the indirect insertion tests, both stainless steel and titanium mini-screws suffered deformation, showing that angling can be an important factor in mini-screw failure rates. The change in angle during the insertion movement increased deformation rates independently of alloy type, increasing the risk of failure. These results could help orthodontists in choosing mini-screws for extra-alveolar anchorage, which can be performed with direct or indirect insertion. In vivo studies should be conducted to confirm the findings of this study.

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Keywords: *orthodontics - orthodontic anchorage techniques - dentistry.*

Resistência estrutural de mini-aparafusos ortodônticos inseridos para ancoragem extra-alveolar

RESUMO

O risco de fratura ou tensão nos mini-parafusos é maior se o diâmetro, comprimento, tipo de liga ou ângulo de inserção for selecionado de forma inadequada. Os objetivos deste estudo foram testar a resistência estrutural de dois tipos de mini-aparafusos ortodônticos –um feito de aço inoxidável e outro de titânio– de uma marca internacional e avaliar a eficácia de dois outros mini-parafusos de titânio de origem brasileira, durante um procedimento de ancoragem extra-alveolar. Os mini-aparafusos analisados foram: Bomei aço inoxidável e Bomei titânio / Taiwan, Morelli titânio e Neodent titânio / Brasil. Foram realizadas experiências em mandíbulas de porcos para simular o processo de ancoragem extra-alveolar. Foram utilizados dois processos de inserção (Direta a 30º, e Indireta, começando a 60º e terminando a 30º com movimento gradual contínuo). A deformação foi avaliada utilizando Microscopia Óptica e Microscopia Eletrônica de Varredura. Os dados foram avaliados utilizando análise estatística não paramétrica Kruskal-Wallis e teste post-hoc Tamhane. Diferenças estatísticas significativas na deformação foram observadas entre os mini-parafusos utilizados nas inserções extra-alveolares, tanto para os procedimentos diretos como indiretos. Nos testes de inserção indireta, tanto os mini-parafusos de aço inoxidável como os de titânio sofreram deformação, mostrando que a angulação pode ser um fator importante nas taxas de falha dos mini-parafusos. A mudança no ângulo durante o movimento de inserção aumentou as taxas de deformação independentemente do tipo de liga, aumentando o risco de falha. Estes resultados podem ajudar os ortodontistas na escolha de mini-parafusos para ancoragem extra-alveolar, que pode ser realizada com inserção direta ou indireta. Estudos in vivo devem ser conduzidos para confirmar os resultados deste estudo.

Palavras-chave: *ortodontia - técnicas de ancoragem ortodôntica - odontologia.*

INTRODUCTION

Skeletal anchorage has been widely used in orthodontics because it does not allow movement of the reaction unit, providing satisfactory results for anchorage control with less discomfort to the patient[1]. To be considered ideal, a skeletal anchorage system must have certain characteristics, such as easy installation, resistance to orthodontic forces, simple removal, small size, and being ready for early activation to minimize treatment time and maximize efficiency[2].

According to the literature, among the means for achieving skeletal anchorage, the orthodontic mini-screw technique has shown the most significant clinical applicability compared to other skeletal anchorage systems such as integrated bone implants or mini-plates[3].

Orthodontic mini-screws have distinct shapes and sizes. Most of them are manufactured in titanium alloy grade V (Ti-6Al-4V) specified by the ASTM F136 standard[4]. Skeletal anchorage can also be achieved using AISI 316L Austenitic stainless steel[5] Skeletal anchorage can be interradicular or extra-alveolar. In interradicular anchorage, mini-screw insertion requires positioning guides with local radiographs and correct root inclination. Direct insertion, on a site with low bone thickness, should be performed avoiding inclination movements[6].

In extra-alveolar anchorage, mini-screws are inserted into a great bone thickness. Inclination and torsions movements are usually necessary to avoid contact with dental roots. Extra-alveolar anchorage has been widely used because it is efficient in the treatment of certain deformities, especially for retractions of the entire upper or lower dentoalveolar complex[7]. However, mini-screws must be used adequately, considering the loads involved and their mechanical properties and structural resistance[8]. It is therefore essential to know the magnitude of the torques involved, and local anatomical structures. Gingival tissue and bone density must be taken into account as factors that can influence the installation of anchorage[9]. Professional experience is important at the time of selecting and using mini-screws, to minimize mini-screw losses and iatrogenic injury. Dental surgeons still have doubts about which mini-screw system to use, due to the high use of titanium, mainly in extra-alveolar anchorage, which requires a significant level of torque and angulation to avoid root contact.

The aim of this study was to test the structural strength of mini-screws of different brands using an *in vitro* model. Pig mandibules were used to test the ability of mini-screws to withstand insertion/removal torques without suffering deformation or fracture during the extra-alveolar insertion procedure.

MATERIALS AND METHODS

Fourty orthodontic mini-screws with the same diameters and lengths were used: 20 made of stainless steel (OBS Bomei/Taiwan, 2.0mm in length, 12 mm diameter (Gold standard) and 20 made of titanium OBS Bomei/Taiwan (2.0 x 12 mm). We also tested another 40 titanium mini-screws of two popular Brazilian brands: 20 Morelli/Brazil (1.5 x 10 mm) and 20 Neodent/Brazil (1.6 x 11 mm), in the largest diameters and lengths available, but with smaller diameter and lengths than gold standard. We evaluate their capabilities in extra-alveolar inserts, since they are designed and manufactured for interradicular insertion. Fig. 1 shows the geometry of the tested mini-screws, and Table 1 shows the main characteristics of the mini-screws used in the extra-alveolar insertion tests.

Experimental Setup for Extra-Alveolar Anchorage Tests

Extra-alveolar anchorage was simulated in the region of the buccal shelf with great bone thickness in pig mandibles. The density and bone metabolism of pig mandible are similar to those of human bones[10], which makes them compatible for in vitro analysis. Eighty fresh mandible semi-arches of adult pigs with approximate weight and size were obtained from a slaughterhouse (Frigorífico São Pedro-Uberlândia/MG-Brasil). During the procedures the mandibles were stored in a refrigerator at 6ºC to 10ºC.

A reference system (positioners made of 0.17” x 0.25” stainless steel wires) was used for positioning the angles (30º and 60º) during the insertion

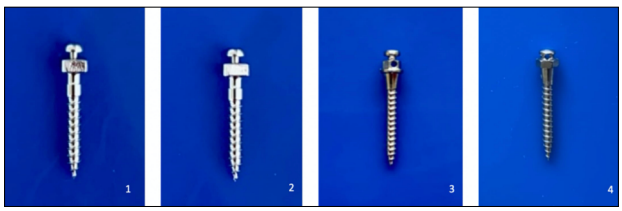

Fig. 1: Geometric configuration of the mini-screws used in the tests. 1: MPO Bomei Titanium; 2: MPO Bomei Stainless Steel; 3: MPO Morelli Titanium; 4: MPO Neodent Titanium.

Table 1. Characteristics of the mini-screws used in the experimental tests.

Manufacturer	External Diameter (mm)	Length (mm)	Alloy	System	Pitch (mm)
Morelli	1.5	10.0	Ti 6Al-4V	Self-Drilling	0.65
Neodent	1.6	11.0	Ti 6Al-4V	Self-Drilling	0.73
Bomei	2.0	12.0	Ti 6Al-4V	Self-Drilling	0.75
Bomei	2.0	12.0	Stainless Steel SS - 316L	Self-Drilling	0.75

procedure (Figure 2a, 2b, 2c). Positioners were fixed between the selected molars to guide the initiation of the process with the appropriate inclination.

The pig mandibles were fixed in a vice supported on a bench. A single experienced surgeon inserted the mini-screws at the mucogingival junction in the posterior region between the 1st and 2nd molars, into the external oblique line (buccal shelf), using the insertion tools provided by each manufacturer. Two insertion procedures were carried out:

- **Direct insertion at 30º:** The mini-screws were positioned at 30º relative to the long tooth axis and inserted in this position up to the end of the thread or beginning of the transmucosal profile (Fig.3a). Ten direct insertion procedures were performed at 30º for each type of screw tested.
- **Indirect insertion at 60º positioning, ending at 30º:** This procedure begins by positioning the mini-screws initially at 60º in relation to the long tooth axis (Fig. 3b). After inserting approximately 2 mm, the mini-screws are gradually inclined to the 30º position, while inserting up to the end of thread or beginning of the transmucosal profile, according to the extra-alveolar technique recommended by Chang[7]. Thereby, there is a gradual bending effect on the mini-screw during the insertion process. Ten indirect insertion procedures were performed at 60º for each type of screw tested.

The mini-screws were then removed from the mandibles by the same surgeon using the same tool as for the insertions.

Optical Microscopy and Scanning Electronic Microscopy (SEM)

Mini-screw strain was analyzed using a CLS 100 Leica optical microscope at magnifications of 1.0, 1.6, 2.5 and 4.0 X, considering head, transmucosal profile, thread and tip.

Strain was then accurately analyzed using scanning electron microscopy of the samples which were mounted on aluminum bases with double-sided carbon tape, in an EVO model MA 10 (Zeiss, Germany) in the high vacuum range. Photomicrographs at 28x magnification of the head, transmucosal portion and thread portion of the mini-screws were used

Statistical analysis

Strain was evaluated only as present or absent, since any level of strain that occurred could compromise

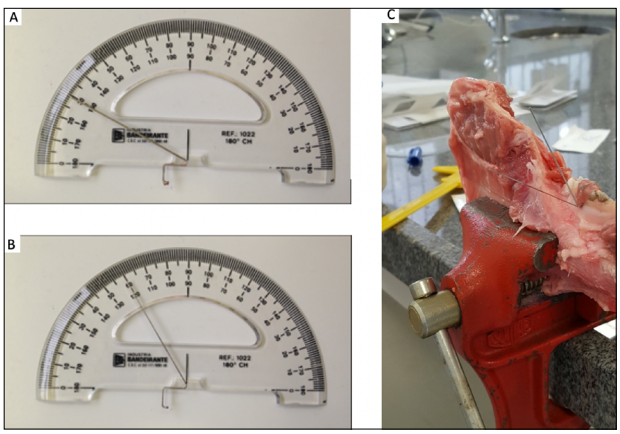

Fig. 2: Positioners made of 0.17" x 0.25" stainless steel wires a) 30°, b) 60°, c) in position between molars.

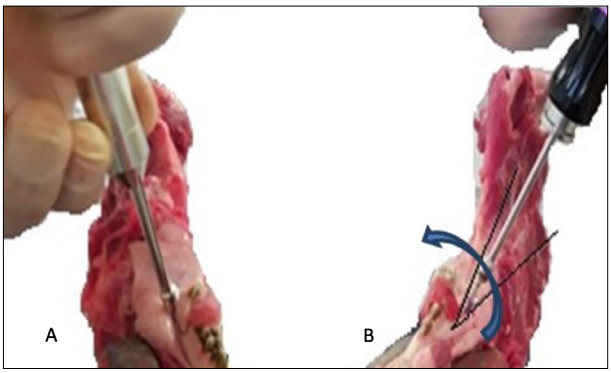

Fig. 3: Insertion of the mini-screws using a directly process (A) and an indirectly process (B).

the quality of extra-alveolar anchorage. Each type of mini-screw was compared to controls without strain considering the regions analyzed. Deformed and undeformed mini-screws were counted to evaluate the level of structural resistance.

Score 0 (zero) was assigned to mini-screws which remained intact and score 1 (one) for mini-screws which suffered any strain or fracture. This procedure was used because from a structural standpoint, any level of strain could lead to early failure of the mini-screw or change the stabilization conditions expected in an extra-alveolar anchorage process. Gold standard 2.0 x 12 mm stainless steel mini-screws recommended in the Chang technique[7] were compared to titanium alloy mini-screws of the same diameter and length, both made by the same manufacturer (Bomei-Taiwan). Two other titanium mini-screws manufactured by Morelli-Brazil (1.5 x 10mm) and Neodent-Brazil (1.6 x 11mm), which were smaller in diameter and length, were also evaluated.

The Kruskal-Wallis non-parametric test was applied. After confirming statistical differences, the Tamhane post hoc test was used to reduce statistical errors and to determine which pairs presented significant statistical differences ($p < 0.05$).

RESULTS

Figs. 4 and 5 show the strain and fractures in the mini-screws evaluated by SEM microscopy. The steel mini-screws did not suffer deformations during the direct insertion process at 30º, showing the greater resistance in this type of fixation.

Taken as a whole, the number of strained titanium mini-screws was high among those subjected to direct insertion, and even higher in those subjected to indirect insertion. Statistically significant difference was found between percentages of strained and non-strained groups. (Fig 6 and Table 2).

Evaluation of strain generated by direct insertion showed that the structural condition was better in stainless steel mini-screws than in titanium mini-screws (Table 3).

With Indirect insertion no statistically significant difference was found between the stainless steel and titanium mini-screws (2.0 x 12 mm), which both showed some deformations.

Contrary to expectations, the longer and larger diameter mini-screws (2.0 x 12 mm) suffered fewer alterations than those of shorter and larger diameters (1.5 x 10 mm and 1.6 x 11 mm), with both insertion procedures.

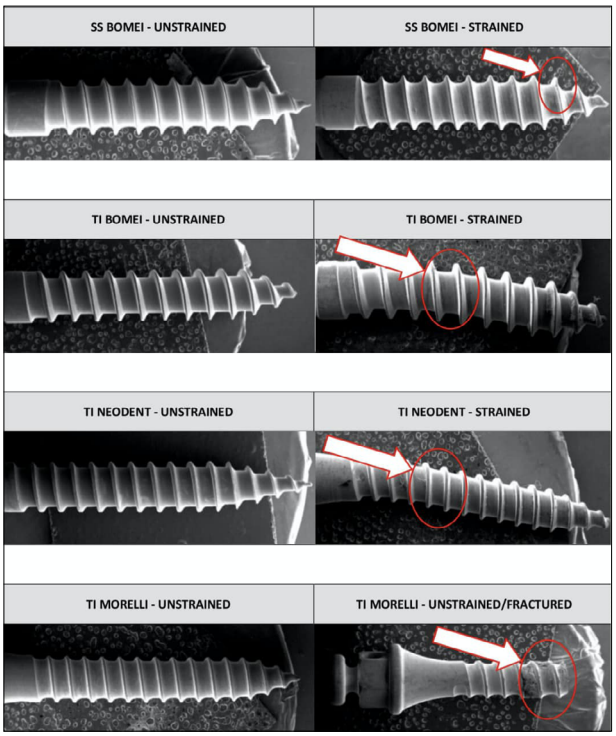

Fig. 4: Mini-screws strained and unstrained under SEM analysis.

DISCUSSION

The aim of this study was to evaluate the performance of Morelli/Brazil mini-screws (Ti 1.5 x10mm) and Neodent/Brazil mini-screws (Ti 1.6 x11mm) in extra-alveolar insertion and to test and compare the

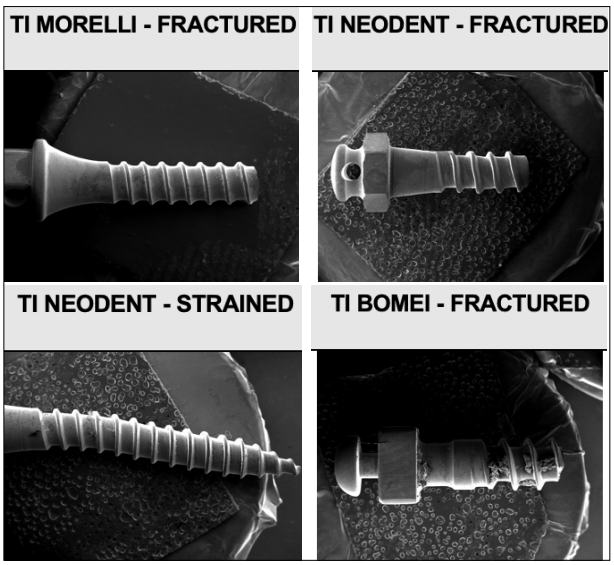

Fig. 5: SEM microscopy of mini-screws inserted by direct technique.

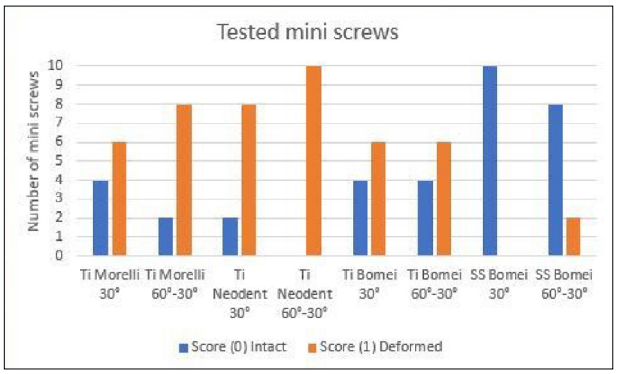

Fig. 6: a) Amounts of strained and strained mini-screw after direct and indirect insertion.

structural resistance of two selected OBS Bomei/ Taiwan mini-screws, Ti 2.0 x12mm and SS 2.0 x12mm, the latter recommended by Chris Chang[7], who was a precursor of the extra-alveolar insertion technique.

Pig mandibles used as a model for extra-alveolar skeletal anchorage were chosen because their bone density and metabolism are similar to those of human bone[10].

We propose to evaluate the outcome of direct and indirect insertion with different mini-screws, to determine how suitable they are for this procedure and to reduce the risks of early failure. When extra-alveolar anchorage is used in orthodontic treatment, several factors should be considered for the choice of the mini-screws, such as the bone thickness of the insertion site, the alloy, the diameter and length and the angle of insertion, and whether to use a direct or indirect process[11-14].

The insertion sites in thick cortical bone, requires high torques for insertion and removal[15]. Appropriate bone density and thickness are critical for the success of the mini-screws[16-18] to ensure the greater stability[19-21] and higher torques[22].

Since torque magnitude, for insertion and removal of orthodontic mini-screws, depends on cortical bone thickness[23,24], areas with greater bone thickness should be selected in order to achieve better stability[25]. However, torque should not be excessive, since higher levels may cause bone tissue damage and necrosis, as well as fractures or strains in the mini-screws, thus compromising stability.

Among the longest and largerst diameter mini-screws, only 35% suffered alterations, while 80% of the shorter and smaller diameter screws showed deformation or fracture. Awareness of the insertion site is therefore important, since bone density and thickness are essential factors for instability and increase of torques.

Table 2. Statistics for direct and indirect insertion using Kruskal-Wallis Test.

Statistical parameter	Deformed condition	
	Direct Insertion	Indirect Insertion
Chi-square	14.04	15.00
Degrees of Freedom	3	3
Significance Level (p<0.05)	0.003	0.002

Table 3. Comparative analysis of direct and indirect insertion using the post hoc Tamhane test.

Direct Insertion	
Mini Screws Compared	**Significance (p<0.05)**
Bomei Steel - Morelli Ti	0.030
Bomei Steel – Neodent Ti	0.001
Bomei Steel – Bomei Ti	0.030
Indirect Insertion	
Mini Screws Compared	**Significance (p<0.05)**
Bomei Steel - Morelli Ti	0.031
Bomei Steel – Neodent Ti	0.001
Bomei Steel – Bomei Ti	0.372

The mechanical properties of materials are also essential for greater safety against failure of mini-screws[26,27]. The chosen material must have sufficient mechanical strength to withstand stresses due to the effect of torsion and bending on the threads during placement and clinical removal, without permanent strain[28,5]. Most orthodontic mini-screws for anchorage are manufactured from titanium alloys or stainless steel. They both meet the biomechanical requirements for anchorage[29]. However, stainless steel mini-screws have proven to be more resistant to failure than titanium[30,31].

In thicker corticals, it was observed that insertion and removal torque are greater for titanium mini-screws than for stainless steel mini-screws. Fracture resistance, measured by maximum fracture torque, was higher in stainless steel mini-screws than in titanium mini-screws, showing that the insertion process is safer with the use of stainless steel mini-screws[4]. Stainless steel has better mechanical properties considering the effects of bending, because of its greater ductility. The torsional resistance of stainless steel provides greater

sensitivity to the professional during insertion, thereby minimizing the risk of fracture. Titanium mini-screws do not provide tactile response during insertion, so it is difficult for professionals to notice when the rupture is about to occur[29]. Thus, ductility is a very important property because in extra-alveolar anchorage, the mini-screws will almost always require tilt adjustments, even when the insertions are made directly at 30º, as shown in this study.

The comparison of mini-screws of the same diameter and length showed that the mini-screws made of stainless steel alloy performed better than the mini-screws made of titanium alloy for the direct insertions at 30º. For indirect insertion, from 60º to 30º, deformations were observed in both alloys, demonstrating that the angular changes interfere in the structure of both titanium and stainless steel mini-screws. In the evaluation of the performance of mini-screws with smaller diameters and lengths, in both insertions procedures the deformations were large, being greater in the 60º to 30º insertions, also demonstrating that changes of angulation should be avoided if possible, thereby improving performance and decreasing failure rates.

Regarding the diameter and length of the mini-screws, they are factors that also affect the stability. The larger the mini-screws, the greater the insertion and pulling forces[32,33]. Greater lengths offer greater resistance to the pullout test, which presupposes greater primary stability[34]. Greater diameters influence the insertion torque[35]. The mini-screws with the largest diameter show greater strength[36-38]. Therefore, an increase in mini-screw diameter can efficiently reinforce the initial stability, and the insertion torque increases with the increasing diameter and length[39,40].

The results of our study are in accordance with the literature, because it was observed that the different diameters (1.5; 1.6; 2.0 mm) and lengths (10; 11; 12 mm) correlate with performance, evaluated in terms of number of strained mini-screws.

The results of this study could help orthodontists in choosing mini-screws for extra-alveolar anchoring, which can be performed using direct (30º) or indirect insertion (starting at 60º and ending gradually at 30º, with continuous tightening and inclination of the mini-screws).

It is advisable to use mini-screws for extra-alveolar anchorage, but attention should be given to bone thickness, the diameter and length of the mini-screws, the type of alloy and its ability to flex, and especially to changes in the angle during insertions, in order to reduce failure rates and ensure that the best choice is made.

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DECLARATION OF CONFLICTING INTERESTS

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Centric relation registration with intraoral central bearing on curved vs. flat plates with rim trays in edentulous patients

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ABSTRACT

The aim of this study was to compare jaw relation record in the completely edentulous patient using acrylic rim trays with curved or flat registration plates or using the manual guidance technique. The study included 17 patients – 11 female and 6 male, average age 70 years. Three jaw relation records were taken during one session, in the same vertical dimension, using acrylic rim trays: (1) with manual guidance (IM), (2) with self-guided recording system with acrylic rim tray and central support using a curved plate (BYC) and (3) with self-guided recording system with acrylic rim tray and central support using a flat plate (BYR). The models were mounted on a Whip Mix 2240 articulator to which a condyle position device (Orthodent) was added to register, at the level of the condyle box and incisal table, the differences among the positions recorded with the different setups (IM, BYC and BYR). The distances were measured on millimeter paper provided by the recording system manufacturer. For statistical analysis, confidence intervals (95%) were calculated for the mean differences and Student's t-test for paired data (significance level: alpha<0.05). On both the mesiodistal plane and the vertical plane at the level of each condyle box and the incisal table, there were statistically significant differences among the three systems (p<0.001). At the level of incisal table, BYC and BYR provided more retrusive records than IM [arithmetic means (standard deviations) in millimeters: 3.82 (2.10) and 4.53 (2.18), respectively). The records obtained with BYR were significantly more retrusive with BYC [arithmetic mean (standard deviation) in millimeters: 1.41 (1.00)]. We reject the null hypothesis that proposes that all three registration systems described are clinically equivalent for establishing a jaw relation record in completely edentulous patients.

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Keywords: *centric relation - complete denture - jaw relation record - edentulous mouth.*

Registro de relación céntrica con apoyo central intraoral en platinas curvas vs. rectas con cubetas rodete en edéntulos

RESUMEN

El objetivo de este estudio fue comparar el registro de la posición intermaxilar en el paciente desdentado total obtenido con el uso de cubetas rodete de acrílico y platinas de registro curva o recta y con la técnica de inducción manual. Se incluyeron en el estudio 17 pacientes, 11 mujeres y 6 varones con edad promedio de 70 años. En cada uno de ellos se obtuvieron en la misma sesión y en la misma dimensión vertical tres registros intermaxilares con cubetas rodete de acrílico: uno con inducción manual (IM), otro autoinducido con el sistema de cubeta rodete de acrílico y apoyo central único utilizando una superficie palatina de registro curva (BYC) y un tercero con este mismo sistema pero con la superficie palatina recta (BYR). Los modelos correspondientes fueron montados en un articulador Whip Mix modelo 2240 al que se le adicionó un dispositivo de posición condilar (Orthodent) para registrar, a nivel de la caja condílea y la platina incisiva, las diferencias existentes entre las posiciones obtenidas con cada una de las variantes de las platinas en las cubetas rodete acrílicas y la posición registrada con inducción manual. Las distancias fueron medidas sobre papel milimetrado provisto por el fabricante del sistema de registro. El análisis estadístico se llevó a cabo mediante el cálculo de intervalos de confianza (95%) para las diferencias medias y prueba de t de student para datos apareados (nivel de significancia: alfa<0,05). Tanto en el plano mesiodistal como en el vertical a nivel de cada caja condilea como en la platina incisiva, se encontró diferencia estadísticamente significativa entre las tres variables de registro (p<0,001). A nivel de las platinas incisivas tanto BYC como BYR proporcionaron registros más retrusivos que IM [medias aritméticas (desviaciones estándar) en milímetros: 3,82 (2,1º) y 4,53 (2,18), respectivamente). Los obtenidos con BYR fueron significativamente más retrusivos que en el grupo BYC [media aritmética (desviación estándar) en milímetros: 1,41 (1,00)]. Rechazamos la hipótesis nula que propone que los tres sistemas de registro expuestos son clínicamente equivalentes para establecer una posición de registro intermaxilar en desdentados totales.

Palabras clave: *relación céntrica - dentadura completa - registro de la relación maxilomandibular - boca edéntula.*

INTRODUCTION

Rehabilitation of completely edentulous patients involves techniques and skills requiring a combination of theoretical and practical knowledge to provide the best possible treatment.

For various reasons, which go beyond specific knowledge of dentistry, edentulism and the need for complete removable dentures are in constant demand for dental care. Demand for this type of treatment is expected to increase due to socioeconomic and demographic circumstances[1], and there is currently a need to provide this kind of treatment to a growing universe of people.

Occlusion has always been one of the key aspects in these treatments[2-7]. In the 21st century, the International Academy of Prosthodontics[8] glossary of prosthodontic terms (GPT-8) recognizes seven meanings for the term Centric Relation, thereby indicating the range of concepts applied to occlusion, jaw relations and their records.

However, beyond the conceptual field, centric relation is used with the ultimate purpose providing treatment that will endow patients with the occlusal ability to perform all functions while maintaining the health of all components[9].

In the clinical/practical field of centric relation, the search for simple, accurate, evidence-based techniques is challenging[10,11]. There is a need for studies on patients to provide clarity on the clinical efficacy of the many proposed variants, to support decision-making during prosthodontic treatments or when teaching prosthodontics[12-18].

Within such a broad topic, the subject of jaw records is central[19-21]. In the search for more precise work protocols, since 2003, the Department of Prosthesis Clinic I at the Buenos Aires University School of Dentistry has proposed and begun to execute a work protocol which includes the use of acrylic rim trays for making definitive impressions and registrations in completely edentulous patients. This technique has been called BOPAYACU, for its initials in Spanish for Palatal Vault and Single Central Bearing Point.

BOPAYACU uses a standardized upper curved plane, onto which the patient performs anteroposterior and lateral mandibular movements as instructed by the clinician, while pressing against it the tracing screw attached to the lower rim tray. This upper curve prevents the mandibular propulsion typical of the fully edentulous patient, and this intraoral upper vault acts as an anterior inclined plane or deprogrammer, enabling the condyles to center and return to their place[9].

In contrast, the method which is most widely known and was formerly used by our department for teaching and for the treatment of completely edentulous patients, was the one proposed by G. Phillips[9,19,21,22]. It involves use of a flat plate connected to one jaw and a central screw attached to the opposite jaw, placed at the height of premolars on the registration rims prepared for such purpose.

Both these techniques are self-guided, without direct operator intervention in the movements, and both use a central bearing which, by directing the interocclusal contact force in the center of both rims, press them against the supporting tissues, thereby contributing to stability.

They differ substantially in the curvature of the surface against which tracing is performed, which also gives direction to the anteroposterior movement performed by the patient while maintaining contact between the two devices.

This difference may or may not have implications in the final position of the intermaxillary record or in the position of the condyles when said registrations have been completed.

The aim of this study was to compare jaw relation records in the completely edentulous patient obtained: 1. with manual guidance technique, 2. with self-guided recording with acrylic rim tray and central support using a curved plate, and 3. the same, using a flat plate (BYR).

MATERIALS AND METHODS

This study was conducted at the Department of Prosthesis Clinic I at the Buenos Aires University School of Dentistry. A detailed work protocol and checklists were prepared to ensure consistency in the clinical steps of the care provided to each patient included in the study. A dental laboratory was trained in the performance of the relevant laboratory steps: making the rim trays, making the plaster casts and trimming for mounting. An informed consent form was submitted to and approved by the Ethics Committee of Buenos Aires University School of Dentistry (# 0037828/2010).

The study included a group of completely edentulous patients who had undergone all the relevant steps up to the clinical instance of taking jaw relation records using acrylic rim trays. Patient inclusion criteria were:

- Having been completely edentulous for at least five years
- Wearing complete dentures installed in the past
- No motor or sensory disability which would prevent them from performing the mandibular movements required for intermaxillary record
- No lesions of the intraoral mucosa at the time the records were taken
- No joint pains or limitation in mouth opening
- Ability to accept and sign the informed consent.

Patient exclusion criteria were:

- Unclear or discordant tracing patterns at the time of registration,
- Not signing consent.

The study included 17 patients: 11 female and 6 male, aged 56 to 78 years, all completely edentulous, who met the inclusion criteria.

For each patient, during one session, in the same position and same vertical dimension, three jaw relation records were obtained with acrylic rim trays: one with manual guidance (MI), one self-guided with the BOPAYACU system using a curved plate (BYC), and one self-guided with the BOPAYACU system using a flat plate (BYR). Then the casts were mounted on an articulator (Whip Mix model 2240).

Impressions and records with rim trays

After the clinical examination, primary impressions were made with rigid Schreinemaker trays for completely edentulous subjects, following the technique used regularly at the service.

Rim trays were designed following the guidelines by Alvarez Cantoni-Fassina[9] and made at a dental laboratory (Figs. 1,2). After clinically verifying the support and the size of bases and flanks, definitive impressions were taken, ensuring proper functional final size, seal and retention.

Rim trays made in this manner provide a unit for impressions and registrations: a rim tray is a single instrument made of a single material, which includes the distinctive parts of custom tray, shaped occlusal rims and both parts of the registration system by single central bearing. In this study, a modification was applied by changing the curved acrylic vault of the upper rim tray for a flat one, with the same inclination as the occlusion plane, without thereby modifying the vertical dimension established in the rims.

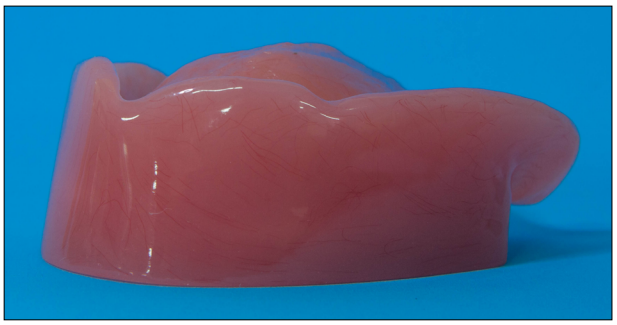

Figure 1: Acrylic upper rim tray

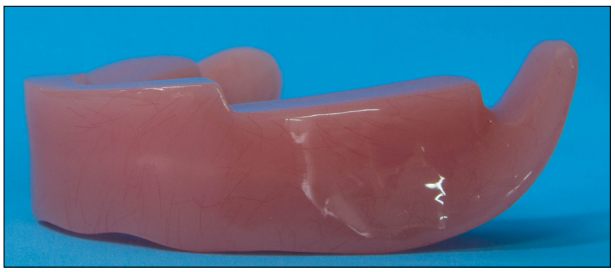

Figure 2: Acrylic lower rim tray

Taking intermaxillary records

Having completed the steps described above in each patient, during the same session, in the same position and same vertical dimension, three jaw relation records were obtained with acrylic rim trays: one with manual guidance (MI), one self-guided with the BOPAYACU system using a curved plate (BYC), and one self-guided with the BOPAYACU system with flat plate (BYR).

Based on these records, the casts were mounted on an articulator (Whip Mix model 2240), to which was added a condylar positioning device (Orthodent) to record variations at the level of the condyle box and incisal table. Distances were measured on millimeter paper provided by the manufacturer of the registration system.

Each patient's head position was standardized for taking the three records, so that any differences between records could not be attributed to differences in head position.

The patient was seated in a dental chair (Dabi Atlante, Brazil) with the backrest tilted at 45 degrees and patient head in a comfortable position, beyond cervical variations in each patient, ensuring that the clinical projection of the Frankfort plane was perpendicular to the backrest. Once a comfortable position was found for each patient, it was maintained throughout the record-taking session.

To ensure maintenance of the vertical dimension while centric relation was being recorded, care was

taken at intraoral level to ensure that the thickness of the registration material placed between the rims in manually guided closing did not exceed 2 mm.

Extra-orally, prior to taking the record and with the rims in the mouth and a 2 mm thick spacer placed in the anterior sector, two marks were made on the skin: one at the level of the wing of the nose and another 1 cm below the lip commissure. The distance between marks was measured with a millimeter ruler. After the registration silicone had hardened in each of the three techniques, it was checked that the distance had not varied by more than 1 mm.

First, the manual guidance technique was used. The patient was instructed to practice in order to find the repetitiveness in the arc of closure and, by applying slight pressure on the chin, the patient was guided to a more posterior position of the upper jaw, without causing pain and always seeking repetition of the arc of closure.

Occlusal registration silicone (Futar 2) was applied using an application gun and mix tips. After the record had hardened, it was removed and identified as number 1 for that patient. Then the patient was instructed to put on his/her usual denture and rest in the waiting room for at least 20 minutes.

Second, using the same rim trays, the lower tracing point of the BOPAYACU system, which consists of a screw with a rounded tip, was positioned. The single central support was positioned against the palatal vault preformed using the shaper designed for such purpose, with a minimum separation between rims to ensure that there was no interference by contact between them. The patient was instructed and trained to make the arrow point tracing. Once the graphic record was achieved, the patient was trained to move to the posterior position without manual guidance by the operator, to ensure it was self-guided, and this new position was recorded with the same registration silicone and identified as number 2. Then the patient was instructed to put on his/her usual denture and rest in the waiting room for at least 20 minutes.

Finally, the upper vault of the recording device was modified by adding self-curing acrylic to it and using a glass tile to shape a flat surface. Solid petroleum jelly was applied to the tile surface to facilitate separation of the acrylic once it polymerized. Then the lower screw was lowered to maintain the separation between rims, in order not to clinically modify the vertical dimension. The patient was again trained to perform the movements that generate the self-guided arrow point tracing, to record the starting point of these movements, thereby obtaining on the registration silicone the third measurement for evaluation and comparison (Fig. 3).

Transfer of the recorded positions to an ARCON type articulator

Once the silicone bite forks had been obtained for each clinical option, identified as I (manual guidance), II (record with BOPAYACU) and III (record with modified BOPAYACU), the definitive casts were made and mounted on a Whipp Mix 2240 articulator using the fork from record I.

For each clinical case and record, we used a single, new set of plates, which was kept until the end of the study. Thus, each of the recording techniques for all 17 clinical cases were mounted on the same articulator, by the same operator.

Graphic registration in the articulator

An Orthodent brand condylar positioning device (CPI, according to the manufacturer) was added at the level of the condyle box on the Whipp Mix 2240 articulator. Once each clinical case was mounted on the articulator, it was labelled and placed in each condylar box of the corresponding CPI device. A set of millimeter graph paper stickers was added to each in order to record the marks at the level of each and of the incisal table (Figs. 4, 5, 6).

After obtaining the records by interposition of Bausch Arti-Fol metallic 12 µm articulating paper, each sticker was removed and placed on a registration card prepared for each case.

The measurements taken at the level of the condylar guidance boxes were recorded according to the plane of movement, mesiodistal and vertical,

Figure 3: Silicone jaw relations

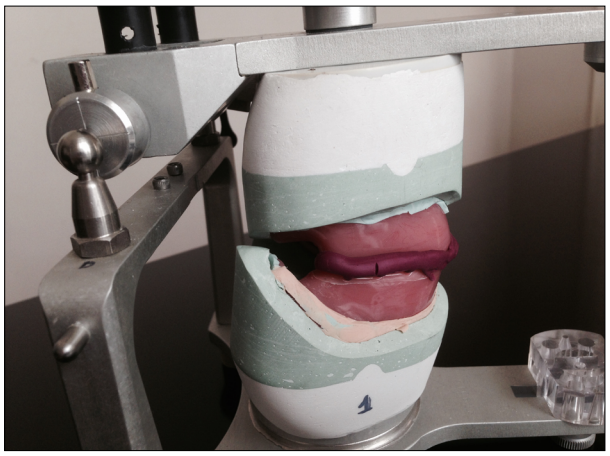

Figure 4: Rim trays attached to casts and mounted on the articulator

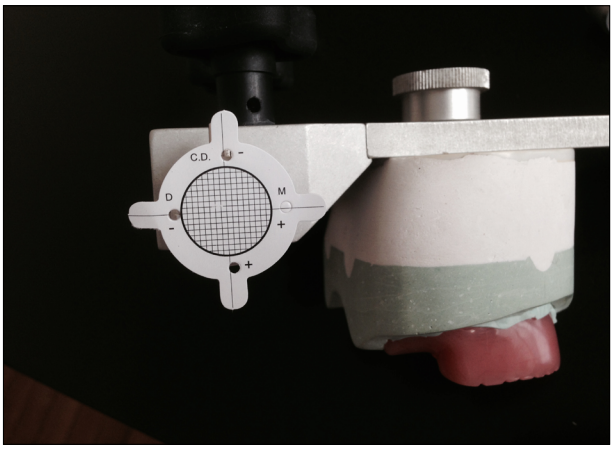

Figure 5: Device for measuring variations at condyle level, placed on the articulator

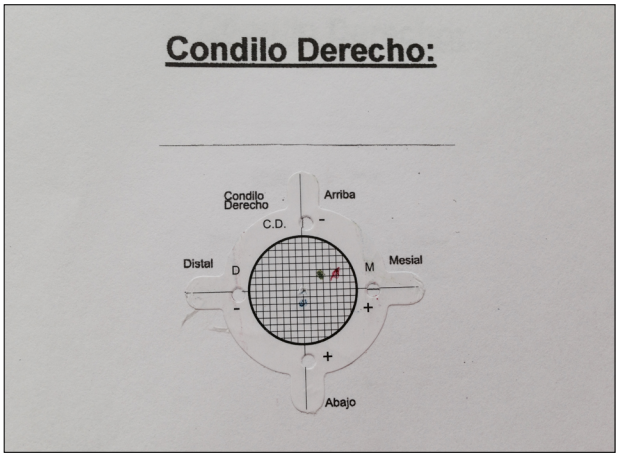

Figure 6: Record of variations in position on millimeter paper (Cóndilo derecho: Right condyle; Mesial: Mesial; Distal: Distal; Abajo: Under; Arriba: Above).

and the side. The records obtained at the level of the incisal table were recorded as anteroposterior and indicated following the same classification criterion according to distance between marks and individualized according to color.

When all records were obtained, the differences in millimeters were measured using a 5x magnifying glass and observing the distance between each colored point recorded on the millimeter graph paper.

The recorded values were uploaded to an Excel spreadsheet, which served as a data matrix for subsequent sample analysis and statistical treatment, and for hypothesis testing.

Records were abbreviated according to the initial of the color of the point generated on the graph: B = Blue (1st record), manual guidance technique (MI); G = Green (2nd record), self-guided technique with curved or standard BOPAYACU (BYC); and R = Red (3rd record), self-guided technique with flat or modified BOPAYACU (BYR).

Values were recorded using the nomenclature provided in Table 1.

The data were grouped firstly according to condyle analyzed. Then, within each unit of analysis, the differences found between the mesiodistal and vertical planes were divided. At the level of the incisal table, the distances were measured in anteroposterior direction.

Data recording and statistical processing

For each patient and each condyle, we calculated the difference between the positions recorded with each BOPAYACU version, and between these and the position recorded with manual guidance.

For statistical analysis, confidence intervals (95%) were calculated for the mean differences between the values recorded with each BOPAYACU system and the manual technique. Student's t-test for paired data was used to compare the values of the two systems, as well as values per condyle in each patient. Pearson's correlation coefficient was calculated for the values for the two condyles in each patient. Significance level was set for alpha less than 0.05 for all cases.

RESULTS

Self-guided records with curved and flat plate BOPAYACU systems both differed significantly ($p<0.05$) from the record obtained by manual guidance, in agreement with studies published on the subject to date. These differences were found at the level of condyle boxes on both mesiodistal and

Table 1. Identification code for the variables included in the study.

Plane	Condyle	Distance	Code
Mesiodistal plane	Right condyle	dist Bl-Gr	MDDV
		dist Bl-Red	MDDR
		dist Gr-Red	Dif_1
	Left condyle	dist Bl-Gr	IDDV
		dist Bl-Red	IDDR
		dist Gr-Red	Dif_2
Vertical plane	Right condyle	dist Bl-Gr	VDV
		dist Bl-Red	VDR
		dist Gr-Red	Dif_3
	Left condyle	dist Bl-Gr	VIV
		dist Bl-Red	VIR
		dist Gr-Red	Dif_4
Anteroposterior plane	Platina incisiva	dist Bl-Gr	APIV
		dist Bl-Red	APIVR
		dist Gr-Red	Dif_5
Surface	Right condyle	Reg Bl-Gr	SUPDV
		Reg Bl-Red	SUPDR
		Dif Gr-Red	Dif_6
	Left condyle	Reg Bl-Gr	SUPIV
		Reg Bl-Red	SUPIR
		Dif Gr-Red	Dif_7

vertical planes, and at the level of the incisal table (Table 2).

In the right condyle boxes, recorded via the condyle positioning devices, on the mesiodistal plane there was a mean difference among the three records of 1.24 mm to 1.82 mm, with mean standard deviation 1.2 mm. For the left side, the differences were 1.06 mm to 1.82 mm with mean standard deviation 1.1 mm.

On the vertical plane, mean difference was 0.71 mm to 2.18 mm, with mean standard deviation (SD) 1 mm.

In both condyle positioning devices and on both planes, maximum differences in the order of 5 mm and 6 mm were measured, and cases were found in which the differences were 0.0 mm to 0.5 mm.

On the incisal table, mean difference between the manual guidance record and the two BOPAYACU records was 3.82 mm (SD 2.10 mm) for curved plate and 4.53 mm (SD 2.18 mm) for flat plate. The mean difference between the two types of BOPAYACU was 1.41 mm (SD 1.00 mm).

The measurements at condyle level expressed in surfaces showed mean differences of 1.10 mm to 1.69 mm on the right side and 1.46 mm to 2.83 mm on the left side.

On both the mesiodistal plane and the vertical plane at the level of each condyle box and on the incisal table, there were statistically significant differences among the three variables recorded ($p<0.001$). At the level of the incisal tables, both BYC and BYR provided more retrusive records than did MI [arithmetic mean (standard deviation) in millimeters: 3.82 (2.10) and 4.53 (2.18), respectively). BYR records were significantly more retrusive than BYC [arithmetic mean (standard deviation) in millimeters: 1.41 (1.00)].

DISCUSSION

This study analyzed the data collected regarding the changes in position of jaw relation records associated to the use of flat and curved plates in the BOPAYACU device for jaw relation records in completely edentulous patients and comparing them to the manual guidance technique. The average values for anteroposterior variation of the jaw relation record at the level of the incisal table on the articulator suggest that the three systems tested are not equivalent. Mean distance was 3.82 mm (SD 2.1 mm) between manual guidance and BYC; 4.53 mm (SD 2.18 mm) between manual guidance and BYR; and 1.41 mm (SD 1.00 mm) between BYC and BYR.

Based on these observations, we reject the null hypothesis, which posits that registration using rim trays with manual guidance and registration using single central bearing and curved registration plate (BOPAYACU) are equivalent at the level of the incisal table; that registration using rim trays with manual guidance and registration using rim trays with modified BOPAYACU are equivalent at the level of the incisal table, and that registration using rim trays with BOPAYACU and registration using rim trays with modified BOPAYACU are equivalent at the level of the incisal table.

Upon determining the variation on the anteroposterior plane and the vertical plane of the jaw relation record at the level of the condyle box in the articulator, for completely edentulous patients, we found that the values were not equivalent in the three registration systems.

These findings are consistent with those obtained by comparing the condyle sliding surfaces calculated in the tables of the condyle positioning device.

Table 2. Statistical data, confidence interval (in millimeters) for study variables, and significance (alpha) for the differences between the two ways of using BOPAYACU.

Variable	Arithmetic mean	Standard deviation	Median	Maximum	Minimum	CI (95%)		P (alpha)
MDDV	1.53	1.27	2	4	0	0.88	2.18	
MDDR	1.82	1.22	1.5	5	0	1.19	2.45	
Dif_1	1.24	1.16	1	4	0	0.64	1.83	<0.001
IDDV	1.82	0.93	2	4	0.5	1.34	2.30	
IDDR	1.82	1.31	1	5	0.5	1.15	2.50	
Dif_2	1.06	1.09	1	4	0	0.50	1.62	0.001
VDV	2.18	1.24	2	4	0	1.54	2.81	
VDR	1.88	1.28	1.5	4	0	1.22	2.54	
Dif_3	0.71	0.75	0.5	2	0	0.32	1.09	0.001
VIV	2.12	1.52	2	5	0	1.34	2.90	
VIR	2.00	1.37	2	6	0	1.30	2.70	
Dif_4	0.82	0.90	0.5	3	0	0.36	1.29	0.002
APIV	3.82	2.10	3	8	1	2.74	4.90	
APIVR	4.53	2.18	4	8	1	3.41	5.65	
Dif_5	1.41	1.00	1	4	0	0.90	1.93	<0.001
SUPDV	1.69	1.89	1	6	0	0.72	2.67	
SUPDR	1.68	2.31	1	10	0	0.49	2.87	
Dif_abs6	1.10	1.10	1	4	0	0.54	1.67	0.001
SUPIV	1.83	1.64	2	6	0	0.99	2.67	
SUPIR	2.82	1.61	2.5	7	0.75	2.00	3.65	
Dif_abs7	1.46	1.26	1	4.5	0	0.82	2.11	<0.001

Considering these results, the clinician should consider in greater depth the importance of knowing about the intrinsic variations of the devices used for jaw relation records. Because they are not equivalent, the concept of self-guidance becomes an epistemological obstacle to understanding the clinical positions recorded. It is no longer the patient that induces a desired or ideal intermaxillary position, free from intervention by the operator, but the device is an inherent part of the outcome. The fact that the device may have either a curved tracing surface or a flat, slanting surface affixed to the slope of the occlusion plane, has been found to influence the record position measured at the level of the articulator's incisal tables or condyle boxes.

CONCLUSION

Within the limitations of this study, we reject the null hypothesis, which posits that registration using rim trays with manual guidance and registration using rim trays with BOPAYACU are equivalent at the level of the articulator's condyle box; that registration using rim trays with manual guidance and registration using rim trays with modified BOPAYACU are equivalent at the level of the articulator's condyle box, and that registration using rim trays with BOPAYACU and registration using rim trays with modified BOPAYACU are equivalent at the level of the articulator's condyle box.

DECLARATION OF CONFLICTING INTERESTS
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Vertical Dimension of Occlusion: A comparative study between Anthropometric and Knebelman's craniometric methods

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ABSTRACT

The masticatory system changes as time passes. The vertical dimension of occlusion (VDO) undergoes alterations due to temporomandibular joint disorders which in turn may be caused by related muscle modifications or pathological tooth wear. There are many methods to measure VDO. Among these, the anthropometric method and Knebelman's craniometric method have been shown to be the most closely related to facial biotype. The aim of this study was to compare data recorded with those two methods. A descriptive cross-sectional study was performed with a total 200 patients. A vernier caliper was used to measure facial landmarks. Results were analyzed using paired t-test, setting the level of significance at p<0.05. There was no significant difference between the two methods but Knebelman's method had less variability. Results suggest that Knebelman's method should provide more reliability for determining VDO in all the facial biotypes studied.

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Dimensión vertical oclusal: un estudio comparativo entre el método antropométrico y el método craneométrico de Knebelman

RESUMEN

El sistema masticatorio cambia con el paso del tiempo. La dimensión vertical oclusal sufre alteraciones que se atribuyen a trastornos temporomandibulares a su vez causados por modificaciones en la musculatura relacionada o al desgaste patológico de las piezas dentarias. Existen muchos métodos para medir la dimensión vertical, entre los cuales el método antropométrico y el método craneométrico de Knebelman mostraron ser los más vinculados con el biotipo facial. El objetivo de este estudio fue comparar datos registrados con ambos métodos. La comparación fue realizada mediante un estudio de diseño transversal descriptivo con un total de 200 pacientes, usando un calibrador vernier para realizar mediciones entre puntos faciales establecidos. Los resultados fueron analizados por medio de la prueba de t de Student para datos emparejados estableciendo el nivel de significación en P<0,05. No se encontró diferencia significativa entre los métodos, pero el método de Knebelman mostró generar menos variabilidad en sus medidas. Este último método parece proporcionar más confiabilidad para su aplicación en la determinación de la dimensión vertical oclusal en todos los biotipos faciales estudiados.

Palabras clave: *dimensión vertical - oclusión dental - articulación temporomandibular.*

INTRODUCTION

A consequence of advancing age is the presence of alterations in the masticatory system which involve maxillary, mandible, temporomandibular joint (TMJ) and musculature. Within the latter, the masseter muscle determines the vertical dimension of occlusion (VDO) stabilization. Thus, any degradation involving those structures derives in a functional decompensation[1-3].

It is currently known that TMJ alterations are not the only cause of occlusion disorders. Temporo mandibular disorders (TMD) related to changes in vertical dimensions with modified functioning of surrounding muscles generate a posterior position in the condyle that can be established using cone-beam computed tomography (CBCT)[4-7]. Some of these alterations are caused by alveolar ridge resorption and changes in biting forces that modify condyle positions inside the articular fossa[8]. Those changes are related to increased mechanical tooth surface wear and may progress to pathological tooth

wear (PTW) because of parafunctional mandibular movements which modify masticatory muscle tone, and generate facial pain and aesthetic and phonetic disorders[8-14].

These alterations plus missing teeth lead to decreased or increased VDO, which is visualized by changes in the distance between the nasal spine (Subnasale, "Sn") and the most anterior inferior point of the chin (Soft Tissue Menton, "Me"). When the mandible is in physiologic rest position with non-contacting teeth, it is called rest vertical dimension (RVD), which differs from VDO [15-17].

Many methods have been suggested over the past decades to establish VDO: swallowing, phonetic, rest position, maximum closing force, pre-extraction records, Willis, Landa, McGee, Hayakawa, comfort, cephalometric, anthropometric and Knebelman's craniometric method, but none of them can register VDO objectively. Original specifications suggest that Knebelman's method should be applied in normal craniofacial development (mesoprosopic). Later studies found a correlation with leptoprosopic type because of racial characteristics, but they emphasize that there is no applicability for euryprosopic[18-28].

Ricketts defined facial biotype as the morphologic and functional characteristics of the face, reason why, facial biotype is used for treatment plans which depends on the maxillary facial growth pattern [29] where biting force, has been shown to be related to final dental arch form [30].

This study evaluated the VDO determinations obtained by anthropometric and Knebelman's craniometric methods.

MATERIALS AND METHODS

A cross-sectional study was performed with 200 adults, selected from patients attending Catholic University of Cuenca dental clinics located in Azogues, Ecuador. Each participant completed an informed consent form. Bioethical and technical aspects of the project were approved by the Catholic University of Cuenca - Azogues authorities under code 00001CIABEOSA.

Demographics (age, sex) and clinical data (edentulism type and facial biotype) were recorded. Trained, calibrated (Kappa= 0.8) clinicians used both the anthropometric method and Knebelman's craniometric method to determine VDO in all participants. The procedure was conducted under natural light. Patients sat with a straight back. Frankfort plane was established parallel to the floor, occluding for partially dentate patients and reaching rest position for edentulous patients. Once these positions were obtained, anthropometric points were marked using an eye pencil. Facial index was determined using the Mayoral index that uses soft tissues related landmark points (ophryon-menton/zygion-zygion) to register measurements in millimeters. This enabled the distribution of facial types within the sample to be established.

The anthropometric method was applied using a vernier caliper to determine the planes mentioned in Table 1. The measurements were compared to the height of the lower facial third. A ± 2 mm error range between measures was set to define whether vertical dimension was maintained, diminished or increased (Figs. 1 and 2).

Knebelman's Craniometric method was applied using a Knebelman's craniometer, which only considers two measurements, called "READ", from external auditory canal to the lateral corner of the bony orbit and "SET" from Sn (subnasal) to the most anterior undersurface of mandible. Both readings are correlated and contribute to the final result (Figs. 3 and 4).

A template was designed on which to record the measurements, allowing selection of measurements not to be considered for the anthropometric method (Table 1).

Statistical analysis was performed by obtaining averages and standard deviation, and differences between the methods were analyzed using the t-test for paired data. Significance level was established at $P<0.05$.

RESULTS

Tables 2 and 3 show distribution of sex and age, respectively, and tables 4 and 5 show distribution of edentulism type and facial biotype, respectively.

The application of the t-test for paired data showed a significant difference ($p<0.001$) between the two methods (Table 6).

When facial type is considered (Table 7) the difference between methods was not statistically significant in leptoprosopic patients, though it was significant in the other two groups.

DISCUSSION

Losing natural dentition can lead to alterations in the height of the lower facial third due to increased bite

Table 1. Template to record the measurements of each patient

VERTICAL DIMENSION OF OCCLUSSION MEASUREMENTS					
Measurements	**Anthropometric Method**		**Chosen measures**	**Knebelman Method**	
1. Eye outer Cantus -Tragus (mm)					
2. Bipupilar					
3. Eye outer Cantus – outer comissure (mm)					
FACIAL THIRDS					
• Trichion - Glabela					
• Glabela - Subnasale					
• Subnasale– Menton					
	Mean (mm)			Mean (mm)	

FACIAL BIOTYPE DETERMINATION (MAYORAL INDEX)				
Glabela- Menton			❖ Leptoprosopic (>104 mm) ❖ Mesoprosopic (97-104 mm) ❖ Euriprosopic (< 97mm)	
Bicigomátic distance (Zygion – Zygion)				
	Mean (mm)			

force, so reestablishing VDO improves quality of life by enabling the patient to recover the ability to produce certain movements without any limitation. It is thus important for dentists to obtain precise measurements for an adequate VDO. Ayoub[28] reports that interpupillary distance can be used as a VDO determination factor in males. Alhajj, et al[29] report that measurements from the outer canthus of the eye to the oral commissure are more

Fig. 1: Anthropometric method application (Eye outer canthus-Tragus measurement delimitation)

Fig. 2: Anthropometric method application (Eye outer Canthus-Outer commissure measurement delimitation)

Fig. 3: Knebelman's craniometric method (Read position application)

Fig. 4: Knebelman's craniometric method (Set position application)

Table 2. Sample distribution according to gender

GENDER	*N*	%
Male	53	27
Female	147	73
TOTAL	200	100

Table 3. Sample distribution according to age

AGE GROUP (years)	*N*	%
17-25	30	15
26-40	31	15
41-60	72	36
>61	67	34
TOTAL	200	100

Table 4. Sample distribution according to edentulism type

TYPE	*N*	%
Partial Edentulism	139	70
Total Edentulism	61	30
TOTAL	200	100

Table 5. Sample distribution according to facial biotype

BIOTYPE	*n*	%
Leptoprosopic	77	38
Mesoprosopic	98	49
Euriprosopic	25	13
TOTAL	200	100

reliable for VDO prediction for edentulous patients. According to Bajunaid et al.[30], facial landmarks mentioned by Misch (about 12 measurements with a difference range between 1-2 mm) are used to determine VDO in edentulous patients reliably and objectively. Distances between left eye-ear and left angle of the eye to angle of mouth showed accuracy in determining VDO for complete denture wearers, as is also mentioned by Majeed et al[31]. The Constantiniuc[32] comparison between phonetic, rest position and anthropometric methods to establish VDO did not show relevant differences between final measurements, which suggests that these methods could be applied to help determine VDO in edentulous patients. However, according to Batra[33], there is no validation for any method proposed to measure VDO. The use of bone points by cephalometric analysis leads to increased accuracy in measurements because there is no facial or positional manipulation determining freeway space, but a functional method must also be used to improve measurements provided by lateral RX in edentulous patients. Ousehal and Sierpinska[34,35] report that cephalometric studies could lead to an adequate initial VDO, referring to Knebelman's craniometer as an adequate way to determine initial VDO that could be complemented with cephalometric studies. One of the most frequently analyzed concerns is considered by Behrensdorf[36], who says that age and time using dentures affect VDO reestablishment and mandibular movements. According to these conclusions, Fukushima[37] evaluated methods based on condyle position and showed their unreliability due to the effect of muscle action on it. Watarai[38]

Table 6. T test between anthropometric method and Knebelman's craniometric method applied in treated patients.

METHOD	Mean	St. Dev.	Mean difference	p value*
Anthropometric	64.18	5.06	-1.28	<0,001
Knebelman's craniométric	65.26	3.78		

***CI (95%) for the mean difference=** -1.660 / -0.50

Table 7. VDO variability considering facial biotype. (n=200)

BIOTYPE	ANTHROPOMETRIC METHOD		KNEBELMAN'S CRANIOMETRIC METHOD		P value*
	MEAN	SD	MEAN	SD	
Leptoprosopic	66.55	4.99	66.77	3.50	0.750
Mesoprosopic	63.19	4.54	64.52	3.53	0.023
Euriprosopic	60.8	4.07	63.60	4.12	0.019

SD= standard deviation

states that physiologic mandibular rest position could be modified by head or body postures, mental state, muscles and TMJ, emphasizing the difficulties in establishing an accurate position.

On the other hand, Miran[39] claims that the applicability of a specific method to determine VDO depends on accuracy, adaptability, equipment and cost. However, Mahboub[40] states that differences in their application using stone casts, pre-extraction records or cephalograms help in the determination of VDO but are not reliable by themselves.

In addition, some studies showed compatibility with the results obtained in the current study. Talavera[18] compared Willis and McGee methods (using craniometrical measures such as Knebelman's). Alhajj[29] using a method that involves a finger length shaft, obtained mean measurements that are within the range of values recorded in our study. This information suggests the reliability of the method in different scenarios.

After studying and recording facial biotype and VDO, our results showed a small difference between mean measurements obtained with the two methods under study. Knebelman's craniometric method seems to be more reliable during the VDO determination process since its variability is lower. It is also easier to apply and reduces diagnosis time. One of its possible disadvantages may be the cost of the instrument. This is the first local study to record VDO and facial biotype, so it could be used as a baseline for further studies.

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Micro-computed tomographic evaluation of root canal morphology in mandibular first premolars from a Colombian population

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ABSTRACT

Dental anatomy can vary significantly between different populations from different countries. Dental anatomical variations are of great interest to the dental professional, especially to endodontists, since they can influence the outcome of endodontic treatment. The purpose of the present study was to describe the anatomical variations of the root canal in mandibular first premolars in a population from Colombia, using micro-computed tomography. Fifty mandibular first premolars were scanned on a SkyScan 1174 and the microcomputed tomographic images were reconstructed. Anatomy was assessed using three-dimensional models. The parameters used were: Vertucci's classification, area and volume, perimeter, circularity, and major and minor diameter at 1, 2 and 3 mm from the apical foramen. According to the Vertucci's classification, teeth were classified as: types I (40%), V (24%), VII (4%) and III (4%), with 28% not classifiable. C-shaped canals were found in 1.8% of the sample. Mean evaluations at 1, 2, 3 mm of the foramen were as follows, respectively: perimeter 1.07 ± 0.57, 1.27 ± 0.78 and 1.57 ± 0.84 mm; circularity 0.59 ± 0.19, 0.57 ± 0.20 and 0.56 ± 0.22; maximum diameter 0.41 ± 0.23, 0.48 ± 0.33 and 0.60 ± 0.37 mm; minimum diameter 0.24 ± 0.10, 0.26 ± 0.11 and 0.21 ± 0.13 mm. Mean total area and volume were 61.27 ± 16.47 mm^2 and 12.47 ± 4.95 mm^3, respectively. There was wide anatomical variation in mandibular first premolars from Colombian individuals, reinforcing the need for proper anatomical knowledge to establish more effective strategies for endodontic treatment.

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Avaliação microromográfica da morfologia interna de canais de primeiros pré-molares de uma população colombiana

RESUMO

A anatomia dentária pode variar significativamente entre diferentes populações, de diferentes países. As variações anatômicas dentais são de grande interesse para o profissional da odontologia, principalmente para os endodontistas, pois podem influenciar no resultado do tratamento endodôntico. Descrever as variações anatômicas do canal radicular dos primeiros pré-molares inferiores em uma população da Colômbia, usando a micro tomografia computadorizada. Cinquenta primeiros pré-molares inferiores foram digitalizados em um SkyScan 1174 e as imagens tomográficas foram reconstruídas e a anatomia foi avaliada por meio de modelos tridimensionais. Os parâmetros utilizados foram: classificação de Vertucci, área e volume, perímetro, circularidade e diâmetros maior e menor a 1, 2 e 3 mm do forame apical. De acordo com a classificação de Vertucci, os dentes foram classificados em: tipos I (40%), V (24%), VII (4%) e III (4%), sendo 28% não classificáveis. Canais em forma de C foram encontrados em 1,8% da amostra. As avaliações médias em 1, 2, 3 mm do forame foram as seguintes, respectivamente: perímetro 1,07 ± 0,57, 1,27 ± 0,78 e 1,57 ± 0,84 mm; circularidade 0,59 ± 0,19, 0,57 ± 0,20 e 0,56 ± 0,22; diâmetro maior 0,41 ± 0,23, 0,48 ± 0,33 e 0,60 ± 0,37 mm; diâmetro menor 0,24 ± 0,10, 0,26 ± 0,11 e 0,21 ± 0,13 mm. A média da área total e do volume foram 61,27 ± 16,47 mm2 e 12,47 ± 4,95 mm3, respectivamente. Houve uma grande variação anatômica nos primeiros pré-molares inferiores de colombianos, reforçando a necessidade de conhecimento anatômico adequado para estabelecer estratégias mais eficazes para o tratamento endodôntico.

Palavras-chave: *modelo anatômico - microtomografia de raios-X - bicúspide.*

INTRODUCTION

The main objective of endodontic treatment is to prevent or treat apical periodontitis[1]. The persistence or emergence of an apical periodontitis lesion after treatment can be regarded as a failed outcome and may be associated with difficulties encountered during endodontic intervention[2].

Chemomechanical preparation can be considered as the main phase of root canal treatment[3]. It consists of cleaning, shaping, and disinfecting the main canal, through the mechanical action of instruments and the chemical effects of irrigant solutions, creating appropriate intracanal conditions to receive the filling material[4-6].

Anatomical complexities of the root canal system can pose a major challenge during endodontic treatment, so knowledge of internal anatomy and its variations is of utmost importance for success[7,8]. Endodontic instruments and irrigants have limitations in reaching and disorganizing bacterial biofilms located in areas such as isthmuses, ramifications and recesses, which often require special strategies for cleaning and disinfection[1].

Difficult-to-reach irregular areas can harbor remnants of pulp tissue or residual infected debris, which may compromise the treatment outcome[9]. Mandibular premolars can present complex anatomy; according to the Vertucci's classification[7], these teeth can present several anatomical classifications, from type I to V. In addition to these variations, these teeth can also present a C-shaped canal, which is a ribbon-shaped orifice, formed when the canals merge to form a 180° arc and a narrow strip of curved pulp tissue is formed[10]. A C-shaped canals presents a serious challenge for adequate endodontic treatment. Root canal anatomy may vary according to ethnic factors[11], sex[12] and age[13]. Most previous studies were performed in Caucasian populations[14]. Similar investigations in other populations in South America are less frequent, especially in Colombia, where studies on internal dental anatomy are rare. Thus, the purpose of the present study was to describe the root canal morphology of the mandibular first premolar in a Colombian population using micro-computed tomography (micro-CT) as the evaluation method.

MATERIALS AND METHODS

Fifty mature mandibular first premolars with intact crowns, available from the Bank of Human Permanent Teeth of the Santo Tomás University, Bucaramanga, Colombia, were used in this study. Teeth had been extracted for orthodontic reasons unrelated to this study. Consent was secured prior to tooth donation. The teeth evaluated in this study were from patients from the metropolitan region of Bucaramanga, Colombia, including the cities Floridablanca, Girón, Lebrija, and Piedecuesta. The population in this region is miscegenated, as in other Latin American countries. This study was conducted under the principles established in Resolution 08430 of Colombia and approved by the Ethics Committee of Santo Tomás University. Exclusion criteria included teeth with incomplete root formation, root resorption, crown and/or root fractures, previous endodontic treatment and extensive restorations and/or caries.

The teeth were scanned on the SkyScan 1174v2 micro-CT device (Bruker-microCT, Kontich, Belgium) with a 50 kV source at 800 μA, with the following parameters: rotation step of 1.0°, a 360º rotation around the vertical axis, and 17 μm pixel size. The image of each specimen was reconstructed from the apex to the cementoenamel junction with the NRecon v.1.6.9 software (Bruker-microCT), which provided transversal axial sections of the internal structure. After this procedure, three-dimensional models of the dentin and canals were obtained through an automatic segmentation threshold with the CTAn V.1.13 software (Bruker-microCT). Subsequently, the software CTVol v.2.2.1 (Bruker-microCT) was used for visualization and qualitative evaluation of root canal morphology according to the Vertucci classification.

Images of each tooth were evaluated by two observers, and by a third in case of disagreement. The evaluation parameters included: Vertucci's classification, two-dimensional data of root canal perimeter, circularity, and major and minor diameters at 1, 2 and 3 mm short of the apical foramen, as well as three-dimensional data of the total area and volume of the root canals.

RESULTS

According to the Vertucci classification, 40% of the mandibular first premolars were categorized as type I, while 32% were distributed among types III, V and VII (Fig. 1, Table 1). The remaining 28% of the tooth specimens did not meet any of the Vertucci classification types and were considered

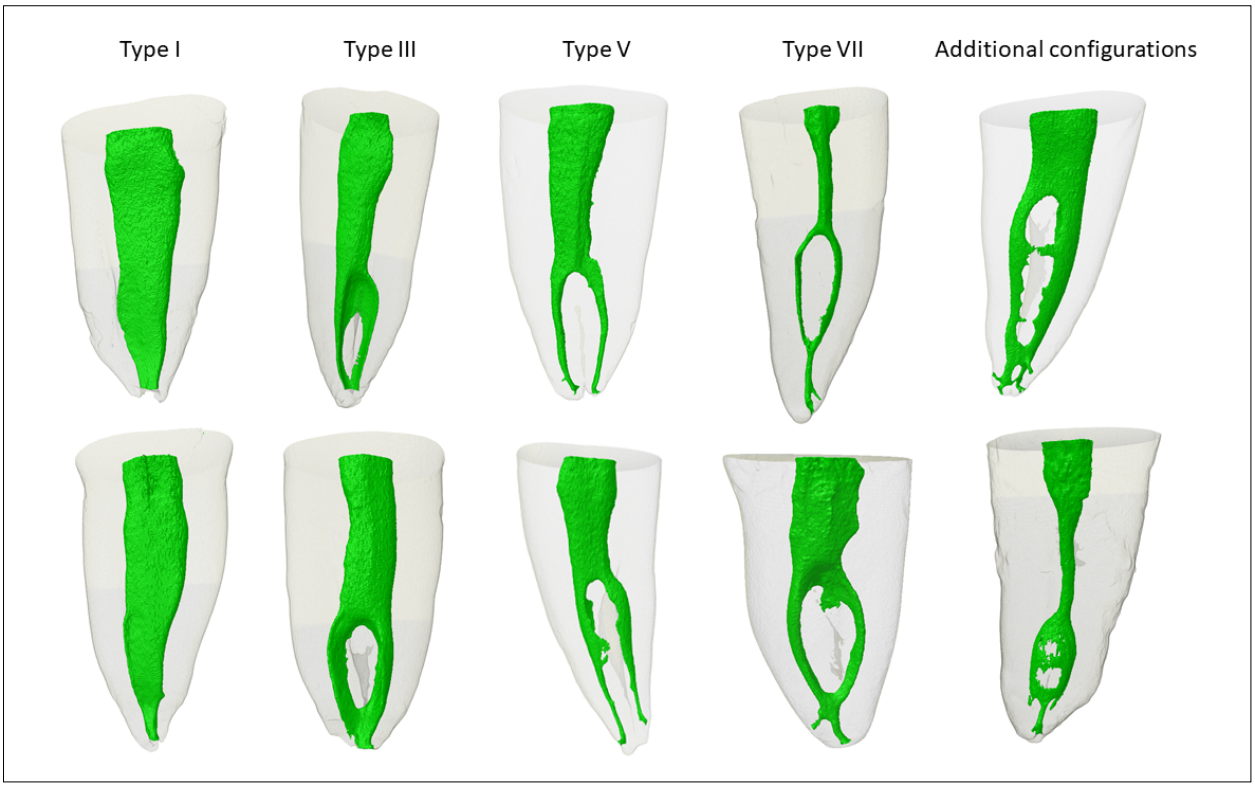

Fig. 1: Types of canals found in Colombian first mandibular premolars according to the Vertucci classification (1984).

as additional configurations. Of these, the most frequent configuration was type 1-2-3 (six teeth), followed by configuration 1-3 (three teeth) (Table 2). C-shaped canals were found in 1.8% of the specimens (Fig. 2). Table 3 shows the data from the two and three-dimensional evaluations.

DISCUSSION

Knowledge of the internal and external anatomy of the different tooth groups is essential for an endodontic treatment with favorable prognosis. Some studies have associated the complexity of the root canal system with the failure of endodontic treatment, usually because of the difficulties in attaining proper disinfection throughout the system irregularities[15,16]. Thus, the aim of this study was to contribute to the knowledge of the anatomy of mandibular premolars, a tooth with recognized complexity in internal anatomy, from a Colombian population that had not been the subject of an anatomical study by micro-CT.

Micro-CT is an excellent non-invasive, non-destructive tool for assessing internal and external

Table 1. Vertucci classification of the root canal morphology of Colombian mandibular first premolars as evaluated by micro-computed tomography

Vertucci classification	Specimens (n)	Frequency (%)
Type I	20	40
Type III	2	4
Type V	12	24
Type VII	2	4
Additional configurations	14	28
Total	50	100

Table 2. Additional configurations of the root canal morphology of Colombian mandibular first premolars as evaluated by micro-computed tomography and not included in the Vertucci classification

Additional configuration	Specimens (n)
1-3	3
1-4	1
1-2-3	6
1-3-2	1
1-2-1-2	1
1-2-1-2-1-3	1
1-2-4-3-4-3	1

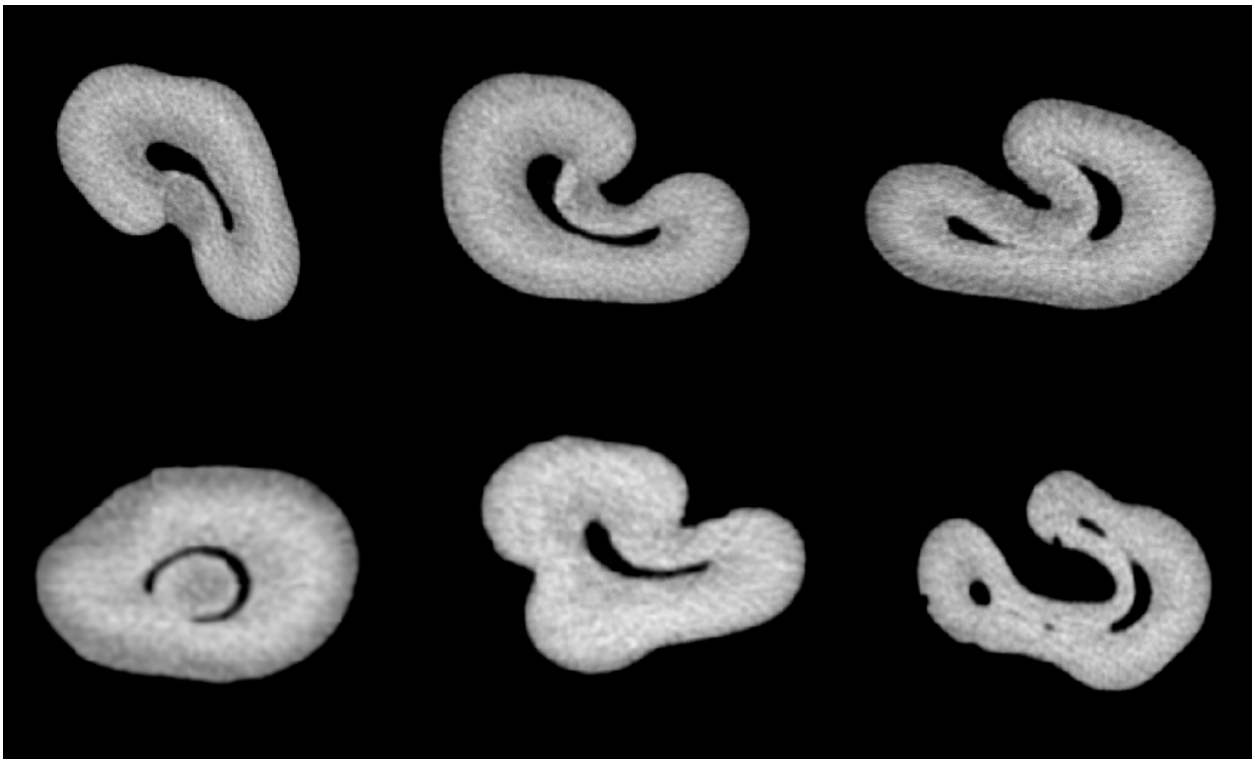

Fig. 2: Cross-sectional representative images of C-shaped canal found in the sample of Colombian individuals.

dental anatomy through the reconstruction of three-dimensional models. In the present study, a pixel size of 17 µm resolution was used, which is efficient to demonstrate the complexity of root canal anatomy[17]. In this study, the Vertucci type I configuration was the most prevalent (40% of cases), in contrast to a systematic review that found a frequency of this configuration almost twice as high in other countries[14].

The prevalence of Vertucci class V canals in the present study of a Colombian population (24%) is similar to prevalence reported in other studies that used micro-CT, including specimens evaluated from populations of China and Saudi Arabia with a frequency of approximately 21%[18,19]. It should be noted that some studies[18,19] did not evaluate data such as apical canal diameter (Table 3), which could be valuable to plan the instrument size and other preparation strategies[20].

Vertucci types III and VII configurations had the lowest frequencies in this study, each occurring in 2% of the sample. Similar findings were reported by Liu et al.[21], who found 2.6% for type III and 0.9% for type VII. Together, types III, V and VII and the additional configurations comprised 60% for the evaluated premolars, highlighting the complex,

Table 3. Data from the total area and volume and other parameters evaluated at 1, 2 and 3 mm from the apical foramen (mean ± standard deviation) of Colombian mandibular first premolars as evaluated by micro-computed tomography

Parameter	Mean ± SD	Range
Area mm2	61.27 ± 16.47	27.53 – 96.51
Volume mm3	12.47 ± 4.95	2.91 – 22.95
Perimeter		
1 mm	1.07 ± 0.57	0.05 – 2.79
2 mm	1.27 ± 0.78	0.20 – 4.08
3 mm	1.57 ± 0.84	0.15 – 4.83
Circularity		
1 mm	0.59 ± 0.19	0.12 – 1.00
2 mm	0.57 ± 0.20	0.15 – 0.93
3 mm	0.56 ± 0.22	0.12 – 0.88
Major diameter		
1 mm	0.41 ± 0.23	0.02 – 1.14
2 mm	0.48 ± 0.33	0.07 – 1.65
3 mm	0.60 ± 0.37	0.06 – 2.13
Minor diameter		
1 mm	0.24 ± 0.10	0.02 – 0.58
2 mm	0.26 ± 0.11	0.05 – 0.63
3 mm	0.21 ± 0.13	0.02 – 0.63

variable anatomy in this group of teeth. These characteristic configurations in the study population may pose greater difficulties for the clinician during endodontic management[18,22].

The results of this study for canal types III, V and VII are similar to those of another study of internal anatomy using a different methodology, also carried out in Colombia[23]. However, Vertucci type I was less frequent in the present study. Just over ¼ of the analyzed samples were considered as an additional configuration that did not fit the Vertucci classification, similar to that reported by Alkaabi et al.[19] in an Arab population.

More than half of the evaluated teeth were not Vertucci class I, i.e., they had more than one canal and/or foraminal exit. This condition can be explained by the diversity of the Colombian population and the presence of external radicular grooves, as mentioned in a previous study[9]. These teeth have at least 2 apical foramina, which can be a challenge for proper cleaning and disinfection, increasing the risk for a poor outcome, as the complexity of the root canal system has been associated with the failure of endodontic treatment[4,15].

The Vertucci classification can be a limitation of this study, as it can be considered to be incomplete for studies of internal tooth anatomy. This is because when the classification was created, there was no availability of a method as accurate as micro-CT. The presence of accessory canals does not allow objective classification, and many additional configurations or biases of interpretation may arise in the assessment.

Only one study[22] with the same group of teeth and methodology in Latin America has been carried out to date, so it is not possible to make a broader comparison with other population groups. Further research with micro-CT, analyzing lower premolars in different regions of Latin America, is necessary for better morphometric comparison of this group of teeth.

CONCLUSION

The internal anatomy of mandibular premolars from the studied Colombian population proved to be more complex when compared with some other populations, with most of the teeth not being classified as Vertucci class I. Knowledge of an anatomical pattern can assist the clinician in planning the endodontic treatment and improve the prognosis. More comprehensive studies including more samples should be conducted on this and other populations to gather more data on internal anatomy.

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DECLARATION OF CONFLICTING INTERESTS

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Effect of treatment time on performance of nano-encapsulated fluoride dentifrices for remineralization of initial carious lesions: an *in vitro* study

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ABSTRACT
The aim of this in vitro *study was to evaluate the influence of treatment time on the remineralization performance of nano-encapsulated fluoride dentifrices on initial carious lesions. Ninety-six human enamel samples were allocated to eight groups (n = 12): 50% NanoF + 50% free NaF, 100% NanoF, 100% NaF (positive control), and placebo (negative control), using two different treatment times (one and five minutes) for each dentifrice tested. After the carious lesion induction, the specimens were submitted to a pH remineralizing cycling model for seven days. Surface microhardness was measured before and after carious lesion induction and after treatment. The percentage of surface remineralization was calculated for each study time. Data were analyzed using two-way ANOVA and ANOVA repeated-measures tests followed by the Bonferroni correction (p < 0.05). Remineralization differences were observed in the dentifrices analyzed according to the treatment time used. NanoF formulations with 50% (one-min treatment) and 100% (five-min treatment) promoted significant remineralization of enamel after the caries challenge when compared to the placebo dentifrice (p < 0.05). Thus, time was considered an important factor for the fluoride release system. Nanotechnology can be a promising system for caries remineralization as it makes fluoride available on the dental surface for a longer time.*

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Keywords: *dental caries - fluorides - nanotechnology - dentifrice.*

Efeito do tempo de tratamento no desempenho de dentifrícios de fluoreto nano-encapsulado para remineralização de lesões iniciais de cárie: um estudo *in vitro*

RESUMO
O objetivo deste estudo in vitro *foi de avaliar a influência do tempo de tratamento no desempenho de dentifrícios fluoretados nano-encapsulados na remineralização de lesões cariosas iniciais. Noventa e seis amostras de esmalte humano foram divididas em oito grupos (n = 12): 50% NanoF + 50% NaF livre, 100% NanoF, 100% NaF (controle positivo) e Placebo (controle negativo) com dois tempos diferentes (um e cinco minutos). Após a indução da lesão cariosa, os espécimes foram submetidos a um modelo de ciclagem de pH por sete dias. A microdureza superficial foi medida antes e após a indução da lesão cariosa e após o tratamento. O percentual de remineralização superficial foi calculado para cada tempo de estudo. Os dados foram analisados pelo ANOVA e ANOVA de medidas repetidas, seguida de Bonferroni (p <0,05). Diferenças de remineralização foram observadas nos dentifrícios de acordo com o tempo de tratamento utilizado. Formulações NanoF com 50% (tratamento de um minuto) e 100% (tratamento de cinco minutos) promoveram uma remineralização do esmalte, após o desafio cariogênico, quando comparado com o dentifrício placebo (p <0,05). Assim, o tempo foi considerado um fator importante para o sistema de liberação de flúor. A nanotecnologia pode ser um sistema promissor de remineralização da cárie, por disponibilizar o flúor por maior tempo na superfície dentária.*

Palavras-chave: *cárie dentária - fluoretos - nanotecnologia - dentifrícios.*

INTRODUCTION

Dental caries has multifactorial etiology involving exposure to acid due to bacterial metabolism[1-3]. It is the most prevalent oral health problem throughout the world, causing a considerable economic burden and a negative impact on quality of life[2,4]. As human enamel cannot be biologically repaired or replaced[5], several studies have been conducted in search of means to protect it by improving techniques and products to ensure better prevention and remineralizing capacity[6-9].

Fluoride is the main factor responsible for the decline in dental caries in recent decades[10,11]. Indeed, fluoride dentifrices are widely used and effective at delivering free or soluble fluoride to the oral cavity[12].

Recent progress has been made in systems for promoting enamel remineralization[6,9]. *In vitro* studies have contributed to the understanding of the chemistry the incorporation of fluoride and its compatibility with the ingredients in dentifrices, and have tested new formulations and mechanisms of caries inhibition[1,13]. The anti-cariogenic role of fluoride has been proven[11,14,15]. Fluoridated hydroxyapatite[2] (mineral crystal of dental tissue) has greater chemical stability and therefore better protects the teeth against acid attacks from cariogenic bacteria and consequent demineralization[1,2]. Thus, local therapies using drug delivery systems have attracted considerable attention as a way to transport and release therapeutic agents or bioactive substances into the oral cavity[16].

The use of nanotechnology has gained prominence in preventive dentistry as a slow-release system for substances that can control and treat oral problems[17-21]. Involving components less than 100 nanometers in size[22], this technology may perform on the molecular level with greater affinity and effectiveness compared to micro or macro components[18,23]. In preventive dentistry, studies have reported better control of biofilm, prevention of caries, remineralization of initial carious lesions and sub-micrometric dental defects, biomimetic enamel synthesis and repair of microcavities[17-20,23,24].

The innovative idea of using nano-encapsulated fluorine ions (NanoF) in a controlled release system may be beneficial to enamel, as it would increase its substantivity and enable the substance to remain in contact with the dental surface for a longer time, leading to a greater protective and remineralizing effect. Therefore, the aim of the present study was to evaluate the influence of treatment time on the performance of nano-encapsulated fluoride (NanoF) dentifrices for the remineralization of initial carious lesions using an *in vitro* pH cycling model. The null hypothesis was that NanoF dentifrices have the same ability to remineralize dental caries as conventional NaF, regardless of treatment time.

MATERIALS AND METHODS

This study received approval from a research ethics committee in Brazil (certificate number: 45917915.6.0000.5188). The donors of the teeth signed a statement of informed consent in accordance with the Declaration of Helsinki and Resolution 466/12 of the Brazilian National Health Board.

Sample size and specimen preparation

Sample size was determined considering remineralizing potential as the primary outcome. Based on a previous study[25] and considering a two-tailed alpha of 0.01 and 80% power, a minimum of seven samples was required per group. Ninety human third molars with indication for extraction were collected, cleaned, and examined for any enamel alterations.

Enamel blocks (3 x 3 x 2 mm) were obtained from the dental crown (buccal and lingual surfaces) and embedded in self-curing acrylic resin using circular molds (16 x 3 mm). A metallographic polisher was used to flatten the outer enamel (with 400, 600 and 1200 grit sandpaper disks) under constant irrigation. The enamel surfaces were polished with wet felt and a 1 µm diamond suspension (Extec Corporation, Enfield, CT, USA) in a rotating polishing machine (PSK- 2V, Skill-Tec Comércio e Manutenção Ltda, São Paulo, SP, Brazil). All samples were submitted to water sonication for five minutes and stored at -20ºC until the day of the experiment.

A total 96 enamel specimens were randomly distributed into eight groups (n=12) according to their initial surface microhardness and time treatments: PC1'(dentifrice with 100% NaF fluoride, 1 minute); PC5'(dentifrice with 100% NaF fluoride, 5 minutes); NC1' (dentifrice without fluoride, 1 minute); NC5' (dentifrice without fluoride, 5 minutes); 50%nF1' (dentifrice with 50% NanoF + 50% NaF free, 1 minute); 50%nF5' (dentifrice with 50% NanoF + 50% NaF free, 5 minutes); 100%nF1' (dentifrice with 100% NanoF, 1 minute) and 100%nF5' (dentifrice with 100% NanoF, 5 minutes), coded by

an independent researcher, as described in Table 1. Enamel surface microhardness was analyzed with a microhardness tester (Shimadzu HMV - AD Easy Test Version 3.0). Five indentations spaced 100µm from each other were made at the center of the enamel surface (Vickers, 100g, 10s). Firstly, a baseline measurement (SH0) was performed, and then new measurements were taken after the formation of the carious lesion (SH1) and remineralizing treatment (SH2), using the same initial parameters. Enamel blocks with initial surface microhardness between 390 and 440 VHN were selected for the study.

Induction of caries lesion

Following the SH0 measurements, the enamel surface of each specimen was divided into three equal parts. One third of the exposed enamel was covered with a double layer of nail varnish (Risque, Niasi, Taboão da Serra, São Paulo, Brazil) to preserve a control area in each specimen. Subsurface enamel demineralization was induced in the other two thirds by immersing each enamel specimen in 32 ml of demineralizing solution [1.3 mM/L $Ca(NO_3)_2.4H_2O$, 0.78 mM/L NaH_2PO_4 H_2O in 0.05 M/L acetate buffer, 0.03 µgF/mL (NaF), pH 5.0] for 16 h at 37ºC [26]. Post-demineralization surface hardness (SH_1) was determined, and the percentage of surface hardness change (SMHC) was calculated [% SMHC= (SH1 – SH0/ SH0) × 100].

Collection of human saliva

Stimulated human saliva was collected from 12 healthy individuals 18 to 35 years old, of both sexes, without system involvement and resident in a city without fluoridated water. Exclusion criteria were having used drugs that interfere in the salivary flow or fluoride products within the last four weeks; using orthodontic appliances; presence of caries and/or erosion lesions and/or periodontal disease. Stimulated human saliva was collected each day of the study. Each volunteer chewed paraffin wax and spat the stimulated saliva into a plastic cup. The samples were stored in a refrigerator at 5 °C until use[27].

Remineralizing pH cycling

Before the remineralization pH cycling model based on Vieira et al.[28], another third of the surface of the enamel specimens was covered with two layers of nail varnish (Risque, Niasi, Taboão da Serra, São Paulo, Brazil), to provide a carious lesion reference area (SH1). In each cycling model (Fig 1), the specimens were submitted to a five-day[28] pH cycling model at 37ºC, and remained in the remineralizing solution for 2 days. The blocks were individually immersed in a remineralization solution (1.5 mmol/L Ca, 0.9 mmol/LP, 150 mmol/L KCl, 0.05 mg F/mL in 0.1 mol/L Tris buffer, pH 7.0) for 18 h. The cariogenic challenge was performed using a demineralizing solution (0.05 mol/L acetate buffer,

Table 1. Dentifrices used in the study according to their composition and manufacturer*.

Group**	Time Treatment	Composition	Manufacturer ***
50%nF1' (50% NanoF + 50% NaF free)	1 minute	50% NanoF + 50% NaF (1450 ppm fluoride)	SAVOY, Jundiaí, São Paulo, Brazil
50%nF5' (50% NanoF + 50% NaF free)	5 minutes	50% NanoF + 50% NaF (1450 ppm fluoride)	SAVOY, Jundiaí, São Paulo, Brazil
100%nF1' (100% NanoF)	1 minute	100% NanoF (1450 ppm fluoride)	SAVOY, Jundiaí, São Paulo, Brazil
100%nF1' (100% NanoF)	5 minutes	100% NanoF (1450 ppm fluoride)	SAVOY, Jundiaí, São Paulo, Brazil
PC1' (Positive control - 100% NaF)	1 minute	100% sodium fluoride - NaF (1450 ppm fluoride)	SAVOY, Jundiaí, São Paulo, Brazil
PC5' (Positive control - 100% NaF)	5 minutes	100% sodium fluoride - NaF (1450 ppm fluoride)	SAVOY, Jundiaí, São Paulo, Brazil
NC1' (Negative control - no fluoride)	1 minute	No fluoride	SAVOY, Jundiaí, São Paulo, Brazil
NC5' (Negative control - no fluoride)	5 minutes	No fluoride	SAVOY, Jundiaí, São Paulo, Brazil

*All experimental dentifrices included the following ingredients: water, carboxymethyl cellulose (binder), sodium lauryl sulfate (surfactant), hydrated silica (abrasive), 70% sorbitol and glycerin (humectants), and methylparaben (preservative).
**1' – one-minute treatment and 5' – five-minute treatment.
***The patent pending (BR 102018070679-9 A2) belongs to NANOVETORES TECNOLOGIA S.A.

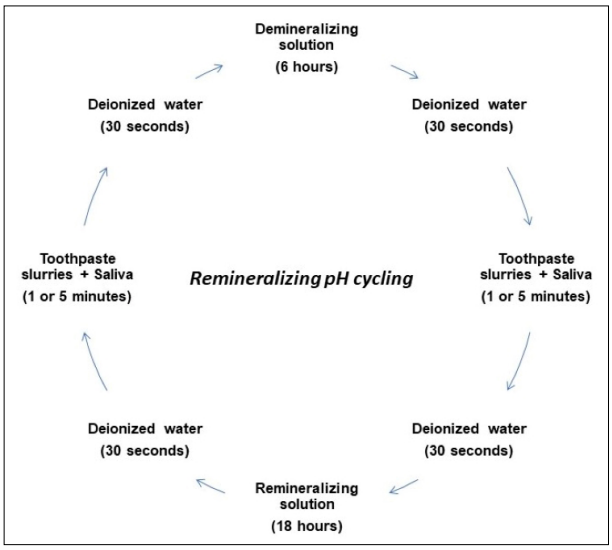

Fig. 1: Flow chart of the remineralizing pH cycling model.

pH 5.0, containing 1.28 mmol/L Ca, 0.74 mmol/LP, and 0.03 mg F/mL) for six hours per day. The treatment regime consisted of immersing the enamel samples in dentifrice slurries twice a day (1:3 w/w; 2 mL/enamel specimen) for one or five minutes under agitation. The treatments were performed before and after the demineralizing solution. In order to activate the salivary amylase of the NanoF groups, a volume of approximately 10% of human saliva was added to all the dentifrice slurries. Samples were rinsed with deionized water between steps. The demineralizing and remineralizing solutions were refreshed every day. All eight groups were submitted to the same pH cycling, differing only in treatment times (one or five minutes). At the end of the seventh day of pH cycling, the specimens were washed again with deionized water under sonication for 5 min and stored in a humidity-controlled environment to prevent drying until further analysis.

After the remineralizing pH-cycling, the nail varnish was removed and enamel surface microhardness was again determined (SH_2) as baseline parameters. The values were averaged (µm) and the percentage surface microhardness recovery was calculated as following formula: $\%SMHR = (SH_2-SH_1)/(SH_0-SH_1) \times 100$.

Statistical analysis

Data were statistically analyzed using the SPSS, version 21.0 (SPSS, Inc., Chicago, IL, USA). Normal Distribution and homogeneity of variances were tested using the Shapiro-Wilk test and Levine test, respectively. Since data demonstrated homogeneity of variances and Gaussian distribution, no data transformation was necessary. Assumptions of analysis of variance were checked before the use of two-way factorial analysis of variance (ANOVA) model to investigate surface microhardness remineralization in the different groups for the dependent variable ($\%SMH_R$), and ANOVA Repeated Measures, followed by Bonferroni, to analyze the variables SH_0, SH_1, SH_2 within the same group at the different analysis times. The level of significance considered was 5% ($p<0.05$).

RESULTS

Table 2 shows the mean and standard deviation values for the variables SH_0, SH_1, and SH_2 for all test groups. Comparison according to the variables SH_0, SH_1 and %SMHC showed no difference between groups ($p> 0.05$). However, for the SH_2 variable, differences were observed between groups ($p <0.001$). The 5-minute PC had the best remineralization value and the 50%nF1', 50%nF5' and 100%nF5' performed similarly to the 1-minute positive control.

Individual analysis of each dentifrice remineralization showed no significant difference between SH_0 and SH_1. However, there were differences between the SH_1 and SH_2 variables, for the 50%nF1', PC1', PC5' and NC5' groups (ANOVA repeated-measures, $p<0.05$).

Fig. 2 shows the comparison of the groups using the percentage surface microhardness recovery (%SMHR) variable. Differences were observed between groups ($p<0.05$). The PC groups presented the best remineralization values, indicating the same pattern observed for the SH_2 variable. The 50%nF1' and 100%nF5'performances were similar to the PC groups (1' and 5'). No remineralization recovery was detected in the negative control (1 or 5 minutes) or in the 50%nF5' groups.

DISCUSSION

Studies in the field of cariology have fostered progress in the treatment of dental caries[29]. Nano-scale drug delivery systems have gained ground in this respect and nanotechnology has been investigated as a means to enhance fluoride substantivity in the oral environment[13,30,31], prolonging its effect with a small amount of product[32-34]. Thus, nano-encapsulated fluoride systems may constitute a promising

Table 2: Mean and standard deviation (SD) of SH_0, SH_1 and SH_2 variables of all the groups, according to the different treatment times (one and five minutes).

GROUPS	SH_0 (SD)*	SH_1 (SD)*	SH_2 (SD)*
50%nF 1'	432.9 (24.36) ^{a,A}	17.51 (1.67)^{a,B}	25.54 (5.4)^{a,C}
50%nF 5'	391.56 (14.16) ^{a,A}	28.69 (0.84) $^{)a,B}$	25.86 (1.27)^{a,B}
100%nF 1'	438.64 (16.18) ^{a,A}	15.23 (1.79) ^{a,B}	14.5 (3.49)^{b,B}
100%nF 5'	390.36 (12.31) ^{a,A}	25.12 (4.33) ^{a,B}	27.48 (2.88)^{a,B}
PC 1'	433.79 (28.8) ^{a,A}	16.07 (0.86) ^{a,B}	24.74 (0.98)^{a,C}
PC 5'	390.00 (12.85) ^{a,A}	29.31 (4.93) ^{a,B}	36.16 (4.57)^{c,C}
NC 1'	439.42 (19.86) ^{a,A}	16.50 (4.46) ^{a,B}	14.25 (2.1)^{b,B}
NC 5'	394.13 (7.43) ^{a,A}	28.03 (5.62) ^{a,B}	15.46 (3.32)^{b,C}

*Similar lowercase letters represent no significant difference between groups for each column (ANOVA, p>0.05). Different capital letters represent significant difference within the same group, for each line, for different analyses (ANOVA repeated measures, p<0.05).

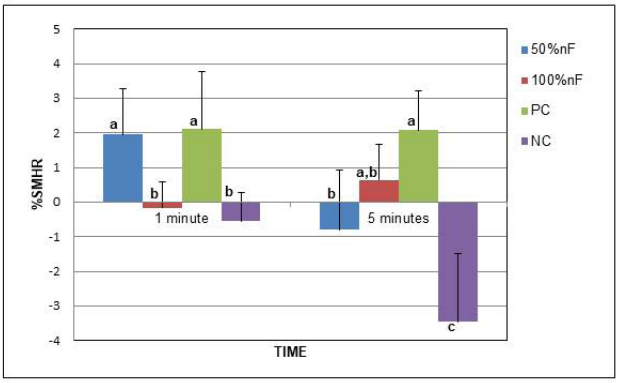

Fig. 2: Mean and standard deviation (SD) of %SMHR for all groups, according to the different treatment times (one and five minutes).

treatment for carious lesions. In the present study, the nano-encapsulated fluoride (NanoF) system was created as an innovative method to act as a controlled release system to increase fluoride substantivity and bioavailability in the oral cavity. The composition of NanoF is basically sodium fluoride (NaF) encapsulated in a natural biopolymer (hydroxypropyl guar) as the drug carrier. Guar gum is a molecular weight carbohydrate polymer derived from the natural seed of the guar plant (*Cyamopsis tetragonoloba* (L.) Taub). Its particle size is about 200 nm. Fluoride release is not dependent on pH, but on enzymatic trigger (amylase) following a mechanism of stimuli-controlled release, which is why fresh human saliva was added to the pH cycling model. Also, a previous clinical study[35], using the same dentifrices, reported a delay in the fluoride release in saliva within the first 60 minutes after brushing and reported that dental biofilm showed a more prolonged incorporation of fluoride ions when compared to conventional NaF dentifrice[35].

It was important to study treatment time (1 or 5 minutes) because the NanoF controlled-release dentifrices were created to deliver fluoride ions more slowly. As expected, there were differences in the potential for remineralizing carious lesions according to treatment time. Also, as the literature lacks studies involving the same NanoF, we suggested some hypotheses to explain the role of these dentifrices in the process of remineralization of the decayed enamel.

Fluoride is known for its anti-caries and remineralizing action in its free form and at appropriate concentrations, with a high salivary dose-response level[36]. As fluorine is an electronegative element and thus highly reactive, the pH, ionic concentration, and/or the composition of the vehicle may modulate its action[36]. Based on this concept and the similar remineralization values found in the 50%nF1' and PC1' groups, it was evident that the free NaF was responsible for the initial performance before NanoF had had sufficient time to exert an effect. However, the unsatisfactory remineralizing performance observed for the 50% nF group after five minutes of treatment may have been due to the solvation of fluoride. As the release of NanoF occurred gradually, complexation may have occurred between conventional NaF and the gradually released NanoF, preventing the effective performance of the fluoride in the remineralization process. Thus, it can be said that the 50% nF5' dentifrice behaved similarly to a dentifrice with a low fluoride content.

Analysis of the 100% nF groups showed that the double molecular polymer of the NanoF required a longer treatment time to release fluoride ions more effectively. During the one-minute treatment, the dentifrice resulted in the absence of protective/remineralizing action. However, an increase of about 75% in %SMHR was found for the five-minute treatment, indicating the importance of the time factor for the release of NanoF. No significant difference was found between the 100%nF5' and PC groups, despite the lower value for %SMHR.

A higher concentration of fluoride increases its capacity to diffuse through biofilm and exert an effect on the dental surface, promoting a gain in remineralizing capacity[37]. Nevertheless, a low concentration of fluoride may promote remineralization, provided that it is employed in an acidic vehicle[35,38,39]. Thus, the poor remineralization found in the 50%nF5' and 100%nF1' groups may also be explained by the neutral pH of the dentifrices used. Treatment time, an acidic environment, and sites of greater fluoride concentration are important factors. Promising results were found in a randomized clinical trial using the same experimental dentifrices, which demonstrated an increase in fluoride substantivity, enabling higher concentrations in dental biofilm over time[35,38,39]. When fluoride is presented only in saliva, dilution and swallowing lead to the return to the initial salivary concentration after 40 to 60 minutes of contact with the dentifrice. Thus, the incorporation of fluoride into the biofilm as a reservoir for subsequent release is more desirable for effective remineralization.

A major problem associated with the delivery of fluoride is the short time of action[21]. In the present study, salivary amylase is believed to have broken down the polymer, releasing the nanofluoride for fast, lasting action. Based on these findings, NanoF is a promising nano-encapsulated fluoride system that can be adjusted to promote faster fluoride release, achieve optimal salivary levels, and enable adhesion to biofilm with a prolonged performance.

DECLARATION OF CONFLICTING INTERESTS

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Endodontic treatment during the COVID-19 pandemic - perception and behaviour of dental professionals

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ABSTRACT
This study evaluated the impact of COVID-19 on the endodontic treatment routine. It was a cross-sectional study using an online questionnaire applied to endodontists to collect information about practical modifications during endodontic treatment to protect professionals and patients against the COVID-19 outbreak. A total 1105 participants from Brazil participated in the survey. More than 90% of respondents identify the high risk of COVID-19 infection to dentists and the need to change some clinical practices. Most respondents (60.1%) are partially following social isolation. The need for a change in Personal Protective Equipment (PPE) during dental appointments was mentioned by 97.1% of respondents. The use of minimal adequate PPE during the pandemic period was associated with the area of residence and marital status of participants. Only 30% of respondents say they use the minimal adequate PPE. Most respondents will change cavity access preparation to reduce virus dissemination. Other changes in endodontic appointments were described in the survey: greater attention to biosafety measures, duration of dental appointments, and duration of intervals between appointments. Endodontists still need to identify the best arrangement for performing their procedures safely during the COVID-19 pandemic. Specific guidelines require detailed information for each specialty and its procedures.
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Keywords: *biosafety - coronavirus - dentistry - endodontics - SARS-CoV-2.*

Tratamento endodôntico durante a pandemia de COVID-19 – Percepção e comportamento dos profissionais de odontologia

RESUMO
Este estudo transversal foi realizado por meio de um questionário online. O questionário foi aplicado a endodontistas e coletou informações sobre modificações práticas durante o tratamento endodôntico para combater o surto de COVID-19 e proteger profissionais e pacientes. Um total de 1105 participantes do Brasil participaram da pesquisa. Mais de 90% dos entrevistados identificam o alto risco de infecção por COVID-19 para os dentistas e a necessidade de mudar algumas práticas clínicas. A maioria dos entrevistados (60,1%) segue parcialmente o isolamento social. A necessidade de mudanças dos Equipamentos de Proteção Individual (EPIs) durante as consultas odontológicas foi referida por 97,1% dos entrevistados. O uso de EPIs mínimos adequados durante o período pandêmico foi associado à área de residência e ao estado civil dos participantes. Apenas 30% dos participantes afirmou usar os EPIs mínimos durante a pandemia. A maioria dos entrevistados mudará a preparação do acesso à cavidade para reduzir a disseminação do vírus. Outras mudanças nas consultas endodônticas foram descritas na pesquisa: maior atenção às medidas de biossegurança, duração das consultas odontológicas e intervalos entre as consultas. Os endodontistas ainda precisam distinguir a melhor maneira para realizar seus procedimentos com segurança, durante a pandemia de COVID-19. Diretrizes específicas requerem informações detalhadas para cada especialidade e seus procedimentos.

Palavras-chave: *biossegurança - coronavirus - odontologia-endodontia - SARS-CoV-2.*

INTRODUCTION

Coronavirus disease (COVID-19) has emerged as the largest global pandemic and has already affected several countries, including Brazil[1]. Even though the disease sometimes appears as an asymptomatic or symptomatic clinical condition[2], the diagnosis is most commonly associated with fever, dry cough, fatigue and difficulty in breathing[3]. COVID-19 can progress to other serious conditions with severe respiratory symptoms, including dyspnea and pneumonia, which can lead to death[4,5].

Although the mortality associated with COVID-19 is low, it has high spreading potential[6] and high levels of transmissibility[6-8]. It spreads mainly from person to person through contact transmission, including oral, nasal and eye membrane contact; or direct transmission, including sneezing, coughing and droplet inhalation[9]. Droplets containing the virus can settle on surfaces, where the virus can remain viable for days, causing significant concern among healthcare professionals and support services, including dental professionals[10].

Dental care settings consistently involve the risk of COVID-19 infection due to the specificity of the procedures conducted in them. Dental professionals are constantly exposed to pathogenic microorganisms, including viruses and bacteria[11]. The exposure to saliva and blood, the use of sharp instruments, and the droplets and aerosols generated during dental treatment can contaminate instruments and environmental surfaces in dental practice, or infect the professional directly[12-14].

The American Dental Association (ADA) recently issued guidance to prevent infection in the COVID-19 pandemic during emergency and non-emergency dental procedures[15]. Infection control in dental practice includes the use of Personal Protective Equipment (PPE), and cleaning and disinfecting the dental care environment as part of the clinical routine needed to prevent cross-infection[16].

The available information on how dentists should work during the COVID-19 pandemic is very helpful in preventing infections. However, this information refers to urgent and emergency procedures or dental procedures in general, but does not provide more specific explanations on actions required for each specialty, e.g., endodontics. Thus, the aim of this study is to evaluate, through the application of a questionnaire, the impact of COVID-19 on endodontic treatment routine among Brazilian dentists, including possible changes in the technique and/or in preventive measures.

MATERIALS AND METHODS

The institutional Research Ethics Committee approved this study (CAAE: 31064820.6.0000.5052). Participants who accepted to take part in the survey signed an informed consent form (ICF) online.

This was a cross-sectional study using an online questionnaire with a convenience sample. The questionnaire design, pilot study and strategies for enrolling dentists were previously discussed, and it was decided that a convenience sample enrolled using social media would be valid to acquire a large number of respondents. The questionnaire was posted on the Google Forms platform, where it was available for one week, after which it was closed.

Respondents had to be dentists who regularly performed endodontic procedures in clinical practice (there was no need to be a specialist in endodontics). The submission was considered only when the 'submit' button was clicked at the end of the questionnaire.

This study was conducted from May 2 to May 6, 2020. The questionnaire was distributed by email as well as being posted on several social media platforms, such as Facebook and WhatsApp. It comprised 20 closed-ended questions designed to collect information about practical modifications established against the COVID-19 outbreak during endodontic treatment to protect professionals and patients (Fig. 1).

The questionnaire was divided into two sections. The first section recorded personal data such as age, sex, years' experience in dental practice, area of residence, and professional setting (private or public) of dental practice. The second section included questions about dentists' perception and behavior regarding endodontic treatment during the COVID-19 pandemic. For data analysis, we considered minimal PPE before COVID-19 pandemic to be the use of gloves, surgical masks, protective eyewear, surgical caps, gowns or lab coats. During the pandemic, minimal PPE includes gloves, N95 masks, protective eyewear, surgical caps, disposable gowns or lab coats, and shoe covers.

After responding to the questionnaire, respondents were requested to provide their registration number in the Regional Council of Dentistry (RCD) to prevent duplication of data.

Endodontic treatment during the COVID-19 pandemic - perception and behaviour of dental professionals

Sex	□ female	□ male		
Year of birth	Year			
Professional experience	□ Up to 10 years	□ 11 to 20 years	□ More than 20 years	
In which Brazilian state do you live?		State		
Marital Status	□ Single	□ Married	□ Divorced, separated or widowed	
Professional setting	□ Private	□ Public	□ Both, public and private	
Highest education level	□ PhD/MS	□ Specialist degree	□ DDS	
Have you already tested positive to COVID-19 or been in direct contact with someone who tested positive?			□ Yes	□ No
What is the risk of COVID-19 infection to dentists?		□ High	□ Moderate	□ Low
Do you believe it will be necessary to change some clinical practices in endodontic treatment due to the pandemic?			□ Yes	□ No
Are you following the recommendation of social isolation?		□ Yes, totally	□ Yes, partially	□ No
In comparison to the most affected countries, how do you expect the infection would affect our country?		□ Brazil would have a lower or similar number of COVID-19 infected people in comparison to the most affected countries □ Brazil would be one of the most affected countries in the world.		
During the COVID-19 pandemic, will you change the PPEs used during dental appointments?			□ Yes	□ No
Which PPE did you usually wear before the COVID-19 pandemic?				select
Which PPE do you think it is necessary to wear during the pandemic?				select
Before the COVID-19 pandemic, did you perform rubber dam isolation before or after endodontic access cavity preparation?			□ Before access	□ After access
During the COVID-19 pandemic, do you perform rubber dam isolation before or after endodontic access cavity preparation?			□ Before access	□ After access
Before the COVID-19 pandemic, how did you perform endodontic access cavity?		□ Using low-speed handpiece □ Using high-speed handpiece with water cooling □ Using high-speed handpiece with irrigation syringe □ Using high-speed handpiece without water cooling		
During the COVID-19 pandemic, how are you performing endodontic access cavity?		□ Using low-speed handpiece □ Using high-speed handpiece with water cooling □ Using high-speed handpiece with irrigation syringe □ Using high-speed handpiece without water cooling		
Which changes in dental practice do you consider during the COVID-19 pandemic?		Changes		

Fig. 1: Questionnaire sent to Brazilian dental professionals

Data were collected and analysed by SPSS 25.0 for Windows (IBM corp., SPSS, Inc., Chicago, IL, USA). Descriptive statistics were performed, and chi-square and Fisher's exact tests were used to test the significance of possible associations. The level of significance was 5% ($p < 0.05$).

RESULTS

A total 1105 questionnaires were answered from all of Brazil's 25 states. Mean respondent age was 37.79±10.35 years. Female respondents accounted for 66.2% (732) and male 33.8% (373) of the total. Regarding time elapsed since graduation in dentistry, 44.2% (488) of respondents graduated in the last 10 years, 30% (332) graduated between 11 and 20 years ago, and 25.8% (285) graduated more than 20 years ago.

The percentage of respondents by regions of Brazil was 263 (23.8%) from the south, 335 (30.3%) from the south-east, 406 (36.7%) from the north-east, 54 (4.9%) from the mid-west and 47 (4.3%) from the north. Regarding clinical practice, most respondents worked only in private practice (72.5%), whilst 5.7% worked exclusively in public practice, and 21.8% worked in public and private practice (Table 1).

Ninety-seven respondents (8.8%) had already tested positive for COVID-19 or had been in direct contact with someone who tested positive. A total 1011 (91.5%) respondents identify the high risk of COVID-19 infection to dentists, while 1073 (97.1%) recognise the need to change some clinical practices in endodontic treatment due to the pandemic.

Most respondents (60.1%) are partially following social isolation. At the time the questionnaire was applied, 777 (70.3%) of respondents believed that Brazil would have a lower or similar number of COVID-19 infected people in comparison to the most affected countries, while 328 (29.7%) believed that Brazil would be one of the most severely affected countries in the world.

When asked if during the COVID-19 pandemic they would change the PPE used during dental appointments, 1073 (97.1%) answered affirmatively. Table 2 shows the use of minimal adequate PPE before and during the pandemic period, and how it differed according to respondent background characteristics.

Fig. 2 illustrates the changes in endodontic cavity access during the pandemic. Most respondents (75.93%) agree that the use of a rubber dam before cavity access preparation diminishes the risk of virus dissemination. Most participants (56.83%) said that they would still use the high-speed handpiece during COVID-19 pandemic for cavity access preparation. Other changes in endodontic appointments were also considered in the survey, including greater attention to biosafety measures, duration of dental appointments, and duration of intervals between appointments (Fig. 3).

Table 1. Respondent demographics

	n	%
Sex		
Female	732	66.2
Male	373	33.8
Professional experience		
Up to 10 years	488	44.2
11 to 20 years	332	30.0
More than 20 years	285	25.8
Area of residence		
South-east	335	30.3
South	263	23.8
North-east	406	36.7
Mid-west	54	4.9
North	47	4.3
Marital status		
Single	402	36.4
Divorced, separated or widowed	78	6.9
Married	625	56.6
Professional setting		
Private	801	72.5
Public	63	5.7
Both, public and private	241	21.8
Highest education level		
PhD/MS	322	29.1
Specialist degree	646	58.5
DDS	137	12.4

DISCUSSION

Since the beginning of the pandemic, other studies have aimed at measuring the impact of the disease on dental professionals. However, ours is the first study

Table 2 – Association between the respondents' demographic and professional characteristics and the use of adequate PPE before and during the COVID-19 pandemic.

	* Minimal PPE before COVID-19 pandemic n (%)	P value	** Minimal PPE during COVID-19 pandemic n (%)	P value
Total	888 (80.4)		255 (23.1)	
Sex				
Female	617 (84.3)	**.000**	170 (23.2)	.940
Male	271 (72.7)		85 (22.8)	
Professional experience				
Up to 10 years	392 (80.3)		100 (20.5)	
11 to 20 years	273 (82.2)	.462	85 (25.7)	.184
More than 20 years	223 (78.2)		70 (24.7)	
Area of residence				
South-east	266 (79.4)		67 (20.0)	
South	210 (79.8)		76 (28.9)	
North-east	322 (79.3)	.247	89 (21.9)	**.037**
Mid-west	48 (88.9)		16 (29.6)	
North	42 (89.4)		7 (14.9)	
Marital status				
Single	328 (81.6)		68 (16.9)	
Divorced, separated or widowed	64 (82.1)	.630	19 (24.4)	**.001**
Married	496 (79.4)		168 (26.9)	
Professional practice				
Private	637 (79.5)		174 (21.7)	
Public	58 (92.1)	.054	15 (23.8)	.186
Both, public and private	193 (80.1)		66 (27.4)	
Highest education level				
PhD/MS	272 (84.5)		69 (21.4)	
Specialist degree	511 (79.1)	.071	152 (23.5)	.670
DDS	105 (76.6)		34 (24.8)	

* Gloves, surgical masks, protective eyewear, surgical caps, gowns or lab coats ** Gloves, N95 masks, protective eyewear, face shield, surgical caps, disposable gowns or lab coats and shoe covers.

to emphasize the need for changes in endodontic practice. Surveys using questionnaires are a popular method for determining the views of a sample of healthcare professionals or patients[17].

Genetic and epidemiological research has found that the COVID-19 outbreak began with the transmission of the virus from animal to human, followed by the spread from human to human[18,19]. Further studies have demonstrated that not only is the virus transmitted by symptomatic patients, but also by asymptomatic persons or even those in the incubation phase[18,20,21]. This difficulty of identifying whether or not individuals have the virus makes the regulation of disease transmission extremely challenging. This has led to the constant recommendation for people to practice social isolation as much as possible during the pandemic period. However, in China, the demand for urgent dental treatment decreased by only 38% during the pandemic period[22], reflecting the fact that urgent

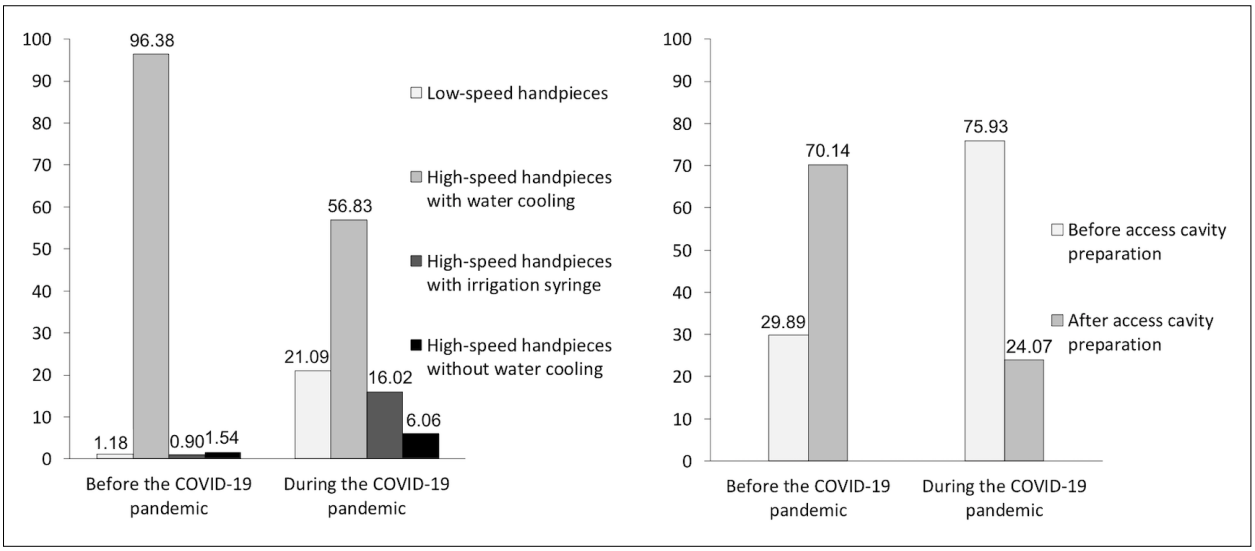

Fig. 2: Professional choices regarding cavity access preparation before and during the COVID-19 pandemic

dental services will always be essential, making it difficult for dental care providers to practice social isolation. In addition, the interruption of dental care during this period puts pressure on the emergency departments at hospitals, which are already dealing with the pandemic[23].

Although at this point Brazil is an epicenter of coronavirus infection, the present study was conducted from May 2 to May 6, 2020, when Brazil only had 90,000 cases of COVID-19 and 6,000 confirmed deaths. The information from the survey showed that most dentists were not in total social isolation, and were still performing different types of procedures, although the ADA (American Dental Association) had recommended that dentists should postpone elective treatments to avoid contact, focusing only on urgent and emergency care[24].

It has been suggested that dental professionals are at higher risk for COVID-19 infection than any other professional[11], requiring strategies to prevent infection, including pre-exposure and post-exposure prophylaxis. The results of the present study demonstrated that dental professionals are aware of this, with most respondents (91.5%) identifying the high risk of COVID-19 infection to dentists, and 97.1% saying they would change the PPE used during dental appointments during the pandemic. In fact, considering the severity of the COVID-19 pandemic, and in the light of the strong commitment of several dental associations, it is essential to provide simple, clear guidelines for managing dental patients and protecting dentists against risks[25].

COVID-19 transmission can be expected via contact with droplets and aerosols generated during dental clinical procedures[26]. The guidelines recommended for dentists and dental staff by the Centers for Disease Control and Prevention (CDC), the ADA and the World Health Organization (WHO) to control the spread of COVID-19 include PPE, hand washing, detailed patient evaluation, rubber dam

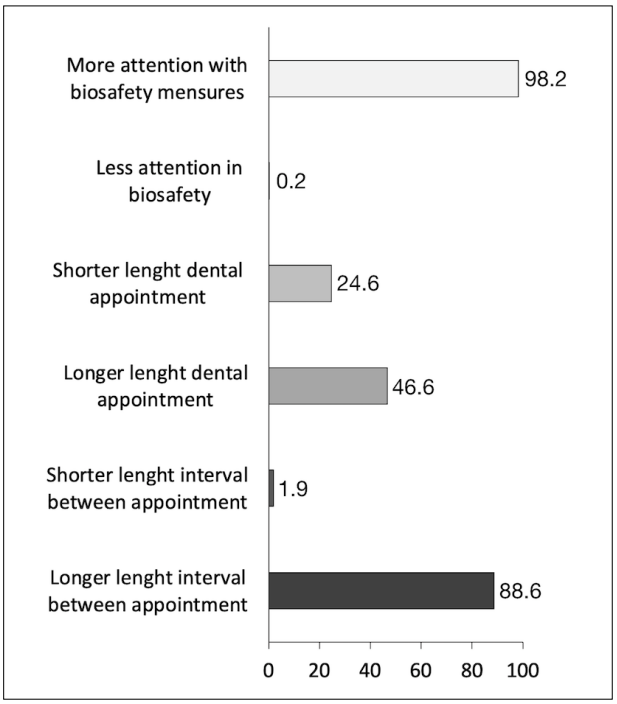

Fig. 3: Changes in endodontic dental practice during the COVID-19 pandemic according to questionnaire respondents

isolation, mouthwash before dental procedures, and disinfection of every exposed surface[27].

Our results showed that before the pandemic, more than 80% of dental professionals used adequate PPE. The use of adequate PPE was more frequent among women, reiterating the concept that women tend to be more careful. This is somehow expected, since in general, women get less sick, suffer fewer accidents and die later because they are more careful and prevent more than men do. However, this association has not been demonstrated considering the pandemic period only, because it is still too recent.

Even though more than 97% of the respondents are aware of the need to change the PPE they use during the pandemic, at the time the survey was carried out, less than 30% of respondents said they used adequate PPE. Single respondents seem to be less worried than married respondents about using appropriate PPE during the pandemic, possibly because they usually live alone and worry less about other family members to whom they could transmit the disease if they became infected. We also found that the percentage of professionals using adequate PPE is higher in the southern and central-western Brazilian regions. It is interesting to note that at the moment, these areas present the lowest number of COVID-19 infected people.

It has already been recognized that the use of high-volume saliva ejectors could reduce the production of droplets and aerosols in dental procedures, thereby protecting dentists from infection. Furthermore, face shields and protective eyewear are essential whenever high- or low-speed drilling are used. The use of rubber dam isolation reduces the generation of droplets and aerosol mixed with patient saliva and/or blood in 1 m diameter of the surgical field by 70%[28]. Opportunely, the current study showed that 75.93% of respondents consider that rubber dam isolation before endodontic access cavity reduces the risk of virus transmission. In addition, the use of a high-speed handpiece with water-cooling to perform the access cavity was seen as a concern. Some of the respondents intend to change the technique during endodontic procedure, using low-speed handpiece or discarding the use of water-cooling. However, the use of high speed without water-cooling is not recommended, and in these cases, the damage to dental structure should also be considered.

Nearly all participants believe that biosafety in dental practice during the pandemic should receive more attention. This seems to be a consequence of extensive media coverage regarding the need to reinforce hygiene methods such as constant handwashing, use of a mask, coughing into a bent elbow, and keeping a distance, among others[29]. Almost half the respondents believe that longer dental appointments are one the changes that should be implemented in clinical practice during the COVID-19 pandemic. Even though there is no published data regarding the adequate length of dental appointments, it seems reasonable to believe that longer dental appointments may allow the dental professional to better complete the endodontic procedures, avoiding repeated contact with the patients on different days. Moreover, it has been recommended that the interval between dental appointments should last long enough for possible contagious droplets to stop floating in the air and settle on the surfaces for subsequent disinfection of the entire dental office and replacement of all PPE[30]. In the current study, almost 90% of respondents believe that the interval between dental appointments should be longer during the pandemic, which is in agreement with the recommendations.

The effects of COVID-19 are becoming worse day by day. As dentistry is at the top of professions at risk of contamination, many dentists are anxious and afraid to work. The general population, however, still demands emergency and non-emergency dental services. It is therefore essential that dentists should have appropriate guidelines for the protection of both parties. Several points can and should be analyzed regarding dental practice during the COVID-19 pandemic. Considering the high level of risk to which dentists are exposed, it is crucial that clearer and more detailed protocols should be developed so that professionals know how to act to prevent the virus from spreading. This risk can be reduced if dentists act appropriately in all specialties and when performing any type of procedure.

In conclusion, dentists who perform endodontic treatment still need to be able to identify the best arrangements for performing their procedures safely during COVID-19 pandemic. Specific guidelines require detailed information for each specialty and its procedures.

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DECLARATION OF CONFLICTING INTERESTS
The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Cross-cultural adaptation of a quality of life questionnaire for individuals with oral potentially malignant disorders in the Brazilian context

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ABSTRACT

The aim of this article is to describe the cross-cultural adaptation of a quality of life (QoL) questionnaire for individuals with potentially malignant oral diseases (OPMD) in the Brazilian context. This methodological study consisted of the following stages of content validation process: (1) Conceptual and item equivalence: stage during which a comprehensive literature review on the construct was performed; (2) Semantic equivalence. The extensive literature review showed that the questionnaire enables evaluation of QoL, and that domains and items are also considered and relevant to the Brazilian context. Semantic equivalence was evaluated as satisfactory by a committee of judges. The scope of the domains was analyzed according to the agreement rate and presented results equal to or greater than 84%. The general Content Validity Calculation (CVC) was 0.84 for clarity and 0.92 for representativeness. Of the 20 items, 18 presented CVC values above 0.8. The indicators for content validation, pre-test and operational equivalence indicate that the Brazilian version of the OPMD QoL questionnaire is a promising instrument and a tool that seems valid to evaluate the quality of life of people with oral potentially malignant disorders. As a next step, it is important to measure equivalences to evaluate the psychometric properties of this instrument.

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Keywords: *validation study - questionnaires - quality of life - leukoplakia - cheilitis.*

Adaptação transcultural do questionário de qualidade de vida para indivíduos com desordens orais potencialmente malignas para o contexto brasileiro

RESUMO

Este artigo objetiva adaptar transculturalmente um questionário de qualidade de vida para indivíduos com doenças orais potencialmente malignas para o contexto brasileiro. Este estudo metodológico consistiu nas seguintes etapas do processo de validação de conteúdo: (1) Equivalência conceitual e de itens - etapa em que foi realizada uma ampla revisão da literatura sobre o construto; (2) Equivalência semântica. A extensa revisão da literatura mostrou que o questionário permite avaliar a QV e que os domínios e itens também são considerados e relevantes para o contexto brasileiro. A equivalência semântica foi avaliada pelo comitê de juízes de forma satisfatória. A análise do escopo dos domínios foi realizada pela taxa de concordância e apresentou resultados iguais ou superiores a 84%. Para a análise de clareza e representatividade, o resultado do cálculo geral do Cálculo de Validade de Conteúdo (CVC) foi de 0,84 e 0,92 respectivamente. Dos 20 itens, 18 apresentaram valores de IVC acima de 0,8. Os indicadores de validação de conteúdo, pré-teste e equivalência operacional indicam que a versão brasileira do QoL-OPMD é um instrumento promissor e que parece válido para avaliar a qualidade de vida de pessoas com doenças orais potencialmente malignas. Como próximo passo, é importante avaliar as propriedades psicométricas desse instrumento.

Palavras-chave: *estudos de validação - questionários - qualidade de vida - leucoplasia - queilite.*

INTRODUCTION

Oral Potentially Malignant Disorders (OPMD) are clinical manifestations that present a risk of progression to cancer and can significantly impair quality of life (QoL)[1,2]. Mouth cancer is a public health problem in several countries around the world, with an estimated annual incidence of 274,000 new cases and 128,000 deaths. In Brazil, for the years 2020-2022, there were an estimated 11,180 cases in men and 4,010 in women. Among all cancers, it ranks 5th for men and 13th for women[3].

The World Health Organization highlights the importance of prevention and early detection as decisive conditions for controlling oral cancer, and identifies the main OPMD as: leukoplakia, erythroplasia, oral lichen planus, actinic cheilitis and oral submucosal fibrosis[4].

Recent studies indicate that patients with OPMD may present physical impairment and limitations, as OPMD cause pain, difficulties and limitations in speech, functional limitations in mouth opening and discomfort to eat[1,5], which may compromise patients' quality of life (QoL). Improvement in QoL has thus become one of the expected outcomes of care practices and public policies in the fields of health promotion and disease prevention[6,7].

To measure the degree of impairment of this important health indicator, the Quality-of-life questionnaire for patients with oral potentially malignant disorders (OPMD QoL questionnaire) was developed and validated in India to measure QoL in patients with OPMD by specifically assessing subjective perceptions of the impacts of OPMD on aspects of their daily lives. This questionnaire is a subjective indicator that aims to ascertain the measure of disability, discomfort and disadvantage attributed to the oral condition, through self-assessment[2].

Developed by Jyothi Tadakamadla *et al.*[2], the questionnaire includes 20 items distributed among four factors: difficulties in diagnosis, physical impairment and limitations, social and psychological well-being and effects of treatment. The OPMD QoL questionnaire was validated in its context of origin, presenting favorable psychometric indices. Internal consistency measured by Cronbach's alpha coefficient was satisfactory (α=0.93). Stability measured through the re-test and verified by the intra-class correlation coefficient was also satisfactory (r=0.85). No other adaptations of this instrument were identified in other contexts.

An extensive literature review using the terms *Quality of Life* and *OPMD* in *Lilacs, Scielo* and *Medline* databases only found seven articles for analysis, thus confirming the existing gap due to low production. All these publications came from foreign journals, and the studies were conducted in India. Of the seven studies evaluated, only two addressed the production and validation of instruments whose objective was specifically to evaluate quality of life (QoL) in patients with OPMD.

The absence of tools in Portuguese for ascertaining quality of life in people with OPMD, and the need for a comprehensive approach in oral health practices that would include a subjective care perspective, create an imperative need in Brazil for a new measurement instrument that would be useful both clinically and epidemiologically. Thus, the aim of this article is to present the process of cross-cultural adaptation of a quality of life questionnaire for individuals with oral potentially malignant disorders (OPMD QoL questionnaire) in the Brazilian context.

MATERIALS AND METHODS

This research project was registered in the Brazil Platform and submitted for evaluation to the Human Research Ethics Committee (REC) at the State University of Feira de Santana, being approved under opinion number 3.578.351 and CAAE: 156224619.1.0000.0053.

The cross-cultural adaptation proposed herein is part of the universalist model of Herdman et al.[8] and Reichenheim and Moraes[9]. Our methodological study focused on adapting the quality of life questionnaire for individuals with oral potentially malignant disorders (OPMD QoL questionnaire).

The OPMD QoL questionnaire consists of 20 items to assess QoL. The answers are scored on a five-point Likert scale (0-4), in which 0= not at all; 1= a little; 2= somewhat; 3= quite a bit; 4= very much. The summary score of the questionnaire ranges from 0 to 80, with a higher score indicating lower quality of life[2].

Conceptual and item equivalence

After securing permission from the author of the original instrument, a comprehensive literature review was conducted on the construct *quality of life* in patients with OPMD and the relevance of this topic, from the domains and items of the OPMD QoL questionnaire, to the Brazilian context.

Semantic equivalence assessment

Semantic equivalence was assessed in seven stages: translation, synthesis, retranslation, synthesis, evaluation by the author of the original instrument, review by a committee of expert judges and evaluation by the target audience through pre-test.

The instrument was translated individually by two Brazilian translators, qualified in English, who were sent a letter of invitation by e-mail. The first two translations of the questionnaire (T1 and T2), they were synthesized by consensus among the study researchers to ensure that the items would be understood by the target population (synthesis of translations).

The synthesis version of the questionnaire in Portuguese was retranslated into English by two qualified translators whose mother tongue was English. This provided two versions of the questionnaire called back-translations – B1 and B2. The two back-translations were evaluated once again by the research team and a single version was produced (synthesis of back-translations), thus generating another synthesis (preliminary version), which was forwarded to the author of the original instrument for evaluation. Subsequently, it was initially evaluated by a group of 13 judges comprising professional specialists in the fields of health and linguistics.

The expert judges for this stage were found by searching the Lattes Curriculum platform for scientific productions related to questionnaire preparation, cross-cultural adaptation and psychometric assessment of health instruments. Subsequently, the Snowball technique was used to find other judges up to the maximum number established for this research. In the Snowball technique, the judge contacted through the Lattes Curriculum suggested another researcher who could respond to the instrument. A defined protocol was followed for the indicated researcher, in the following sequence: (1) Invitation letter; (2) Informed Consent Form for expert judges and (3) Evaluation instrument for expert judges.

A specific evaluation instrument was created for the analysis to be performed by the 13 expert judges. It was organized in two stages: (1) evaluation of the domains and their content regarding the scope of the construct, and (2) evaluation of each item in the OPMD QoL questionnaire regarding clarity and representativeness in the Brazilian context. The judges used a scale of 1 to 4 to assess the level of adequacy of language clarity and representativeness for the Brazilian context (evaluation of clarity, 1= very unclear; 2= unclear; 3= clear; 4= very clear; evaluation of representativeness, 1= not representative; 2= requires thorough review to be representative; 3= requires little review to be representative; 4= representative).

In the first stage, the judges verified whether the structure of the domain and its content were adequate and whether the content in each domain was representative in the Brazilian context. The agreement rate was calculated as the ratio between the number of judges who agreed and the total number of judges who participated, multiplied by 100, following the recommendations of Alexandre *et al*.[10].

Then the clarity and representativeness of the 20 individual items were analyzed using the content validity calculation (CVC). The aim of the CVC is to identify items that may not be appropriate to the objectives of the instrument. It analyzes the agreement among the expert judges. For this study, the CVC was calculated for each item in the instrument (CVC_f) and for the instrument as a whole (CVC_t). CVC was considered appropriate if it was above 0.70[11].

After analyzing the evaluation performed by the specialists, adjustments were made, obtaining the preliminary version of the OPMD QoL questionnaire to be evaluated by the target population through the pre-test stage, of which the aim was to identify any problems of interpretative order. Participants in the pre-test were a group of patients with a clinical diagnosis of OPMD and histopathological epithelial dysplasia or oral lichen planus. They were interviewed by a dentist who had been previously trained for the application of the sociodemographic data collection questionnaire and a specific evaluation instrument for the target population that also contained the OPMD QoL questionnaire.

The interviewer held individual conversations with the participants. Each item was read, and the participant was asked to paraphrase what he/she understood about it and to identify any words or terms he/she did not understand. The participant answered the question first, and the interviewer marked one of the four options provided in the instrument according to the following scores: 0= not at all; 1= a little; 2= half the times; 3= a lot;

4= completely. After this stage, the participant responded by checking YES or NO, if he/she considered the item important to evaluate his/her quality of life and for the clarity of the item. The pre-test instrument included a blank field for suggestions or observations. In case of misunderstanding of any word or item, the participant was asked to suggest expressions that would improve understanding.

After completing the pre-test, the group's agreement rate was calculated as the percentage of each domain according to the following formula: % of agreement = number of participants who agreed, divided by the total number of participants, multiplied by 100.

Operational equivalence

Operational equivalence was analyzed with the same group of pre-test participants, using a specific evaluation instrument. The aim of this phase was to obtain information for refining the adapted instrument by asking about presentation format, questions, instructions, application location, application mode and OPMD QoL questionnaire response alternatives.

RESULTS

Tables 1, 2, 3 and 4 show the original version, syntheses of the translations and back-translations, version evaluated by the judges and final version of the OPMD QoL questionnaire.

Regarding the analysis of the scope of the domains, the agreement rate was calculated, providing the following results: (I) for the difficulties in diagnosis domain, the agreement rate was 92%; (II) for the physical impairment and limitations domain, the agreement rate was 88%; (III) for the psychological and social well-being domain, it was 84% (IV) and for treatment effects on daily activities, it was 84%.

To analyze the clarity and representativeness of the items, the CVC was calculated initially for each item and, at the end, for the whole instrument. The general CVC of the OPMD QoL questionnaire was 0.84 for clarity and 0.92 for representativeness. Table 5 shows the CVC results for clarity and representativeness of the 20 items.

In the pre-test phase, the analysis of participant sociodemographic data showed: mean age 61.5 years; 83.3% (n=10) self-reported as brown or black and 16.7% (n=2) as white; 50% (n=6) of the respondents had only incomplete primary school, 25% (n=3) secondary school, 16.6% (n=2) complete primary school and 8.3% (n=1) without schooling. Regarding type of OPMD, the following indices were identified: 50% (n=6) oral leukoplakia; 24.9% (n=3) lichen planus (one erosive and two reticular); 16.6% actinic cheilitis (n=2); 8.3% (n=1) erythroleukoplakia, no patient presented oral submucosal fibrosis. Regarding habits, 75% (n=9) were nonsmokers and 25% (n=3) smokers; 83.4% (n=9) non-alcoholic and 16.6% (n=3) alcoholics.

The average response time of the instrument was

Table 1. Original version, synthesis of translations and back-translations, version evaluated by the judges and final version of the OPMD QoL questionnaire (domain 1 - difficulties in diagnosis).

Original Version (items 1 to 3)	Synthesis of translations (T1 and T2)	Synthesis of back-translations (B1 and B2)	Version evaluated by the judges	Final version of the QOL-OPMD
How difficult was it for you to get your mouth condition diagnosed?	*Que dificuldade você teve para ter o diagnóstico da sua condição bucal?*	How difficult was it for you to have your oral condition diagnosed?	*Que dificuldade você teve para ter o diagnóstico da sua condição bucal?*	*Foi difícil para você conseguir o diagnóstico da sua condição bucal?*
How much did the need to visit many doctors for getting your mouth condition diagnosed affect daily life activities?	*A necessidade de visitar muitos médicos para ter o diagnóstico de sua condição bucal afetou quanto as atividades da sua vida diária?*	To what extent did the need to visit many doctors in order to get a diagnosis for your oral condition affect your daily routine?	*A necessidade de visitar muitos médicos para ter o diagnóstico de sua condição bucal afetou quanto as atividades da sua vida diária?*	*A necessidade de visitar muitos dentistas para ter o diagnóstico de sua condição bucal afetou as atividades da sua vida diária?*
How stressful was it for you to take a variety of treatments before being diagnosed with your mouth condition?	*Quão estressante foi para você se submeter a uma variedade de tratamentos antes de ser diagnosticado com a sua condição bucal atual?*	How stressful was it to undergo a variety of treatments before being diagnosed with your current oral condition?	*Quão estressante foi para você se submeter a uma variedade de tratamentos antes de ser diagnosticado com a sua condição bucal atual?*	*Foi estressante para você se submeter a vários tratamentos antes de ser diagnosticado com a sua condição bucal atual?*

Table 2. Original version, synthesis of translations and back-translations, version evaluated by the judges and final version of the OPMD QoL questionnaire (domain 2 – physical impairment and limitations).

Original Version (items 4 to 10)	Synthesis of translations (T1 and T2)	Synthesis of back-translations (B1 and B2)	Version evaluated by the judges	Final version of the QOL-OPMD
How much pain and agony does your mouth condition cause you?	*Quanta dor e agonia a sua condição bucal causa em você?*	How much pain and agony does your mouth condition cause you?	*Quanta dor e agonia a sua condição bucal causa em você?*	*Sua condição bucal causa dor em você?*
How much burning sensation do you experience while having spicy food?	*Quanta sensação de queimor você experimenta quando come comida picante?*	How much burning sensation do you feel when eating spicy food?	*Quanta sensação de queimor você experimenta quando come comida picante?*	*Quanta sensação de queimação você sente quando come comida picante, quente ou ácida?*
How difficult is it for you to open your mouth widely?	*Quão difícil é para você abrir a sua boca de forma bem ampla?*	How difficult is it for you to open your mouth wide?	*Quão difícil é para você abrir a sua boca de forma bem ampla?*	É difícil para você abrir a sua boca de forma bem ampla?
How much is your oral condition causing you to limit your desired foods?	*Quanto a sua condição bucal limita você de comer os alimentos que deseja?*	To what extent does your oral condition stop you from eating what you want?	*Quanto a sua condição bucal limita você de comer os alimentos que deseja?*	*Sua condição bucal limita você de comer os alimentos que mais gosta?*
How much is your mouth condition limiting you from enjoying your meals?	*Quanto a sua condição bucal limita você de aproveitar as suas refeições?*	To what extent does your oral condition stop you from enjoying your meals?	*Quanto a sua condição bucal limita você de aproveitar as suas refeições?*	*Sua condição bucal limita você de aproveitar suas refeições?*
How much does your mouth condition affect your taste sensation?	*Quanto a sua condição bucal afeta seu paladar?*	To what extent does your oral condition affect your sense of taste?	*Quanto a sua condição bucal afeta seu paladar?*	*Sua condição bucal afeta o sabor dos alimentos?*
How much dryness do you feel in your mouth?	*Quão seca você sente a sua boca?*	How much do you feel that your mouth is dry?	*Quão seca você sente a sua boca?*	*Você sente a sua boca seca?*

10 minutes, and the mode of administration was the interview. The instrument was well accepted by the participants. It is important that no respondent considered participation to be tiring, possibly because the instrument is considered short, with only 20 items. All items were well understood and considered useful for QoL assessment by the respondents. No participant suggested modifications to the instrument.

All participants considered the answer options for the 20 items in the questionnaire using the 5-point Likert scale (in which the 0= not at all; 1= a little; 2= somewhat; 3= quite a bit; 4= very much) to be adequate and there was no suggestion of change.

The respondents were also asked to evaluate whether the 20 items in the OPMD QoL questionnaire were relevant/important for assessing quality of life and whether they were clear enough to understand. All respondents (100%) considered all items to be both important and clear (i.e., checked YES for all items for both questions).

In the operational equivalence stage, the participants answered the questions related to questionnaire format, their opinion of the items, the instructions provided during the interview, the place of pre-test, in relation to the way the instrument was applied and finally the participant's opinion on the alternatives of the questionnaire. The participants considered all these points to be adequate, thus generating 100% positive responses.

DISCUSSION

The cross-cultural adaptation (CCA) of the OPMD QoL questionnaire followed the stages of content validation recommended by the literature, demonstrating the importance of each item in the instrument in measuring quality of life of people with OPMD[8-12].

Thus, the first stage of the OPMD QoL questionnaire CCA process, in the evaluation of conceptual and item equivalence[8,9], was the extensive, in-depth literature review, which showed that the original instrument and its conceptual bases were also relevant and applicable in the Brazilian context.

Table 3. Original version, synthesis of translations and back-translations, version evaluated by the judges and final version of the OPMD QoL questionnaire (domain 3 – psychological and social well-being).

Original Version (items 11 to 17)	Synthesis of translations (T1 and T2)	Synthesis of back-translations (B1 and B2)	Version evaluated by the judges	Final version of the QOL-OPMD
How frustrated are you because of your oral condition?	*Quão frustrado você está por causa de sua condição bucal?*	How frustrated do you feel because of your oral condition?	*Quão frustrado você está por causa de sua condição bucal?*	*Sua condição bucal te deixa frustrado?*
How depressed or low do you feel because of your mouth condition?	*Quão deprimido ou para baixo você se sente por causa da sua condição bucal?*	How depressed or low do you feel because of your oral condition?	*Quão deprimido ou para baixo você se sente por causa da sua condição bucal?*	*Você se sente deprimido ou para baixo por causa de sua condição bucal?*
In general, how much is your mouth condition affecting your relationship with family and friends?	*Em geral, quanto a sua condição bucal está afetando seu relacionamento com a família e os amigos?*	In general, to what extent is your oral condition affecting your relationship with Family and friends?	*Em geral, quanto a sua condição bucal está afetando seu relacionamento com a família e os amigos?*	*Sua condição bucal está afetando seu relacionamento com a família e os amigos?*
How much is your mouth condition affecting your satisfaction with life?	*Quanto a sua condição bucal está afetando a sua satisfação com a vida?*	To what extent is your oral condition affecting your life satisfaction?	*Quanto a sua condição bucal está afetando a sua satisfação com a vida?*	*Sua condição bucal está afetando a sua satisfação com a vida?*
How scared are you about the possibility of your oral condition turning into cancer	*Quão assustado você está com a possibilidade de sua condição bucal se transformar em câncer?*	How frightened are you with the possibility of your oral condition turning into cancer?	*Quão assustado você está com a possibilidade de sua condição bucal se transformar em câncer?*	*Você está assustado com a possibilidade de sua condição bucal se transformar em câncer?*
How scared are you about the outcome of this condition affecting your life?	*O quanto assustado você está com o resultado dessa condição afetar a sua vida?*	How scared are you about the outcome of this condition affecting your life?	*O quanto assustado você está com o resultado dessa condição afetar a sua vida?*	*Você está com medo dessa condição afetar sua vida?*
How embarrassing is it for you to eat foods at parties, functions, or other social gatherings?	*O quanto é embaraçoso para você comer alimentos em festas, eventos ou outras reuniões sociais?*	How embarrassing is it for you to eat foods at parties, functions, or other social gatherings?	*O quanto é embaraçoso para você comer alimentos em festas, eventos ou outras reuniões sociais?*	*É desagradável para você comer alimentos em festas, eventos ou outras reuniões sociais?*

Table 4. Original version, synthesis of translations and back-translations, version evaluated by the judges and final version of the OPMD QoL questionnaire (domain 4 – effects of treatment on daily life).

Original Version	Synthesis of translations (T1 and T2)	Synthesis of back-translations (B1 and B2)	Version evaluated by the judges	Final version of the QOL-OPMD
How much pain do you experience with treatment of your oral condition?	*Quanta dor você sente com o tratamento de sua condição bucal?*	*Quanta dor você sente ao tratar sua condição bucal?*	*Quanta dor você sente com o tratamento de sua condição bucal?*	*Você sente dor devido ao tratamento de sua condição bucal?*
How satisfied are you with the effectiveness of treatment for your mouth condition?	*Quão satisfeito(a) você está com a eficácia do tratamento para a sua condição bucal?*	*How satisfied are you with the result of the treatment for your oral condition?*	*Quão satisfeito(a) você está com a eficácia do tratamento para a sua condição bucal?*	*Você está satisfeito com o resultado tratamento da sua condição bucal?*
How much are your treatment appointments affecting your daily schedule?	*Quanto as suas consultas de tratamento estão afetando a sua programação diária?*	*Até que ponto suas consultas estão afetando sua rotina diária?*	*Quanto as suas consultas de tratamento estão afetando a sua programação diária?*	*Suas consultas de tratamento estão afetando a sua programação diária?*

Table 5. Values of initial and final CVC for clarity and representativeness and total CVC for clarity and representatives of the OPMD QoL questionnaire.

Item	CVCfc	CVCfr	Item	CVCfc	CVCfr
1	0.74	0.95	11	0.83	0.89
2	0.75	0.91	12	0.83	0.91
3	0.79	0.93	13	0.89	0.93
4	0.77	0.89	14	0.87	0.91
5	0.74	0.88	15	0.89	0.95
6	0.87	0.91	16	0.77	0.85
7	0.85	0.95	17	0.85	0.91
8	0.81	0.83	18	0.91	0.95
9	0.85	0.91	19	0.87	0.95
10	0.95	0.99	20	0.81	0.91
CVCtc = 0.84 / CVCtr = 0.92					

CVCfc = Final Content Validity Calculation of clarity / CVCfr = Final Content Validity Calculation of representativeness / CVCtc = total Content Validity Calculation of clarity of the OPMD QoL questionnaire / CVCtr = total Content Validity Calculation of representativeness of the OPMD QoL questionnaire.

Moreover, the individual evaluation of each item and its inclusion in the questionnaire showed that the questionnaire contemplated all aspects that could influence the quality of life of individuals with OPMD, so the 20 items in the original instrument were maintained, with only language adjustments to ensure the target audience would understand, without conceptual modifications or addition of new items.

The analysis of the instrument performed by the group of expert judges and target population was essential to the adequacy of the OPMD QoL questionnaire items for use in the Brazilian context. The group of judges included specialists in the areas of Stomatology, Oral Pathology, Dental Clinic, Nursing, Medicine and Linguistics, thereby ensuring careful, pluralistic evaluation, offering information that would broaden the universe of suggestions and critiques for semantic improvement of the questionnaire.

Corroborating the need for diversity of qualification among the members of the committee of judges, Beaton *et al*.[12] and Rubio *et al*.[13] highlight that a group of judges should be multidisciplinary, including specialists such as health professionals, language professionals and specialists in methodology, i.e., it should consist of specialists in the area of knowledge of the instrument.

It is important to highlight that the instructions sent to the expert judges for the assessment of the OPMD QoL questionnaire, in a specific instrument[13], were of paramount importance for adequate evaluation of the scope of the domains, clarity and relevance of the items. The judges considered that all four domains in the OPMD QoL questionnaire were comprehensive when considering the concepts and aspects of QoL of individuals with OPMD in the Brazilian context, thus achieving conceptual and item equivalence. For all domains, the agreement rates were higher than or equal to 84%.

For the first domain, *difficulties in diagnosis*, comprising items 1 to 3, the agreement rate was 92%, and it was therefore considered adequate. It is important to highlight that OPMD is a group of chronic disorders that have an increased potential for malignant transformation, so early diagnosis is fundamental for maintaining patients' health[1,14]. However, a study conducted by Tadakamalda *et al*.[2,6], revealed that the pre-diagnosis phase was very traumatic for most of the interviewed patients, as they had to perform many visits and undergo different types of treatment, usually without relief.

Corroborating the issue of difficult diagnosis of OPMD, a study conducted by Silva *et al*.[15] and Fanaras[16] found that some professionals had little knowledge on what to do about those disorders. However, younger participants, students and more recent graduates demonstrated better knowledge of those diseases.

The second domain, *physical impairment and functional limitations*, comprises items 4 to 10 of the OPMD QoL questionnaire, which together propose to evaluate subjective issues such as pain, burning sensation, difficulty in opening the mouth, limitations in eating and dryness sensation. For this domain, the agreement rate among the judges was 88%, so it was considered adequate.

Tadakamadla *et al*.[6], in a study on 150 people diagnosed with OL, OLP and OSF, using the OPMD QoL questionnaire, found as the most recurrent complaints the sensation of burning, difficulty in mouth opening and roughness in the cheek mucosa and pain. The same study showed that patients with OLP have the worst QoL scores, especially for the domain of physical impairment and functional limitations, when compared to patients with OL and OSF.

Corroborating this perspective, Warnakulasurya[17] found a decline in the QoL of patients with OLP,

and the physical dimension and social disability presented higher values, thus, values such as these demonstrate that patients with OPMD who present pain require specific individualized treatment in an attempt to revert the picture to the asymptomatic profile.

The third domain, *psychological and social wellbeing*, comprises items 11 to 17, which aim to evaluate issues related to frustration, depression, satisfaction, relationship with family and friends and concern about the malignant potential of disorders. For this domain, the agreement rate among the judges was 84%.

It is important to report that a healthy oral cavity enables the individual to perform routine activities without physical and psychological limitations; however, any disorder of the oral cavity can reduce self-confidence and relational capacity, thus compromising the quality of life of affected individuals[18].

Corroborating the above, Gondivkar *et al*.[1], observed that, in a group of 305 people with OPMD, more than half presented affected social interaction and 82% showed psychological impact associated mainly with the risk of malignancy of the lesions. The same study also found that the greater the progression of the disease, the greater the psychological and social impacts on affected patients. Tadakamadla *et al*.[2], observed that fear associated with the possibility of malignant transformation was predominant among the interviewed patients, because the term cancer is associated with fear and stigma, since it is considered a fatal disease for most people.

The fourth and final domain, *effect of treatment on daily life*, comprises the last three items, 18 to 20, which seek to assess the individual's satisfaction with the treatment of his/her oral condition, as well as to measure the impact on daily life. The agreement rate among judges was 84%.

OPMD usually requires long-term treatment involving multiple treatment sessions and follow-up visits, with which patients often find it difficult to comply. Socioeconomic status greatly influences the maintenance of periodic visits. Furthermore, adverse effects such as nausea, swollen mouth, bad taste and smell, difficulty in spray application, dry mouth, sore throat, redness and occurrence of pseudomembranous candidiasis were reported by patients treated for some OPMD[2,6,19].

As for OPMD QoL questionnaire items, some adjustments were made to the wording, including replacing certain words or expressions with others that were better understood or more suitable for the target culture. Changes in the wording of items are expected in this process of cultural adaptation of instruments in order to maintain semantic equivalence[8-10,20].

For the content validity calculation (CVC) related to clarity, only five items (1 (0.74), 2 (0.75), 4 (0.77), 5 (0.74), 16 (0.77)) scored slightly below 0.78, thus requiring change, adopting the suggestions by the judges and the author Hernandez-Nieto[11]. This decision was based on the assumption described by Alexandre *et al*.[10], in which the authors recommend changes in the wording of the items with the aim of improving understanding by the target population.

In the representativeness analysis, all items had a final CVC value greater than 0.78. Nevertheless, it is important to highlight that item 8- *How much is your mouth condition limiting you from enjoying your meals?* – presented the lowest CVC value (0.83). It is worth noting that, although some judges suggested removing this item, the research committee decided to keep it, taking into account that through this question, the intention of the original instrument was to capture other subjective aspects related to the habit of eating and not just chewing. The question raised by some judges was the fact that this item may be similar to the previous item *(7- How much is your oral condition causing you to limit your desired foods? -)*.

In the evaluation phase by the target population during the pre-test, the participants who answered the questionnaire considered that it was fully understood and easily answered. Moreover, all participants considered that all 20 items were important for assessing quality of life. Semantic analysis showed that 100% of the pre-test participants considered that all items of the specific instrument for this stage were adequate, without suggesting any changes. This may be explained by the low level of education of the participants, of whom approximately 75% had only the elementary level of education.

The results of the operational equivalence phase showed that the participants responded affirmatively that the form, the instructions provided during the interview, the place where the pre-test was held, the way the instrument was applied and the response alternatives were adequate, thus generating 100% acceptance.

Despite its contributions, this study has some limitations, such as the performance of the committee of judges through electronic correspondence, which limited their discussions with the research team, and the low level of education of the pre-test participants, which may have reduced the possibilities of suggestions and improvements in the operational equivalence stage.

The results presented in this study demonstrate good acceptability of the OPMD QoL questionnaire to the respondents and, above all, may reflect effective cultural adequacy of the instrument for the target population. Thus, the final version of the instrument maintained the format and sequence of the items presented in the original version and was able to provide empirical validity.

Thus, the present study adapted the Quality-of-life questionnaire for patients with oral potentially malignant disorders to the Brazilian context by following the steps recommended by the literature. The questionnaire meets the equivalences proposed in the process of cross-cultural adaptation regarding domains, clarity and understanding of the items, satisfactory instructions and representativeness.

The indicators of content validation, pre-test and operational equivalence indicate that the Brazilian version of the OPMD QoL questionnaire is a promising instrument and a tool that seems valid to evaluate the quality of life of people with oral potentially malignant disorders. As a next step, it is important to measure equivalences to evaluate the psychometric properties of this instrument.

DECLARATION OF CONFLICTING INTERESTS
The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Impact of oral conditions on the quality of life of adolescents in a rural area of Brazil

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ABSTRACT

The aim of this study was to assess the impact of oral conditions on the oral health-related quality of life (OHRQoL) of early adolescents aged 11 to 14 years in the rural population of a Brazilian municipality. Adolescents attending public schools in the municipality participated in the study. OHRQoL was measured using the short Brazilian version of the Child Perceptions Questionnaire (CPQ11-14) with four domains: oral symptoms, functional limitations, emotional wellbeing and social wellbeing. A higher score indicates a more negative perception of the adolescent regarding his/her OHRQoL. Oral conditions such as dental caries, malocclusion and dental trauma were evaluated using DMTF index, Dental Aesthetics Index (DAI) and Andreasen criteria, respectively. The variables sex and age, number of siblings, parents' schooling, family monthly income, number of times of tooth-brushing/day and visits to the dentist/year were also evaluated. Descriptive analysis and regression models were performed. Of the 202 participants, 94 (46.5%) were female and 108 (53.5%) were male. Adolescents from low-income families (p=0.042) and with more severe malocclusion (p=0.037) scored higher in the CPQ11-14. Those with severe malocclusion scored higher in the emotional wellbeing domain (p=0.009). Females scored higher than males in the oral symptoms domain (p=0.002). Adolescents from low-income families scored higher in the social wellbeing domain (p=0.006). Malocclusion negatively affected the OHRQoL of adolescents from a Brazilian rural area, mainly regarding emotional wellbeing. Negative repercussions were also observed among females and adolescents whose families had lower income.

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Keywords: *quality of life - oral health - rural population - adolescent.*

Impacto de condições bucais na qualidade de vida de adolescentes em uma área rural do Brasil

RESUMO

O objetivo deste estudo foi avaliar o impacto de condições bucais na qualidade de vida relacionada à saúde bucal (QVRSB) de adolescentes de 11 a 14 anos da população rural de um município brasileiro. Adolescentes que estudavam em escolas públicas do município participaram do estudo. QVRSB foi avaliada usando a forma curta da versão brasileira do Child Perceptions Questionnaire (CPQ11-14) com quatro domínios: sintomas bucais, limitações funcionais, bem-estar emocional e bem-estar social. Um escore mais alto indica uma percepção mais negativa do adolescente com relação à sua QVRSB. Condições bucais, tais como cárie dentária, má oclusão e traumatismo dentário foram avaliados com o índice CPOD, Índice Estético Dental (IED) e os critérios de Andreasen, respectivamente. As variáveis sexo e idade dos adolescentes, número de irmãos, escolaridade dos pais, renda familiar mensal, número de vezes de escovação dos dentes/dia e visitas ao dentista/ano também foram avaliadas. Análise descritiva e modelos de regressão foram realizados. Dos 202 participantes, 94 (46,5%) eram meninas e 108 (53,5%) eram meninos. Adolescentes cujas famílias tinham uma renda mais baixa (p=0,042) e com má oclusão mais severa (p=0,037) obtiveram escores mais altos no CPQ11-14. Aqueles com má oclusão severa obtiveram um escore mais alto no domínio de bem-estar emocional (p=0,009). Com relação ao sexo do indivíduo, meninas tiveram um escore mais alto no domínio sintomas bucais (p=0,002). Adolescentes cujas famílias tinham uma renda mais baixa obtiveram um escore mais alto no domínio de bem-estar social (p=0,006). A má oclusão afeta negativamente a QVRSB de adolescentes de uma área rural brasileira, principalmente o bem-estar emocional. Repercussões negativas também foram observadas entre meninas e adolescentes cujas famílias tinham uma renda mais baixa.

Palavras-chave: *qualidade de vida - saúde bucal - população rural - adolescente.*

INTRODUCTION

Oral health-related quality of life (OHRQoL) was defined by Locker and Allen in 2007 as the effect of oral conditions on aspects of everyday life that are important to people. Those effects are of sufficient magnitude, whether in terms of frequency, severity or duration, to affect the individual's perception of his/her life in general[1]. It is a complex, dynamic construct that includes multidimensional indicators of health and wellbeing[2]. OHRQoL not only measures the influence of oral conditions on a person's physical, emotional and social wellbeing, but also evaluates the repercussions of dental treatment on his/her life[3,4].

Oral conditions can affect the quality of life of children and adolescents and may have impact on their daily activities, including eating, sleep, speech, communication, and social interaction, as well as on their self-esteem[5]. The presence of dental caries[6], malocclusion[7], and dental trauma[8] has been associated with a negative impact on child and adolescent quality of life. The impact of these and other oral disorders on child and adolescent quality of life has been measured by epidemiological research, which, in addition to clinical measurements, uses individuals' self-perception of their health and of how oral conditions impact their daily lives[9].

The literature contains few epidemiological studies on rural populations[10,11], in particular regarding the evaluation of oral health[12]. It is important to assess the rural population because it has different social characteristics from the urban population. Thus, the aim of this study was to assess the impact of oral conditions on the quality of life of early adolescents, aged 11 to 14 years, in the rural area of a Brazilian municipality.

MATERIALS AND METHODS

This study was approved by the Human Research Ethics Committee of the Federal University of Minas Gerais, registered under protocol number 07939819.1.0000.5149. The adolescents who agreed to participate in the study signed a voluntary informed assent form and their parents/guardians signed a voluntary informed consent form authorizing their children to take part in the study.

Participants, eligibility criteria, study setting and period of data collection

This cross-sectional study was conducted with early adolescents aged 11 to 14 years, of both sexes, living in the rural area of Paula Cândido, Minas Gerais, Brazil. Data were collected between April and November 2019. Adolescents who had undergone or were undergoing orthodontic treatment and those with syndromes or cognitive impairment were excluded from this study.

Sample size calculation

According to the Municipal Health Department, Paula Cândido has 255 adolescents aged 11 to 14 years who live in rural areas. Thus, the sample for this study was defined by calculating the sample for finite populations[13]:

$$n = \frac{N \times \hat{p} \times \hat{q} \times z^2_{\alpha/2}}{\hat{p} \times \hat{q} \times z^2_{\alpha/2} + (N-1) \times e^2}$$

where N is the number of individuals in the finite population, p is the sample proportion (proportion of the impact of oral conditions on quality of life), q is the complement of the proportion (1-p), z is the confidence level (95% confidence interval), and e is the margin of error.

Considering the number of early adolescents aged 11 to 14 years living in rural areas as 255, a 50% prevalence of the impact of oral conditions on their quality of life, a confidence level of 1.96 (95% confidence interval), and a margin of error of 5%, the minimum sample comprised 154 adolescents. As data would be collected in multiple settings (schools in Paula Cândido), an effect factor of 1.2 was employed to mitigate imprecision. Thus, the minimum sample size was 185 participants. To compensate for possible losses, 20% was added. Thus, the final sample was 222 adolescents from rural areas.

Data collection

OHRQoL was assessed using the short Brazilian version[14] of the Child Perceptions Questionnaire (CPQ11-14), which consists of a set of questions that measure how oral conditions impact quality of life[15].

The short version of the CPQ11-14 consists of 16 questions distributed across four domains: oral symptoms, functional limitations, emotional wellbeing, and social wellbeing. Each question addresses the frequency of events in the past three months and a five-point response scale is used with

the following options: never = 0; once/twice = 1; sometimes = 2; often = 3; and every day/almost every day = 4. A higher score indicates more negative perception regarding the impact of oral conditions on quality of life[14,15].

Oral health status was clinically evaluated by one trained examiner at public schools in Paula Cândido. Calibration for dental caries, dental trauma and malocclusion was performed before data collection. A lecturer in Pediatric Dentistry coordinated the calibration of the examiner. For the three oral conditions, inter- and intra-examiner agreement were above 0.70. The examiner wore personal protective equipment (disposable gloves, mask, white coat, cap, and protective glasses) and used equipment for clinical examination such as mouth mirrors, wooden spatulas, and clinical probes. A note-taker recorded appropriate notes on a clinical record sheet.

Dental caries was evaluated according to the decayed, missing and filled teeth index (DMFT), following World Health Organization (WHO) criteria[16]. Participants were examined and the number of teeth with dental caries, missing teeth, and filled teeth were recorded. Malocclusion was assessed according to the Dental Aesthetic Index (DAI). This index assesses 10 occlusal characteristics according to three components: dentition (number of missing incisors, canines, and premolars), crowding and/or spacing (crowding and/or spacing in the upper arch, crowding and/or spacing in the lower arch, greater upper anterior irregularity, greater lower anterior irregularity and diastema between the upper central incisors) and occlusion (overjet, anterior crossbite, open bite and anteroposterior molar relation). The value found for each occlusal characteristic was multiplied by its specific weight and added up. To this sum, the value 13 was added to obtain the final DAI score. According to their DAI score, the adolescents could be assigned to one of four groups of malocclusion severity: normal or mild malocclusion (DAI $\leq$ 25), definitive malocclusion (26 $\leq$ DAI $\leq$ 30), severe malocclusion (31 $\leq$ DAI $\leq$ 35) or very severe malocclusion (DAI $\geq$ 36)[17]. The analysis of dental trauma was based on the Andreasen classification,[18] through which only the upper and lower incisors were evaluated. The teeth were classified as: without evidence of dental trauma, teeth with mild trauma (fracture involving only enamel), teeth with severe trauma (fracture involving dentin and fracture involving dentin and pulp) and teeth with trauma that had been filled[19].

Directed acyclic graph

A directed acyclic graph (DAG)[20] was used to identify potential confounding factors for the association between oral conditions and the OHRQoL. The confounding variables sex[21] and age[22], number of siblings[23], parents' schooling[22], family income[23], number of tooth-brushing times/day[24] and visits to the dentist/year[25] were incorporated into the model (Fig. 1).

Statistical analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS, version 23.0, SPSS Inc., Armonk, USA). A descriptive analysis was performed. For the assessment of the association of oral conditions with the total score and the scores of the CPQ11-14 domains, regression models were used to control the influence of confounding variables. For the regression models, sex and dental trauma were analyzed as categorical variables. Age, number of siblings, parents' schooling, family income, number of tooth-brushing times, visits to the dentist, DMFT and malocclusion were analyzed as quantitative variables. The level of statistical significance was $p<0.05$.

RESULTS

Of the 222 adolescents from rural areas, 202 were evaluated. Of these, 94 (46.5%) were male and 108 (53.5%) female. Mean age was 12.64 years (± 1.09). The regression models showed that

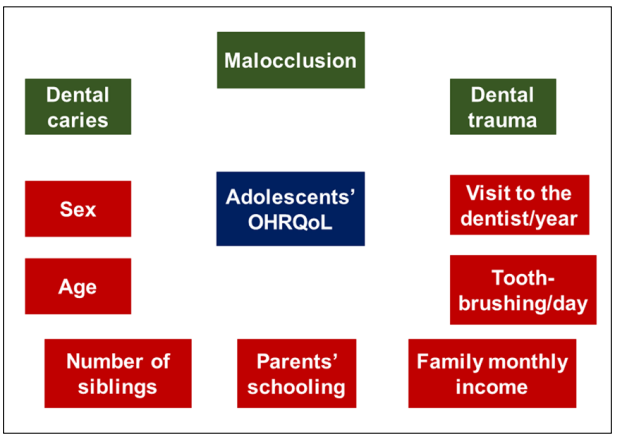

Fig. 1: Directed acyclic graph

Table 1: Regression model evaluating the association of the total CPQ11-14 score with oral conditions and confounding variables

	Total CPQ11-14 score	
	Coefficient (SE)	***p*-value**
Sex (female*/male)	-1.91 (1.17)	0.105
Age (years)	0.39 (0.54)	0.473
Number of siblings	0.46 (1.19)	0.698
Parents' schooling (in years)	-0.88 (1.24)	0.480
Family income (BMW)	-1.83 (0.89)	**0.042**
Number of tooth-brushing times (daily)	-0.97 (1.26)	0.438
Visits to the dentist (during the year)	-1.74 (1.18)	0.142
DMFT	-0.04 (0.19)	0.801
DT (without trauma*/enamel/dentin-pulp)	0.51 (1.09)	0.638
Malocclusion (DAI)	0.15 (0.07)	**0.037**

BMW=Brazilian minimum wage, DMFT=decayed, missing and filled teeth index, DT=dental trauma, DAI= dental aesthetic index, SE=standard error, *p*-value in bold indicates statistical significance. Sex and DT were analyzed as categorical variables. Age, number of siblings, parents' schooling, family income, number of tooth-brushing times, visits to the dentist, DMFT and malocclusion were analyzed as quantitative variables.
*Reference variable

adolescents whose families had a lower monthly income (p=0.042) and adolescents with more severe malocclusion (p=0.037) had a higher total CPQ11-14 score (Table 1). Adolescents with more severe malocclusion also scored higher in the emotional wellbeing domain (p=0.009). Finally, females scored higher than males in the oral symptoms domain (p=0.002), and adolescents from lower income families scored higher in the social wellbeing domain (p=0.006) (Table 2).

DISCUSSION

The aim of this study was to assess the impact of oral status on the OHRQoL of adolescents in rural areas. The results showed that subjects whose families had a lower monthly income and subjects with more severe malocclusion had a more negative perception of their OHRQoL. The main negative effect of malocclusion was on the emotional wellbeing domain. There is growing awareness that oral disorders can significantly impact young people's physical, social, and psychological wellbeing. Malocclusion may have undesirable emotional and social effects[26]. This negative impact is likely due to the aesthetic impairment that dental and/or skeletal discrepancies can cause on the individual's face. An altered smile or an unfavorable facial appearance can cause psychological discomfort in young people affected by malocclusion, inhibiting them from maintaining social interactions with their peers. The fear of maintaining interpersonal relationships may occur due to the adolescent's growing apprehension of being a target of pejorative comments related to his/her dentofacial characteristics, which can produce feelings of inferiority and low self-esteem[7,27].

According to the results of this study, the main negative impact of the family income variable is on the social well-being domain. Socioeconomic factors during adolescence can lead to inequalities in general and in the oral health of individuals from less privileged social strata[23]. The impact of socioeconomic factors such as family income on an adolescent's social wellbeing and OHRQoL can be explained by material or psychosocial mechanisms[28,29]. Regarding the material issues, low family income can be one of the main barriers to access dental care services, where the individual can be advised regarding preventive measures and treated[23]. Psychosocial issues, on the other hand, address each individual's experience related to social inequality, under psychological stress and lack of social protection, which can contribute to worsening his/her oral health and ultimately impair his/her OHRQoL[30,31].

The results of the present study also demonstrated that female adolescents from rural areas had a more negative perception of the oral symptoms domain compared to male adolescents. Previous studies have highlighted the individual's sex as a variable of significant influence on adolescents' quality of life[32,33]. Differences related to sex can influence the way the individual responds to the presence of health issues/oral conditions or to a medical/dental treatment, which reflect on the quality of life and its domains[34]. In addition, several articles in the literature show that women are more concerned with health and oral health and seek more dental treatment than men, as the presence of any oral disorder has more negative repercussions on their quality of life[21,35,36].

This study presents some limitations due to its cross-sectional design, as it was not possible to directly determine cause-and-effect relationship between the

Table 2: Regression model evaluating the association of CPQ11-14 domain scores with oral conditions and confounding variables

Oral symptoms	Coefficient (SE)	*p*-value
Sex (female*/male)	-1.08 (0.35)	0.002
Age (years)	-0.02 (0.16)	0.871
Number of siblings	0.46 (0.35)	0.195
Parents' schooling (in years)	0.52 (0.37)	0.162
Family income (BMW)	-0.07 (0.26)	0.787
Number of tooth-brushing times (daily)	-0.18 (0.37)	0.628
Visits to the dentist (during the year)	-0.35 (0.35)	0.311
DMFT	0.06 (0.05)	0.238
DT (without trauma*/enamel/dentin-pulp)	-0.04 (0.32)	0.881
Malocclusion (DAI)	0.02 (0.02)	0.337
Functional limitations	**Coefficient (SE)**	***p*-value**
Sex (female*/male)	-0.36 (0.35)	0.308
Age (years)	0.17 (0.16)	0.289
Number of siblings	0.34 (0.36)	0.341
Parents' schooling (in years)	0.07 (0.37)	0.850
Family income (BMW)	0.45 (0.27)	0.100
Number of tooth-brushing times (daily)	-0.02 (0.38)	0.949
Visits to the dentist (during the year)	-0.67 (0.36)	0.064
DMFT	-0.01 (0.05)	0.785
DT (without trauma*/enamel/dentin-pulp)	-0.05 (0.33)	0.864
Malocclusion (DAI)	0.02 (0.02)	0.360
Emotional well-being	**Coefficient (SE)**	***p*-value**
Sex (female*/male)	-0.33 (0.46)	0.472
Age (years)	0.16 (0.21)	0.439
Number of siblings	-0.34 (0.46)	0.463
Parents' schooling (in years)	-0.93 (0.48)	0.057
Family income (BMW)	-0.61 (0.35)	0.081
Number of tooth-brushing times (daily)	-0.65 (0.49)	0.188
Visits to the dentist (during the year)	-0.27 (0.46)	0.548
DMFT	-0.03 (0.07)	0.644
DT (without trauma*/enamel/dentin-pulp)	0.25 (0.43)	0.552
Malocclusion (DAI)	0.07 (0.02)	**0.009**
Social well-being	**Coefficient (SE)**	***p*-value**
Sex (female*/male)	-0.13 (0.33)	0.686
Age (years)	0.07 (0.15)	0.619
Number of siblings	-0.01 (0.33)	0.992
Parents' schooling (in years)	-0.54 (0.34)	0.121
Family income (BMW)	-0.69 (0.25)	**0.006**
Number of tooth-brushing times (daily)	-0.12 (0.35)	0.734
Visits to the dentist (during the year)	-0.43 (0.33)	0.190
DMFT	-0.06 (0.05)	0.225
DT (without trauma*/enamel/dentin-pulp)	0.36 (0.30)	0.233
Malocclusion (DAI)	0.03 (0.02)	0.079

BMW=Brazilian minimum wage, DMFT=decayed, missing and filled teeth index, DT=dental trauma, DAI= dental aesthetic index, SE=standard error, *p*-value in bold indicates statistical significance. Sex and DT were analyzed as categorical variables. Age, number of siblings, parents' schooling, family income, number of tooth-brushing times, visits to the dentist, DMFT and malocclusion were analyzed as quantitative variables
*Reference variable

independent variables and the OHRQoL outcome[37]. Another limitation is the fact that the sample is restricted to rural adolescents aged 11 to 14 years from a single Brazilian municipality. Thus, the external validity of these findings is limited and the results of this study should be used with caution when applied to rural populations with different characteristics from the population investigated herein.

In view of the results obtained, the present study may be useful for proposing and designing public health policies and services aimed specifically at populations living in rural areas. Rural populations, in general, use fewer health services[38]. The difficulties encountered by this population, such as lack of transportation, great distances to healthcare facilities, lack of means of communication, and low income[23] can make it difficult for adolescents to access oral health services[27]. Thus, public policies are essential to facilitate their access to dental care services. Such services should also focus on guiding and advising their users, in addition to providing prevention measures and early treatment of certain oral disorders, such as malocclusion, to improve oral health status and provide better quality of life for adolescents living in rural areas[39].

CONCLUSION

Malocclusion affected the quality of life of adolescents from the rural area of a Brazilian municipality, with a significant negative effect on their emotional well-being. A negative impact was also observed among individuals from lower income families and among females.

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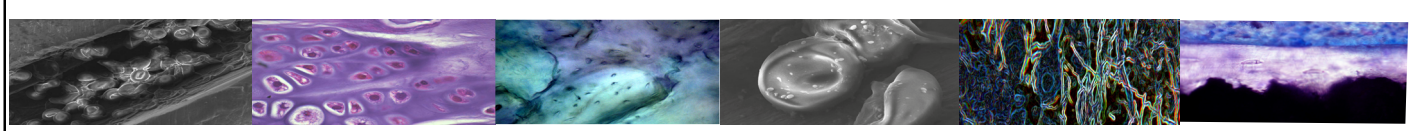

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