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CONTENTS / *ÍNDICE*

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A partir del Volumen 27 (2014) AOL se edita en formato digital con el *Sistema de Gestión de Revistas Electrónicas* (Open Journal System, OJS). La revista es de acceso abierto (Open Access). Esta nueva modalidad no implica un aumento en los costos de publicación para los autores.

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Follow-up of first permanent molar restorative treatment with and without Molar Hypomineralization

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ABSTRACT

The selection and long-term stability of restorative materials for teeth affected by Molar Hypomineralization (MH) are controversial. The aim of this study was to compare need for treatment and status of restorations performed on first permanent molars in patients with and without MH. Retrospective design based on the clinical records of 153 patients who had received comprehensive care in 2014 at the Clinic of the Children's Comprehensive Dentistry Department (FOUBA) by 3 pediatric dentists (Kappa MH 0.94), and who attended periodical follow-up visits for at least 24 months. Need for treatment in first molars, and type and longevity of treatment were recorded (modified Ryge criteria /USPHS, Kappa 0.78). Results were compared between patients with MH and without MH. The Kruskal Wallis test was used to compare follow-up time, the asymptotic test was performed to compare proportions, and relative risk (RR) was calculated to compare need for treatment.

Mean follow-up times for the 595 teeth analyzed were 61.7±20.1 months for Group without MH, and 57.5±23.9 months for Group with MH (p=0.0504). The percentages of teeth requiring at least one treatment were 7.2% in Group without MH and 27.5% in Group with MH (RR = 3.80, p < 0.001). Of the teeth treated in Group with MH 23.1% required retreatment, while none of the teeth in Group without MH did. The need for treatment was approximately 4 times higher in molars affected with MH, with greater probability of retreatment.

Keywords: *dental enamel - molar incisor hypomineralization - therapeutics.*

Seguimiento del tratamiento restaurador de primeros molares permanentes con y sin Hipomineralización Molar

RESUMEN

La selección y estabilidad a largo plazo de los materiales de restauración en piezas afectadas por Hipomineralización Molar (HM) es en la actualidad un tema de controversia. El objetivo de este estudio fue comparar las necesidades de tratamiento y el estado de las restauraciones realizadas en primeros molares permanentes en pacientes con y sin HM. Diseño retrospectivo sobre las historias clínicas de 153 pacientes que habían recibido atención integral en 2014, en la Clínica de la Cátedra de Odontología Integral Niños (Facultad de Odontología de la Universidad de Buenos Aires) por 3 odontopediatras (Kappa HM 0,94) y que asistieron a las recitaciones periódicas durante un mínimo de 24 meses. Se registraron las necesidades de tratamiento, tipo y longevidad de los mismos en primeros molares (criterios Ryge modificados /USPHS, Kappa 0,78). Se compararon los resultados entre los pacientes con y sin HM. Se utilizó test de Kruskal Wallis para comparar tiempos de seguimiento, test asintótico de comparación de proporciones y se calculó riesgo relativo (RR) para comparar la necesidad de tratamiento. Los tiempos medios de seguimiento de las 595 piezas analizadas resultaron de 61.7±20.1 y 57.5±23.9 meses en los grupos sin HM y con HM respectivamente (p=0.0504). Los porcentajes de piezas que requirieron al menos un tratamiento fueron 7.2% en el grupo sin HM y 27.5% en el grupo con HM. (RR = 3.80, p < 0.001) De las piezas con HM tratadas, el 23.1% requirieron retratamiento, mientras que en el grupo sin HM ninguna pieza lo necesitó. La necesidad de tratamiento fue aproximadamente 4 veces mayor en los molares afectados con HM con más probabilidad de retratamiento.

Palabras clave: *esmalte dental - hipomineralización molar incisiva - terapéutica.*

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INTRODUCTION

Hereditary, environmental and local factors can cause structural defects in the enamel of primary and/or permanent teeth. According to the amelogenesis period affected, these alterations may be quantitative if they act during the protein matrix secretion phase, or qualitative if they occur during the maturation or mineralization processes.

Until two decades ago, the three most frequent enamel developmental defects reported in the literature were amelogenesis imperfecta, endemic fluorosis and hypoplasia.

In recent years, research – particularly research in pediatric dentistry – has been focusing on Molar Hypomineralization (MH), which affects almost 1 out of every 5 children. MH presents as an anomaly in the translucence of the adamantine structure, with clinically observed white/yellow/brown demarcated areas without alteration of enamel thickness, which can sometimes disintegrate, giving rise to rapid progression of caries lesions. Worldwide prevalence, based on 141 studies in 47 countries, is currently 19%, with first permanent molars being the most frequently affected teeth. This rate makes MH the most frequent enamel anomaly, with 17 million new cases per year. MH can impact nutrition and hygiene due to molar hypersensitivity, even when the enamel is intact, as well as causing esthetic problems if anterior teeth are involved, affecting patients' quality of life. The etiology of MH remains uncertain[1].

Results of previously published studies by teachers from the Children's Comprehensive Dentistry Department, School of Dentistry, University of Buenos Aires, reported prevalence of 15.9 % for the Autonomous City of Buenos Aires in 2010[2].

Leppaniemi et al., Muratbegovic et al. and Da Costa-Silva et al. reported an association between presence of opacities and caries lesions[3-5].

Using spectrometry, Jälevik et al. found greater presence of carbon, especially from proteins, Mangum et al. and Farah et al. using biochemical approaches for isolating and characterizing proteins, found they may be up to 21 times higher, with a 20% reduction in mineral content[6-8].

Bozal et al. analyzed the ultrastructure and mineral composition of the enamel surface of a molar with clinical diagnosis of MH, with and without acid etching treatment, using SEM and ionic analysis (EDS). They concluded that the enamel, including clinically intact adamantine surface, presents severe ultrastructural alterations and changes in ionic composition, affecting the acid etching pattern, which could interfere with bonding mechanisms[9].

Because these characteristics make the behavior of opacities unpredictable, with probable collapse of the enamel and loss of restorations, the selection and long-term stability of the restorative materials in affected teeth is currently subject to controversy.

The aim of this study was to compare the need for treatment and the status of restorations performed on first permanent molars in patients with and without MH.

METHODS

This was a retrospective study based on the clinical records of 153 patients born from 2003 to 2006, who had received comprehensive care during 2014 at the clinic of the Children's Comprehensive Dentistry Department of the School of Dentistry of Buenos Aires University. Care was provided by 3 pediatric dentists (Kappa MH 0.94), and the children attended periodical follow-up visits for at least 24 months. The project was approved by the Ethics Committee of the School of Dentistry of Buenos Aires University (006/2019 CETICA-FOUBA).

Need for treatment, and type and longevity of treatments in first permanent molars were recorded (modified Ryge criteria /USPHS, Kappa 0.78). Results were compared between patients with MH and without MH groups.

An asymptotic test for comparing proportions was applied, and the relative risk (RR) calculated with 95% confidence interval (CI 95%) to compare need for treatment. Since teeth had different follow-up times, they were compared previously with a Kruskal Wallis test to determine the validity of the comparison of need for treatment between groups.

RESULTS

The sample consisted of 595 molars (236 with MH and 359 without MH). Mean follow-up times were 61.7±20.1 months for group without MH, and 57.5±23.9 months for group with MH ($p = 0.0504$), (Fig. 1).

The percentages of teeth requiring at least one treatment were 7.2% in Group without MH and 27.5% in Group with MH (RR = 3.80, CI 95%: 2.50-5.81. $p < 0.001$), (Fig. 2).

In Group with MH, 23.1% of the treated molars required retreatment. No retreatment was recorded in Group without MH.

In Group with MH, the treatments selected were glass ionomers (GI) and steel crowns (Cw), while in group without MH, they were mainly composite resins (CR). Fig. 3 shows the distribution of treatments.

The highest proportion of failures in Group with MH occurred in restorations with glass ionomers, although differences were not significant ($p= 0.53$).

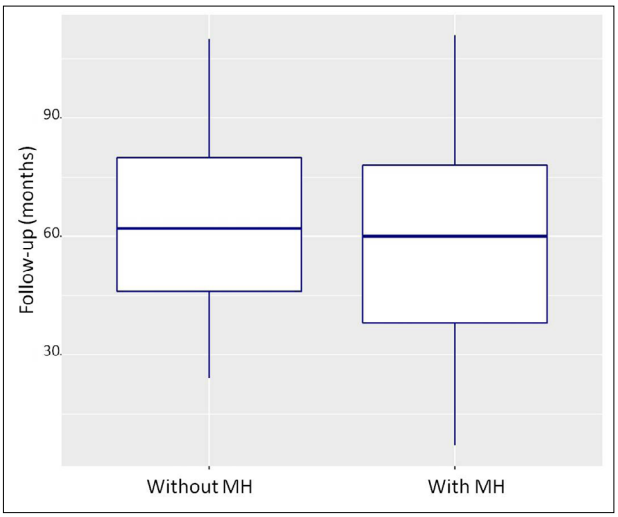

Fig. 1. Comparison of follow-up times for without MH and with MH groups.

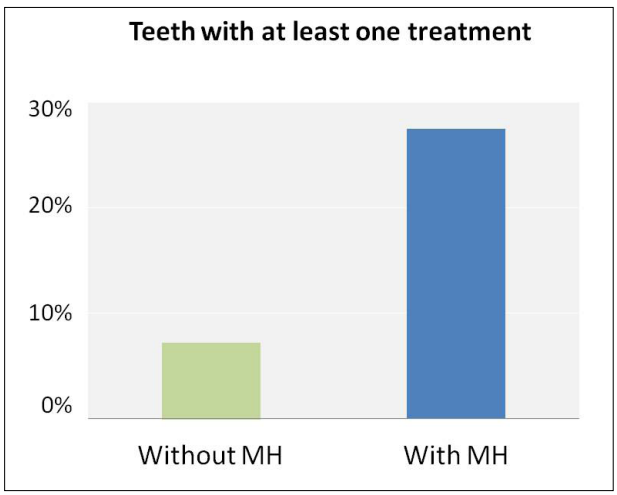

Fig. 2. Comparison of need for treatment

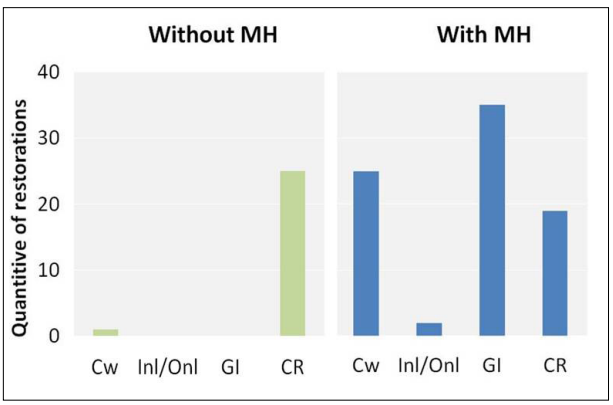

Fig. 3. Quantity of restorations and treatment type in each group

DISCUSSION

The ultrastructural alterations of the hypomineralized enamel interfere with and alter the bonding mechanisms of restorative materials[9]. Because of the small amount of available information regarding the longevity of restorative practices, the selection and stability of materials is currently a subject of research. Lygidakis et al.[10] agree that choosing a treatment is complex, and depends upon factors such as severity of the defect, dental eruption stage, patient age and cooperation, social context, and the expectations of the child and parents. Options include preventive practices for mild MH, restoration (GI, CR or steel crown) or tooth extraction for moderate/severe MH.

The literature reports a higher frequency of treatment in teeth with MH than in unaffected teeth[11]. In the current sample, over a follow-up period of several years, need for treatment was 4 times higher in the group with MH. This rate is lower than the one found by Jälevik and Klinberg[12], who conducted a cross-sectional study in 2002 on 9-year-old patients, concluding that children with MH received dental treatment 10 times more often than children without MH, and that affected teeth were treated twice, on average. A subsequent study by the same authors on 18-year-olds found a similar rate to the current study[13].

Because teeth in the current study had different follow-up times, these times had to be compared previously in order to determine the validity of the comparison of need for treatment between groups. Although the significance of the difference was borderline, in practice, it is not significant.

Choice of treatment differed between Groups with and without MH based on previous knowledge regarding the bonding difficulties and the sensitivity typical of MH. For Group with MH, the most frequent choices were modified GI and steel crowns, while for Group without MH, the most frequent choice was composite resins.

Ionomers are used for intermediate restoration because studies on conventional GIs report low longevity rates. Linner et al.[11] found that only 7% of GI restorations survived at 36 months, while Durmus et al.[14] found 87% longevity at 2 years for modified ionomers. In the current study, photoactivated resinous GIs were used, with a 74% success rate.

There is a point of controversy regarding the removal of tissue prior to restoration. Although decisions are variable, most dentists prefer conservative, minimally invasive preparations, as used in the current sample[15]. Linner et al.[11] conclude that restorations with non-invasive composite resins, used mainly in younger or less cooperative children, were associated to lower restoration survival rates. These authors found a 30% survival rate at 36 months for conservative resins, which was lower than the rate found in the current sample. Rolim et al.[16], for a similar sample and using

preparations with minimal intervention with composite resins, found a success rate of 81% using total etching with 37% phosphoric acid, and 62% with a self-conditioner. In the current study, all composite resins were used with total etching and achieved a similar success rate, with 89.5%.

Regarding the use of steel crowns, which was high in the current sample for the group with MH, Farías et al. report a 94% success rate at 24 months, which is much higher than the composite resin success rate, in agreement with the results of the current study reflecting a low failure rate for crowns[17].

Ceromer inlays/onlays (Inl/Onl) were used for teeth in which previous treatments failed, due to which they subsequently received endodontic treatment. Gaardmand et al. found a 98% success rate at 36 months in teeth with MH[18].

With regard to need for retreatment, the results of the current study are similar to those of Kotsanos et al., who found three times more retreatments in the group with MH than in the control group[19].

Na Ha et al. conclude that the size of the defect is the factor with greatest impact on the prognosis for the restoration[20].

It is currently hoped that biological materials and resin infiltration may provide a solution for this problem which has high impact on both the patient and the professional.

CONCLUSIONS

In group of patients under study, the need for treatment was approximately 4 times higher in molars affected by MH, with more probability of retreatment.

DECLARATION OF CONFLICTING INTERESTS:
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Immune challenges upregulate the expression of cannabinoid receptors in cultured human odontoblasts and gingival fibroblasts

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ABSTRACT

Odontoblasts and gingival fibroblasts play essential roles in the physiological and pathological processes of dental tissue. Cannabinoid receptors (CB1 and CB2) are involved in analgesia by modulating the función of calcium channels that inhibit the synthesis of some neurotransmitters. A better understanding of the physiology of these receptors would provide the possibility of using them as therapeutic targets in controlling dental pain. The aim of this study was to evaluate the presence and activity of cannabinoid receptors in human odontoblast-like cells (OLC) and human gingival fibroblasts (HGF). CB1 and CB2 transcription was analyzed by real-time PCR, proteins were detected by immunofluorescence, and functional cannabinoid receptors were evaluated by measuring intracellular calcium concentration after stimulation with cannabidiol (CBD) and pre-treatment with a CB1 antagonist, a CB2 inverse agonist and a TRPV1 antagonist. Transcripts for CB1 and CB2 were found in both odontoblasts and gingival fibroblasts. Cannabidiol induced an increase in [Ca2+]i in both cells types, but surprisingly, pre-treatment with selective cannabinoid antagonists attenuated this effect, suggesting a functional communication between specific cannabinoid receptors and other CBD target receptors. In conclusion, human odontoblasts and gingival fibroblasts express functional CB1 and CB2 cannabinoid receptors, which could be modulated to improve the treatment of pain or dental sensitivity.

Keywords: *cannabinoid receptor CB1- cannabinoid receptor CB2 - dental pain - odontoblasts - fibroblasts.*

Los desafíos inmunológicos regulan la expresión de los receptores cannabinoides en cultivos de odontoblastos y fibroblastos gingivales humanos

RESUMEN

Los odontoblastos y los fibroblastos gingivales desempeñan funciones esenciales en los procesos fisiológicos y patológicos de los tejidos dentales. Los receptores cannabinoides (CB1 y CB2) participan en la analgesia mediante la modulación de la función de canales de calcio que inhiben la síntesis de algunos neurotransmisores. Un mejor conocimiento de su fisiología abre la posibilidad de utilizar estos receptores como dianas terapéuticas en el control del dolor dental. Este trabajo tuvo como objetivo evaluar la presencia y la actividad de los receptores cannabinoides en células humanas similares a los odontoblastos (OLC) y en fibroblastos gingivales humanos (HGF). Se analizó la transcripción de CB1 y CB2 por PCR en tiempo real, la detección de las proteínas por inmunofluorescencia y se evaluaron los receptores cannabinoides funcionales midiendo las concentraciones de calcio intracelular, tras la estimulación con cannabidiol (CBD) y el pretratamiento con un antagonista de CB1, un agonista inverso de CB2 y un antagonista de TRPV1. Se encontraron mensajeros para CB1 y CB2 tanto en odontoblastos como en fibroblastos gingivales. El cannabidiol indujo un aumento de la [Ca2+]i en ambos tipos de células, pero sorprendentemente el pretratamiento con antagonistas cannabinoides selectivos atenuó este efecto, lo que sugiere una comunicación funcional entre receptores cannabinoides específicos y otros receptores diana del CBD. En conclusión, los odontoblastos humanos y los fibroblastos gingivales expresan receptores cannabinoides CB1 y CB2 funcionales, que podrían ser modulados para mejorar el tratamiento del dolor o la sensibilidad dental.

Palabras clave: *receptor cannabinoide CB1- receptor cannabinoide CB2 - dolor dental - odontoblastos - fibroblastos.*

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INTRODUCTION

Dental pain, dentinal sensitivity, and periodontitis are caused by structural damage to the enamel, dentin and periodontal tissues, usually triggered by biofilm on these surfaces. Dental pain associated with microbiological, physical, or chemical stimuli is the most frequent cause for dental consultation, and affects patients' quality of life. Currently, non-steroidal anti-inflammatory drugs (NSAIDs) and opioids are frequently used for treating pain[1], but due to the complex pathophysiology of pulpal pain[2], this traditional approach may not be effective, so it is necessary to find an alternative that could be applied locally.

Odontoblasts and gingival fibroblasts are found in the pulp and gingiva, respectively, and are essential to oral health because they respond to external noxious stimuli and participate in mechanotransduction and tissue repair activities by expressing a variety of ion channels and receptors[3,4].

Cannabinoid receptors are ligand-activated G-protein coupled receptors found mainly in the plasma membrane[5,6]. The binding of the ligand generates allosteric changes in the receptor, enabling the activation or inhibition of multiple signaling pathways. In this regard, the cellular response depends on the type of agonist and the biological context[7,8].

The role of CB1 and CB2 in the regulation of pain pathways through the modulation of neurotransmitter release in nerve endings has been widely reported[9]. There is also evidence of the anti-inflammatory effect caused by the activation of both receptors[10]. For these reasons, those receptors have been investigated as therapeutic targets for treating pain and inflammation.

In several pathological conditions, the expression of cannabinoid receptors is regulated to maintain homeostasis and physiological processes, so different cells and tissues express them to mediate antinociceptive and anti-inflammatory effects[11].

In dental tissues, both receptors could have a crucial role in dental sensory transduction, biomineralization, and tissue repair[12], which is why extensive research has been performed in recent years. The expression of both receptors has been demonstrated in murine odontoblasts and gingival fibroblasts[13-15], and CB1 was found in the human odontoblast primary cultures[12]. Furthermore, the presence of CB2 in gingival fibroblasts seems to be involved in controlling the inflammatory environment, such as during periodontitis[16].

The aim of this study was to define the presence and activity of cannabinoid receptors in cultured odontoblast-like cells and primary human gingival fibroblasts, and evaluate the change in CB1 and CB2 expression under inflammatory conditions in odontoblasts. Results will provide important information that could be used to develop new therapeutic options using cannabinoid receptors as a pharmacological target for controlling pain and inflammation.

MATERIALS AND METHODS

Cell culture

Odontoblast-like cells (OLC) were differentiated from dental pulp mesenchymal stem cells using 10 ng/mL TGF-β (Abcam, Cambridge, MA, USA) for three weeks, following the previously established protocol[17]. In addition, human gingival fibroblasts (HGF) were harvested from gingival biopsies taken from periodontal plastic surgery. In brief, small tissue fragments were dissociated with a collagenase (100 U/mL) and dispase (1 mg/mL) mix for one hour, then washed with fresh medium and centrifuged. The cell pellet was seeded in 25 cm^2 culture flasks for seven days and maintained at 37 °C in a 5 % of CO_2 incubator. In both cases, the cells were cultured in DMEM (Hyclone, Thermo Scientific, Bremen, Germany) supplemented with 10% FBS (Gibco; Thermo Fisher Scientific) and antibiotic (100 U/ml penicillin + 100 µg/ml streptomycin) until 70 % confluence was reached, to be distributed on dark 384-well plates after 0.25% trypsin-versene treatment to harvest cells. In addition, 24-well plates seeded with OLC were treated for 24 hours with 2 µg/ml *E. coli* LPS (Sigma-Aldrich, St. Louis, MO) or 40 µg/ml Poly-I:C (InvivoGen, San Diego, CA) to simulate bacterial and viral infection inflammatory conditions, respectively. The project received the endorsement of the Ethics Committee of Facultad de Odontología, Universidad Nacional de Colombia (B.CIEFO-008-2021).

CB1 and CB2 transcription by multiplex RT-qPCR in OLC and HGF

A one-step retrotranscription quantitative polymerase chain reaction (RT-qPCR) using Taqman® Multiplex Real-Time solution hydrolysis probes (Thermo Fisher

Scientific) was used to determine the transcription of CB1 and CB2 using the CFX96 real-time thermal cycler detection system (BioRad; Hercules, CA, USA). Amplification conditions were as follows: 10 min retrotranscription at 55 °C, denaturation for 1 min at 95 °C, 40 cycles of amplification with an alignment temperature of 58 °C, using the following primers and probes (Macrogen®) CB1: Forward 5′-GGTTAGCAAGATACACTCAAGCATGA-3′ Reverse 3′-CTGGAAAAAGGCCCAACAAG-5′, Probe: 6FAM-5'-CAGCTGCTGCTTTCTTCTTCTTACACACCCCGGTCTC-3'-TAMRA; CB2: Forward 5′-GACACACGGACCCCTTTTTTTCTTGCT-3′ Reverse 3′-CCTCGTGGCCCTACCTATCC-5′, Probe: ROX-5'-TGGCCTTGCCCACCTGCACACACAG-3'-TAMRA and β-actin was used as reference gene: Forward 5′-GGATGCAGAAGGAGATCACTG-3′ Reverse 5′-CGATCCACACGGAGTACTTG-3′, HEX: 5′-CCCTGGCACCCAGCACAATG-3′.

CB1 and CB2 transcription during inflammatory stimuli in OLC

RT-qPCR was performed to evaluate CB1 and CB2 gene expression of LPS- and Poly-I:C-stimulated odontoblasts using SYBR Green (Luna® Universal One RT-qPCR Kit, New England BioLabs; USA). Amplification conditions were as follows: 10 min retrotranscription at 55 °C, denaturation for 1 min at 95 °C, 40 cycles of amplification with an alignment temperature of 58 °C using the following primers (Macrogen®) CB1: Forward 5′-GGTTAGCAAGATACACTCAAGCATGA-3′ Reverse 5′ CTGGAAAAAGGCCCAACAAG-3′, CB2: Forward 5′-GACACGGACCCCTTTTTGCT-3′ Reverse 5′ CCTCGTGGCCCTACCTATCC-3′ and Cholinergic receptor beta 2 subunit gene (CHRNB2) was validated and used as reference gene: Forward 5′-CAATGCTGACGGCATGTACGA-3′ Reverse 5′-CACGAACGGAACTTCATGGTG-3′. PCR efficiency was calculated with LinRegPCR sofware (Academic Medical Center, AMC, Amsterdam, Netherlands), and relative quantification was performed with the Schefe method[18] using unstimulated cells as a control.

Detection of CB1 and CB2 proteins in OLC and HGF

An indirect immunofluorescence technique was performed for CB1 and CB2 protein detection. Briefly, the cells (5×10^4 cells/well) were seeded on poly-L-lysine-treated glass coverslips for 24 hours, then some cells were treated for 24 hours with 2 µg/ml LPS or 40 µg/ml Poly-I:C, the cells were fixed with 4 % paraformaldehyde (PFA), and permeabilized with Triton X-100, to subsequently block with 10% goat serum. Cells were incubated at 37 °C with anti-CB1 or anti-CB2 polyclonal primary antibody produced in mouse (Invitrogen, Thermo Fisher Scientific), diluted 1:100 in blocking buffer. The samples were then incubated with Alexa 594 conjugated anti-mouse IgG secondary antibody (Thermo Fisher Scientific) diluted 1:200 in PBS at room temperature. The nuclei were counterstained with DAPI, and the slides were mounted with Prolong®, for observation under the Zeiss Axio Imager A2 microscope (Göttingen, Germany) with the AxioVision software. The presence or absence of the protein in cells and its location were evaluated in three independent experiments ($n = 3$).

Agonists, antagonists, and intracellular Ca2+ measurement

In vitro CB1 and CB2 activity in OLC and HGF was determined by calculating changes in intracellular calcium concentrations ([Ca2+]i) after different stimuli. Firstly, cells were loaded with 2 µM of fluo-4 AM solution (Invitrogen, Thermo Fischer Scientific) at 37 °C for 45 min in the dark. Next, cannabidiol 10 µM (CBD) (Biominerales Pharma, Colombia-Canada) was used for 1 and 10 min cell stimulation, either alone or with a 5-minute pretreatment with different antagonist concentrations (antagonist: agonist combinations, 10µM:10µM; 10µM:1nM; 1nM:1nM; 1nM:10µM), according to a previous viability experiment. A selective CB1 antagonist (AM251, Tocris, Bristol, UK), a selective CB2 inverse agonist (AM630, Tocris, Bristol, UK) and a TRPV1 antagonist (Capsazepine, CZP (Tocris, Bristol, UK) were used. The stock solution at a concentration of 10 mM was prepared in DMSO, and the serial dilutions used were prepared in culture medium.

Ionomycin is a lipophilic molecule that binds to calcium ions and carries them through membranes, so it was used as a positive control. Fluorescence quantitation of the cells was performed at a wavelength of 494/525 nm (excitation/emission) in a spectrofluorimeter (ClarioSTAR, BMG Labtech). For the analysis of results, the data were normalized

with respect to unstimulated cells (F/F0) and plotted at each evaluation time (1 and 10 min).

Data analysis

Data were organized in excel spreadsheets (Microsoft Office 2010) and described as mean and standard deviation (SD). Figures were prepared using GraphPad Prism 7.0 software (GraphPad Software, San Diego, CA, USA). All experiments were performed in triplicate. One-way analysis of variance ANOVA was used to determine the P-value using an alpha value <0.05.

RESULTS

Odontoblasts and gingival fibroblasts express CB1 and CB2, and are upregulated in the inflammatory environment.

Both OLC and HGF express CB1 and CB2 transcripts. For OLC, the mean Cq values were 31.62 and 32.02 (CB1 and CB2, respectively), while for HGF, they were 29.87 and 31.62, showing a similar pattern of receptor transcription (Fig. 1). Odontoblasts stimulated with LPS and Poly-I:C showed increases the cannabinoid receptor transcripts. Stimulation with LPS induced a fivefold rise in CB1 expression levels, while CB2 increased more than tenfold regarding unstimulated cells (Fig. 2). Poly-I:C stimulus caused a significant 4- and 8-fold increase in transcription of CB1 and CB2, respectively, in OLC. Overexpression of CB2 was significantly higher than CB1 transcripts in both LPS and Poly-I:C conditions.

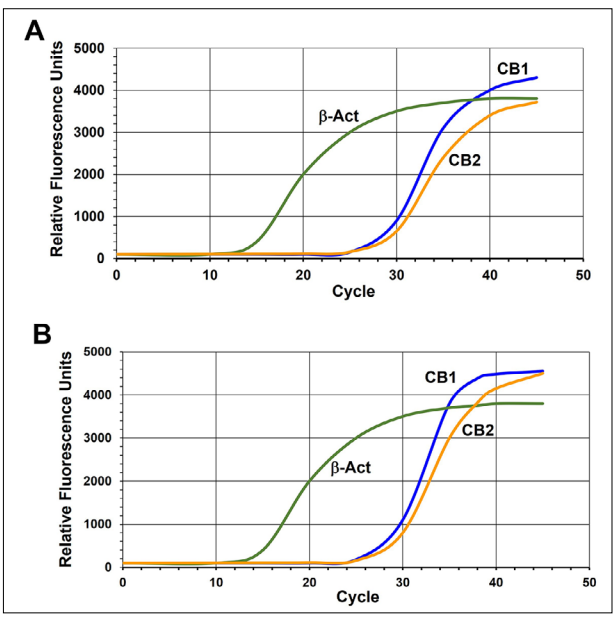

Fig. 1: Multiplex RT-qPCR amplification curves. (A) Human Gingival fibroblasts; (B) Odontoblast-like cells.

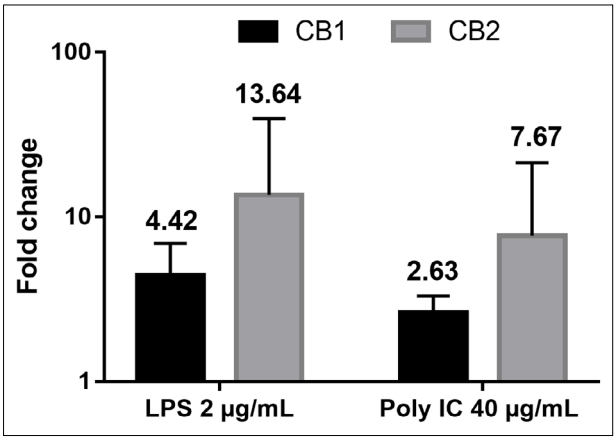

Fig. 2: Relative quantification of CB1 and CB2 mRNA in OLC stimulated with 2 µg/mL of LPS and 40 µg/mL of Poly-I:C.

Odontoblasts and gingival fibroblasts express CB1 and CB2 protein

Additionally, using immunofluorescence, both CB1 and CB2 proteins were identified in HGF (Fig. 3B) and OLC (Fig. 3C). SH-SY5Y and monocyte-derived macrophage cells were used as positive controls, respectively (Fig. 3A). Both receptors were found to be expressed in the cell membrane, nucleus, and cytoplasm, as fluorescence was observed in these areas in both cell types.

CBD increases intracellular calcium concentrations in odontoblasts and gingival fibroblasts

Stimulating gingival odontoblasts and fibroblasts with 10 µM of cannabidiol increased intracellular calcium concentrations compared to unstimulated cells ($p<0.001$). Pre-treatment with AM251, AM630 and CZP antagonists of CB1, CB2 and TRPV1 respectively, inhibited calcium influxes in both cell types (Fig. 4), demonstrating functional receptors in those cells.

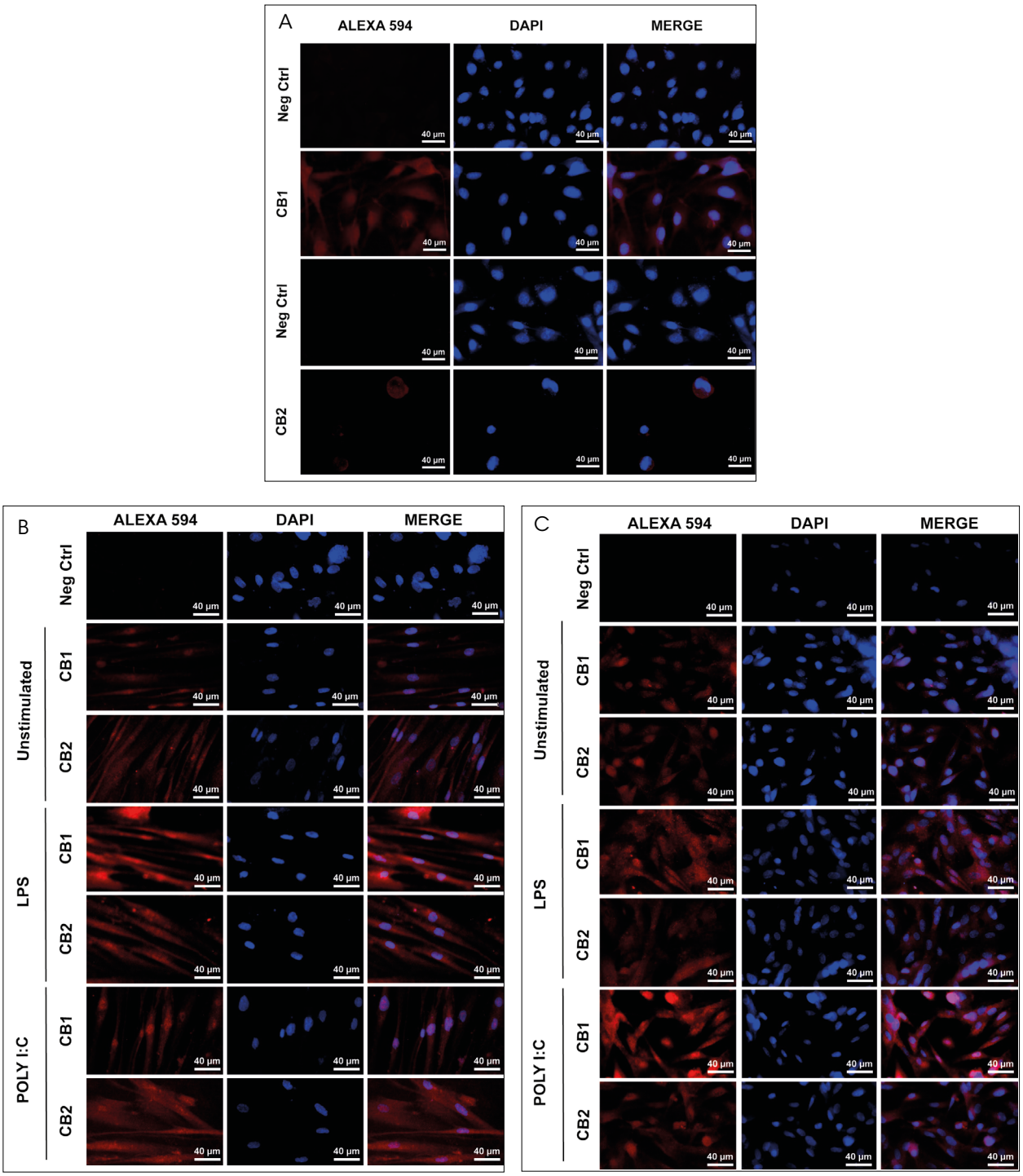

Fig. 3: CB1 and CB2 immunocytochemistry in LPS or Poly I:C stimulated and unstimulated cells. (A) Positive controls, CB1 (SH-SY5Y) and CB2 (Monocyte-derived macrophage cells); (B) Human Gingival fibroblasts; (C) Odontoblast-like cells. Scale bar: 40µm

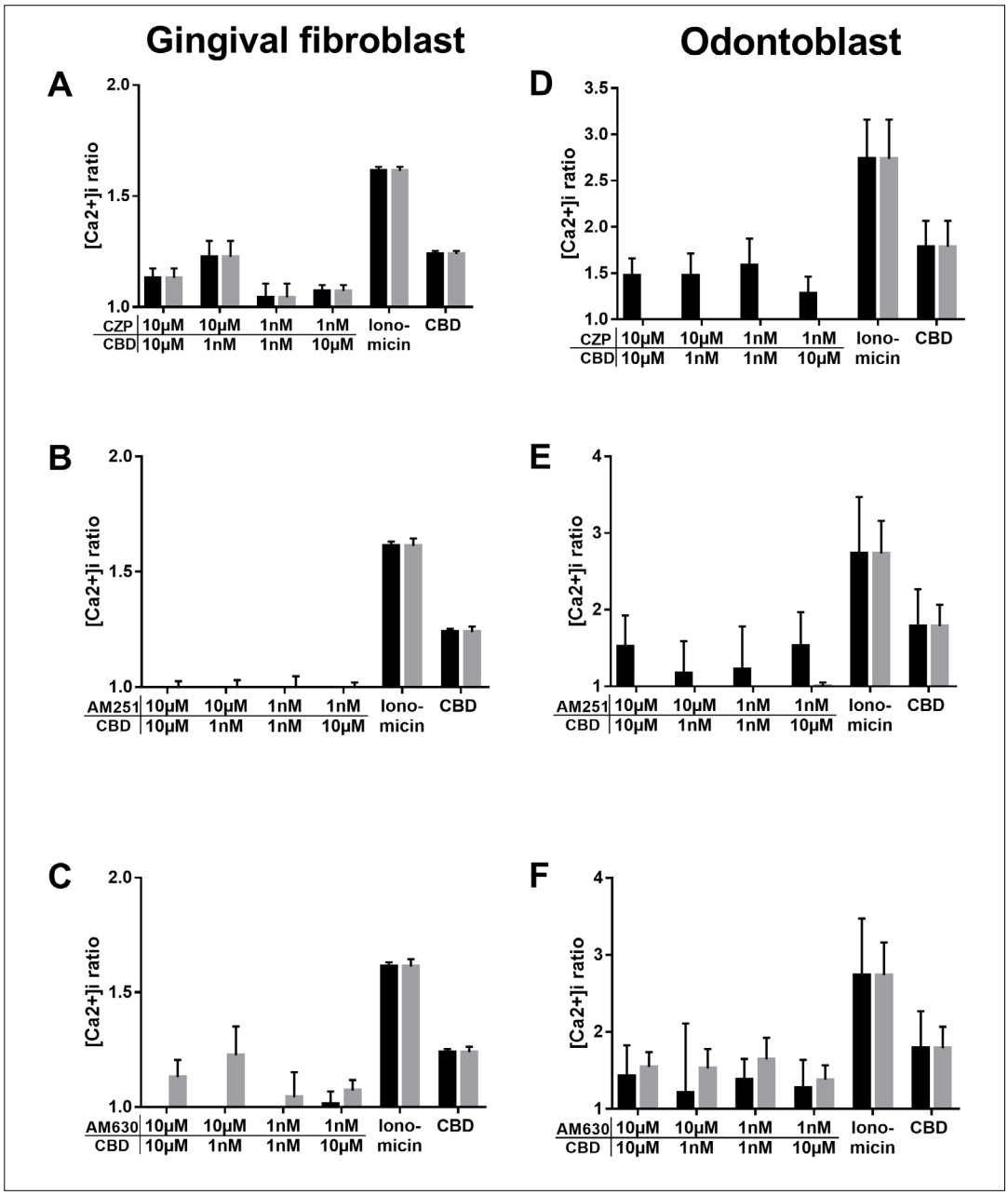

Fig. 4: Change in intracellular calcium concentrations by CBD and antagonists effect. Data are the ratio between specific fluorescence and fluorescence in unstimulated cells (Fi/F0) at one minute (black bar) and 10 minutes (grey bar). A - C: Gingival fibroblasts; D - F: Odontoblasts. P-value = 0.001.

DISCUSSION

This study demonstrated the expression of CB1 and CB2 cannabinoid receptors in human odontoblasts and gingival fibroblasts. An increase in their expression was observed in immune challenges. Additionally, their functionality was evidenced based on observed changes in calcium influx when CB1, CB2 and TRPV1 bind to their respective antagonist or inverse agonist. These cells are involved in response to mechanical, chemical, or infectious challenges in dental or periodontal tissues. Additionally, these receptors were found to be upregulated after inflammatory stimuli.

The expression and function of both receptors in dental tissues have been reported previously. For example, CB1 was found in nerve endings in human dental pulp[19]. Furthermore, immunohistochemistry has found evidence of the presence of CB1 receptors

in mouse odontoblasts[20]. Moreover, CB1 and CB2 expression has been reported in periodontal ligament stem cells, where they are involved in differentiation to osteo- and dentinogenic phenotype through p38, MAPK and JNK signaling in an inflammatory environment[14,21]. The major importance of CB in tissue repair has been highlighted, since they contribute to regulating bone volume and metabolism, bone loss and bone cell function in murine models[15].

CB1 and CB2 were found to be expressed in mouse periodontal tissue and in human periodontal fibroblasts. The activation of these receptors induces their adhesion and migration through the activation of focal adhesion kinase and MAPK activity[21], showing that they participate in the regulation of several signaling pathways and gene expression. Here, under inflammatory conditions, using LPS and Poly-I:C as bacterial or viral challenge surrogates, upregulation of CB1 and CB2 transcripts was found. Higher fluorescence CB receptor reactivity was observed in LPS and Poly I:C treated cells compared to untreated OLC and HFG, which could be interpreted as an increase in the amount of both cannabinoid receptors under inflammatory conditions. Previous research reports that CB2 is expressed in normal conditions but is exceptionally high in pathological inflammation[9]. Maresz, et al, (2005) demonstrated in microglial cells that an inflammatory environment induces cell activation and CB2 overexpression, dependent on IFN-γ and GM-CSF cytokines stimuli[22]. Positive labelling for CB1 and CB2 is observed throughout the cell. In addition to membrane activity, functional cannabinoid receptors have been reported to be located intracellularly in the nucleus, vesicles and mitochondria, where they can activate intracellular signaling[23,24].

Although periodontal ligament cells typically express both cannabinoid receptors, CB1 has higher expression in healthy tissue, even though it is downregulated during bacterial infection, while the CB2 is induced to overexpression. Conversely, sterile inflammation caused significant upregulation of CB1 and CB2[13]. The treatment with CB2 agonists and antagonists was found to regulate the inflammatory response in periodontal ligament fibroblasts. CB2 agonist ligands attenuated p38 and NFκB phosphorylation and simultaneously caused an increase in p-ERK and p-CREB, affecting cAMP concentration and β-arrestin pathways[15,25]. Nakajima et al. (2006) reported that CB1 and CB2 are upregulated in HGF samples from gingivitis and periodontitis. Stimulation of HGF with anandamide reduced LPS-induced secretion of cytokines, and CB1 or CB2 receptor blocking with specific antagonists attenuated that effect, confirming the involvement of cannabinoid receptors in the anti-inflammatory effect of the endocannabinoid anandamide[26].

On the other hand, the functionality of the receptors was evaluated by FLUO 4 AM, quantifying intracellular calcium concentrations, as it is known that activation of CB1 and CB2 leads to inhibition of adenylyl cyclase. This results in the blockade of A-type potassium channels (K^+A) and Ca2+ channels (L-, N-, P-/Q- and voltage-dependent) and increases the opening of inwardly rectifying potassium channels (Kir), causing cells to hyperpolarize[27]. We found that CBD generates an increase in intracellular calcium concentration. In addition, there is evidence that human odontoblasts express functional CB1, since they respond after stimulation with different agonists and antagonists[12]. CBD is the main non-psychoactive component of cannabis and is reported to possess sedative, anti-inflammatory, and antipsychotic effects[28], although several of these effects seem to be mediated by mechanisms independent of cannabinoid receptors. Previous evidence has shown that CBD inhibits several receptors such as TRPV1, GPR55, NMDAR, opioid receptors, adenosine A1 receptors and voltage-dependent calcium channels; and interestingly, it also activates PPAR-ɣ, 5HT1A and the glycine receptor[29,30]. Therefore, changes in intracellular calcium concentrations generated by CBD are due to the activation and blockade of several associated channels. Since calcium channels are involved in the CB1 and CB2 signaling cascade activation, measuring calcium influxes provides indication of whether these receptors are functional.

Thus, the action of CBD through these pathways may be responsible for the suppression of neuronal excitability and pain perception. Still, there is also evidence that CBD inhibits the uptake of dopamine, noradrenaline, GABA, serotonin and anandamide in the synapses[31], which would explain its antinociceptive and neuroprotective effects. Moreover, the anti-inflammatory effect is also explained by COX-2 inhibition, which prevents the

production of arachidonic acid metabolites[32]. Even so, in this work, it was found that when cells were stimulated with CBD and CB1 and CB2 antagonists, intracellular calcium concentrations were reduced, showing that CBD may be acting directly or indirectly through cannabinoid receptors. On the other hand, the selective antagonists used (AM251 and AM630) are also inverse agonists, which means that they have a negative efficacy for a certain signaling pathway, which ends up blocking calcium channels so that when stimulating with CBD, no calcium influx is generated through CB1 and CB2.

Crosstalk is a mechanism of interaction between different intracellular substances or signal transduction pathways[12]. Previous reports have shown that there is coupling between cannabinoid receptors and TRPV1. For example, in odontoblast cells, a stimulus inducing an intracellular calcium increase follows the ion extrusion through the sodium-calcium exchanger channels towards the mineralization front[33], explaining the role of cannabinoid receptors and TRPV1 in calcium apposition in the tertiary dentin and the transduction of external stimuli.

CBD is a TRPV1 agonist and negative allosteric modulator of cannabinoid receptors[34], so the explanation for the decrease in intracellular calcium when CB1 and CB2 antagonists are applied may be crosstalk between both types of receptors, where signaling via TRPV1 may be decreased when cannabinoid receptors are blocked, even at low concentrations of CB1 and CB2 antagonists and high concentrations of CBD.

Such communication between the two receptors or their signaling pathways causes a physiological balance to be maintained under pathological conditions. For example, previous evidence has demonstrated that cannabinoid receptors mediate anti-inflammatory and protective effects in periodontal tissues, while TRPV1 mediates pro-inflammatory effects, which lead to periodontal injury[15]. Further studies should be performed on odontoblasts and gingival fibroblasts to elucidate the signaling pathways that can be activated by CBD and the mechanism by which crosstalk with TRPV1 is generated, so that CBD can be considered a possible treatment for infectious or inflammatory pathologies in the oral cavity.

CONCLUSIONS

This study demonstrated that human odontoblasts and primary gingival fibroblasts express functional CB1 and CB2 cannabinoid receptors, and that under inflammatory conditions, both receptors are overexpressed, especially CB2. On the other hand, it showed that CBD generates calcium influxes, demonstrating that it has biological activity and functional communication with TRPV1. These results show the importance of the expression of these receptors in the physiological and pathological processes in the dental pulp and periodontal tissues, demonstrating their functional expression in these cells and opening new perspectives for searching for alternatives for more effective treatments against dental pain and inflammation.

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DECLARATION OF CONFLICTING INTERESTS

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

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Influence of melatonin associated with the Bio-Gide® membrane on osteoblast activity: an *in vitro* study

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ABSTRACT

Melatonin (MLT) is a hormone responsible for regulating several physiological processes. It has been shown that MLT can be an important mediator in bone formation and stimulation, promoting osteoblast differentiation. In clinical practice, in tissue regeneration procedures, it is necessary to use membranes or barriers, associated with biomaterials, or not. The aim of this in vitro *study was to assess the effect of melatonin on the activity of osteoblastic cells, associated, or not, with a resorbable collagen membrane (Bio-Gideä). For this, mice-derived pre-osteoblastic cells MC3T3 obtained from the ATCC (American Type Culture Collection) were used. Cultured cells were subject to the following treatments: MLT with a concentration of 1mM, a Bio-Gideä membrane and a membrane associated with MLT (Bio-Gideä + MLT). Proliferation and cell viability assays and protein lysate (ELISA test) quantification for the BMP-2 protein were carried out, in periods of 72 hours, 7 days and 10 days. After analyzing the data (one-way ANOVA, alpha=5%) it was observed that when MLT was used in isolation, there was an increase in cell proliferation and viability in osteoblastic cells ($p<0.05$). But, when MLT was associated with resorbable membranes, there was an inverse behavior, both in terms of proliferation and viability ($p<0.05$). In the case of the ELISA test, no secretion of BMP-2 was detected in any of the analyzed groups. It is concluded that MLT has a stimulatory effect on osteoblasts, but, when associated with Bio-Gideä resorbable membranes, it does not show any viable action in osteoblastic cell stimulation.*

Keywords: *biocompatible materials - bone morphogenetic protein 2 - melatonin - osteoblasts.*

Influência da melatonina associada à membrana bio gide® na atividade de osteoblastos: um estudo *in vitro*

RESUMO

*A melatonina (MLT) é um hormônio responsável pela regulação de diversos processos fisiológicos no nosso organismo. Tem sido demonstrado que a melatonina possa ser um importante mediador na formação e estimulação óssea, promovendo a diferenciação dos osteoblastos. Clinicamente, para o procedimento de regeneração tecidual, faz-se necessário a utilização de membranas ou barreiras, associadas ou não a biomateriais. Assim, o objetivo deste estudo in vitro foi avaliar o efeito da melatonina na atividade de células osteoblásticas, associada ou não a uma membrana de colágeno reabsorvível (Bio-Gide®). Para isto foram utilizadas células pré-osteoblásticas MC3T3 do ATCC (*American Type Culture Collection*), de camundongos. As células em cultura foram submetidas aos seguintes tratamentos: MLT na concentração de 1mM, membrana Bio Gide® e membrana associada à MLT (Bio-Gide® + MLT). Foram realizados os ensaios de proliferação e viabilidade celular e quantificação do lisado proteico (teste ELISA), para a proteína BMP-2, nos períodos de 72 horas, 7 e 10 dias. Após a análise dos dados (ANOVA um critério, alfa=5%) pode-se observar que a MLT quando utilizada sozinha, resultou em um aumento na proliferação e viabilidade celular nas células osteoblásticas ($p<0,05$). Entretanto, quando a MLT foi associada à membrana reabsorvível foi observado um comportamento inverso, tanto na proliferação quanto na viabilidade ($p<0,05$). Para o teste ELISA realizado, não houve secreção detectável de BMP-2 para nenhum grupo analisado. Conclui-se que a melatonina possui uma ação estimuladora nos osteoblastos, mas quando associada à membrana reabsorvível Bio-Gide®, não demonstra uma ação viável na estimulação de células osteoblásticas.*

Palavras-chave: *materiais biocompatíveis – proteína morfogenética óssea 2 – melatonina – osteoblastos.*

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INTRODUCTION

The regeneration of lost periodontal tissues is a clinical challenge. Even though some evidence has shown that periodontal regeneration may be obtained, success is still limited, namely in challenging clinical scenarios[1-3]. Another challenging scenario is Guided Bone Regeneration (GBR), in deficient areas that implants be made for subsequent prosthetic rehabilitation. Nevertheless, attaining complete regeneration of periodontal and bone tissues is still unpredictable[4-6]. Yet, both GTR and GBR are procedures applied increasingly followed in the search for optimal results. For applying these surgical techniques, it is necessary to build membranes associated or not associated with biomaterials[7]. Such membranes play the role of protective barriers to prevent invasion of soft tissues[8], by segregating cells with regenerative potential, such as osteoblasts and fibroblasts, from epithelial cells and conjunctive tissue cells with high proliferation potential[9].

Membranes may be classified in two main groups: resorbable and non-resorbable. Resorbable membranes experience biologic degradation, with variable sustainability, that may interfere with bone regeneration[10,6]. Unlike non-resorbable membranes, resorbable membranes gradually go through biodegradation and, thus, this avoids the need for a second surgical procedure for membrane removal.

This property of degradation in resorbable membranes may affect the ability for keeping free spaces and of forming new tissue. Ideally, the rate of degradation should be moderate because fast degradation rates may lead to early mechanical loss. On the other hand, slow degradation rates prevent the development of new tissues[6], thus, the biodegradation rate of collagen membranes have an impact on their efficacy[9,11-13]. This rate may also be influenced by other materials, and, the degradation process must be slow and sufficient for attaining tissue regeneration, before the membrane disintegrates[14].

Bio-Gide® is a porcine resorbable membrane, comprised of a double layer of Type I and III collagen, indicated for tissue regeneration. It may act as a tissular barrier as it promotes the deposition of new bone with a stable absorption rate, without causing tissue inflammation[15,16].

With the intent of enhancing the effects of reabsorbable membranes in bone regeneration, research has been done of additives used on such membranes. Melatonin (MLT) is a synthesized hormone secreted by the pineal gland that regulates several physiologic processes in different parts of the body. In the oral cavity, the main role of melatonin is its antioxidant and anti-inflammatory effects, as well as being a mediator in the process of bone formation and reabsorption. In therapeutic doses, MLT inhibited bone reabsorption and increased bone formation, thus accelerating the mineralization process of the matrix[17,18]. Besides, MLT might play a crucial role in bone growth regulation as, with therapeutic doses, the ability of melatonin for inhibiting osteoclastic activity and promoting osteoblast differentiation was observed[19].

In MLT, one of the mechanisms underlying its ability for regulating bone development is its stimulatory effect on osteoblasts. In *in vitro* human osteoblastic cells, melatonin has the ability of stimulating, in micromolar concentrations (nM), the proliferation and synthesis of Type I collagen, as well as of other proteins of the bone matrix and bone markers (alkalin phosphatase, osteopontin and osteocalcin)[18,20,21]. Yet, the action mechanism of MLT on these markers has not yet been entirely elucidated.

With the aim of enhancing GBR (Guided Bone Regeneration), different biomaterials associated with the use of membranes have been studied[13,14,16]. Thus, being a synthetic hormone that could potentially impact on bone formation, melatonin could be associated with membranes in regeneration processes. Bearing this in mind, this research study aimed at assessing, *in vitro*, the effect of adding melatonin to reabsorbable membranes (Bio-Gide®) in the initial stages of new bone formation.

MATERIALS AND METHODS

The project was approved by the Ethics and Research Committee of the São Leopoldo Mandic College, under Protocol: 2019/0176.

Biomaterials

In this research study Melatonin synthetic hormone - *N*-Acetyl-5methoxytryptamine, Melatonin ($C_{13}H_{16}N_2O_2$) (Sigma, St. Louis, Missouri, EUA), grade of purity > 98%, and a concentration of 1mM, was used, based on the research study by Dalla-Costa *et al*., (2020). The Geistlich Bio-Gide® membrane was used (Gide Compressed 13x25mm (Ref.: 500362 and 308013) (Geistlich Pharma do Brasil, São Paulo, SP, Brazil).

One nM of MLT was diluted in the culture medium and the Bio-Gide® membrane was cut into 5 mm squares.

Study Design

Pre-osteoblastic cells were subject to different treatments, according to the study design shown in Fig. 1: the control group was formed by the Bio-Gide® membrane-only; a second group was formed by MLT-only, and a third group was formed by the Bio-Gide® membrane associated with MLT.

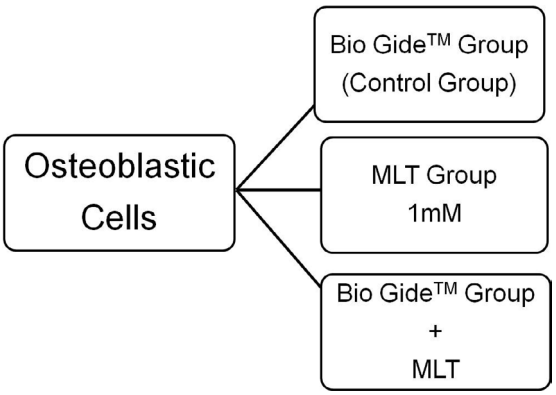

Fig. 1: Schematic Design of the Experimental Groups.

Laboratory Tests

A strain of osteoblastic cells (MC3T3-E1) was obtained from the ATCC (*American Type Culture Collection*, ATCC, VC, USA). The osteoblastic cells were cultivated in the Minimum Essential Medium, with alpha modification (α-MEM) and supplemented with bovine fetal serum (10%) (Cultilab®, Campinas, SP, Brazil) and 1% of an antibiotic-antimycotic solution (Sigma, St. Louis, Missouri, USA).

In all the procedures, laminar flow chapel was used for preserving the sterilization properties of the materials and substances used in cell culture. The cultures were supplemented with 10 mM of βGP (Sigma, St. Louis, MO, USA) and 50μg/mL AA (Sigma), except for the groups with melatonin used in isolation. Cells were kept in a heater at 37ºC, in a humid atmosphere with 95% of air and 5% of carbon dioxide. The culture medium was changed every 2-3 days and the culture progression was assessed through phased microscopy in cultures grown on polystyrene, with a control function.

Cell Culture

A strain of mice osteoblastic cells (MC3T3-E1) was obtained from the ATCC (American Type Culture Collection, ATCC, VC, USA). The osteoblastic cells were cultivated in the Minimum Essential Medium, with alpha modification (α-MEM) and supplemented with bovine fetal serum (10%) (Cultilab®, Campinas, SP, Brazil) and 1% of an antibiotic-antimycotic solution (Sigma, St. Louis, Missouri, USA). In all the procedures, laminar flow chapel was used for preserving the sterilization properties of the materials and substances used in cell culture.

Cells were kept in a heater at 37ºC, in a humid atmosphere with 95% of air and 5% of carbon dioxide. The culture medium was changed every 2-3 days and its progression was assessed through phase-contrast microscopy, according to its growth on polystyrene, that had a control function. Table 1 shows mineral nodule counts evaluated using the Alizarin Red (VA) and Fast Red (FR) tests.

Measuring Total Protein Content

Total protein measurement was done on Days 3, 7 and 10, as per the Lowry *et al*. method. (1951). The culture medium was removed from the wells. Then, these were triple-rinsed with warm PBS (37ºC) and refilled with 2 ml of deionized water. The specimens were submitted to five thermal shock cycles. The procedure in each one of the cycles was: 20 minutes at -20°C, and 15 minutes at 37ºC. At the end of the cycles, 1 ml of the cell lysate of each well was transferred to test tubes, mixed with 1 ml of Lowry solution (Sigma) and left to rest at room temperature for 20 minutes. After this, a 0.5 ml solution of Folin-Ciocalteau's phenol reagent (Sigma) was added to each tube and left to rest again at room temperature for 30 minutes. Immediately after this,

Table 1. Means (standard deviation) of 10-day mineral nodule counts evaluated using the Alizarin Red (VA) and Fast Red (FR) tests.

	crtl	0,01 mm	0,1 mm	1 mm
VA	15.35 (1.88) A	29.93 (6.03) B	68.24 (3.82) C	45.43 (1.45) D
FR	42.42 (1.93) A	29.43 (5.36) B	66.17 (2.46) C	14.64 (1.31) D

the absorbance level of each tube was measured in a spectrophotometer (CE3012, Cecil, Cambridge, England). A wavelength of 680 mm was used and the total protein concentration (in g/ml) in each well was calculated based on a standard curve with bovine albumin (Sigma).

Cell Proliferation Assay

For assessing cell proliferation, a *Trypan* blue vital exclusion method of cell viability was used (at 72 hours, 7 days and 10 days) in the cell culture plates. For this, cells were placed in plates (with a 110 cell/mm^2 density) on the different membranes and, after having reached sub-confluency, they were enzymatically removed from the plates. Then, the cell precipitate resulting from centrifugation was suspended in a 1 ml medium. Ten (10) µL were withdrawn from the cell suspension and 10 µL of *Trypan* blue were added. One (1) µL of this solution was placed in a hemacytometer (Neubauer-Fisher Scientific, Pittsburgh, PA, USA) and then taken in an inverted phase microscope (Nikon, Eclipse TS100) for cell count and observation. The total cell count present in each well at different times was obtained through the following mathematical equation:

$$\text{Total number of cells} = \frac{\text{number of cells counted x initial volume x dilution x 104}}{\text{number of squares used for counting}}$$

Cytotoxicity Assay (MTT)

Cell cultures were tested for cell viability by means of MTT Assays. Such assay assessed the ability of metabolically active cells for reducing MTT, transforming yellow tetrazolium salts (3-(4,5 Dimetiltiazol-2-yl)-2,5-difeniltetrazol bromide) into purple-colored formazan crystals, and, thus, assessed the ability of viable cells to cleave tetrazolic rings present in MTT (3-(4,5-Dimetiltiazol-2-yl)-2,5-difeniltetrazol bromide) through the dehydrogenase enzymes present in the active mitochondria, forming formazan crystals.

In cytotoxicity assays, cells were set on plates with a 110 cells/mm^2 density on the membranes. Ten (10) µl of the MTT solution (5 mg/mL, Sigma-Aldrich, USA), diluted in a DMEM non-serum culture medium, were added to the cell cultures, which were incubated during a 3-hour period, at 37ºC. After this, 100 µl of DMSO (Dimethyl Sulfoxide, LGC, São Paulo, Brazil) were added and kept at room temperature for 15 minutes. After the solubilization of the crystals, counting was done through a ELX800 Microplate reader (Biotek Instruments Inc.) at 590 nm.

Enzyme Immunoassay for BMP-2 Secretions (ELISA)

Quantification of BMP-2 secreted by the osteoblastic cells on plates under different conditions was assessed by means of ELISA. The supernatant was collected and centrifuged at a rate of 5000 g for 15 min, at 4º C. The aliquots of each sample were assessed by means of enzyme immunoassays (ELISA) for determining the levels of Type I collagen, as per manufacturer's instructions (R&D Systems, EUA). The sequence of primers is seen in the table 2.

The reaction was completed by adding 50 µl of sulphuric acid (H_2SO_4) 2 N to the substrate solution in each well. Besides, color was measured with a spectrophotometer (Epoch, Biotek, Winooski, VT, USA) with a 450 nm wavelength. The total amount of Type I collagen was determined in picograms (pg/mL).

All the assays were done in triplicate.

Statistical Analysis

After verifying if data met normal distribution and homocedasticity, the impact of the melatonin added

Table 2. Sequence of primers for collagen Type I (COL-I), osteopontin (OPN) and the endogenous control (GAPDH).

Gen's name	Gen's symbol	Primers Sequency
COL- I	COL1A1	F 5' CCAGAAGAACTGGTACATCAGCAA-3 R 5' GGACATCAGGCGCAGGAA-3'
OPN	OPN	F 5'-TGCTTGGGTTTGCAGTCTTCT-3' R 5'-CCAAACAGGCAAAAGCAAATC-3
Glucose 6 phosphate dehydrogenase	GAPDH	F 5' ACCCACTCCTCCACCTTTGA-3' R 5'-TGTTGCTGTAGCCAAATTCGTT-3'

to the Bio-Gide® membrane on cell proliferation and viability after 72 hours, 7 days and 10 days in plates was examined through variance analyses (ANOVA) against one criterion. For multiple comparisons, a Fisher LSD test was run. Statistical calculations were done in the SPSS 23 program (SPSS INC., Chicago, IL, USA), the result being a significance level of 5.

OUTCOMES

Cell proliferation and viability outcomes were analyzed in the 72-hour, 7-day and 10-day-periods. (Fig. 2 and 3).

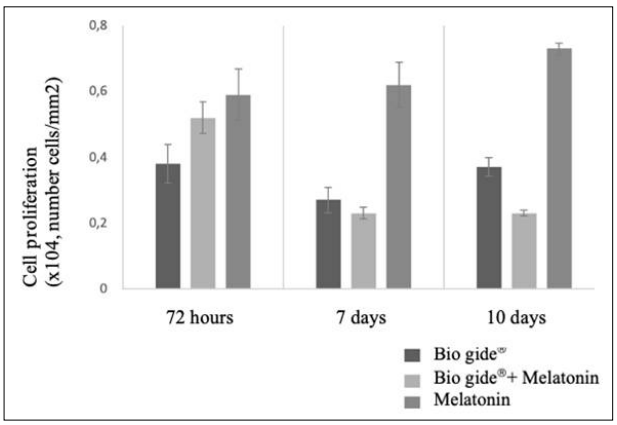

Fig. 2: Column Chart of Cell Proliferation in each of the treatments (Bio Gide®, Bio Gide® + Melatonin, and Melatonin), throughout the different time period assessed (72 hours, 7 days and 10 days). Different letters represent statistical differences between the treatments, within the same time period assessed (ANOVA, alfa=5%).

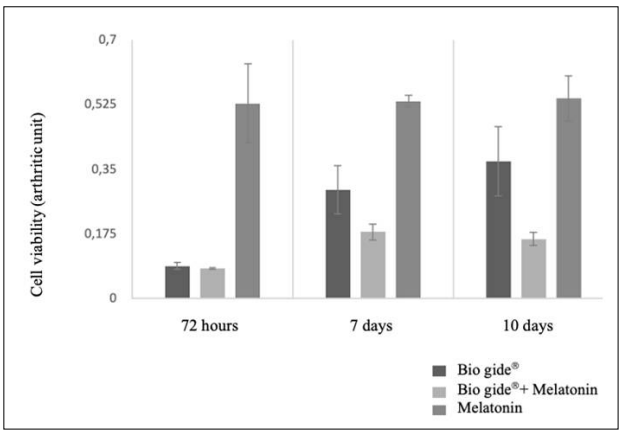

Fig. 3: Column Chart of Cell Viability in each of the treatments (Bio Gide®, Bio Gide® + Melatonin, and Melatonin), throughout the different time period assessed (72 hours, 7 days and 10 days). Different letters represent statistical differences between the treatments, within the same time period assessed (ANOVA, alfa=5%).

Cell Proliferation

In the 72-hour period, a statistical difference was observed in the proliferation of osteoblastic cells ($p = 0,014$), only in the Bio-Gide® group, which had lower proliferation rates, as shown in Fig. 2.

As regards the cells treated with melatonin or with the Bio-Gide® + Melatonin, there was no statistical difference between them ($p>0,05$).

After seven days, proliferation of osteoblastic cells significantly varied ($p<0,001$) depending on the treatment selected. In the group treated exclusively with melatonin, there was a significantly higher proliferation ($p<0,05$), when compared to the two other treatments. On the other hand, treatments with membrane-only or associated membrane did not show any significant difference ($p>0,05$). After 10 days, the association of Membrane and Melatonin (Bio-Gide®+MLT) showed a statistically significant reduction in the number of osteoblastic cells ($p<0,001$), vis a vis the group that only received the Bio-Gide®-only membrane. Yet, when MLT was used in isolation, a higher cell proliferation level was observed, when compared to the two other treatments ($p<0,05$).

Cell Viability

In the 72-hour period, cell viability was statistically higher in the MLT group ($p<0,001$), when compared with the two other treatments, which, in turn, showed no difference between them ($p>0,05$). In the 7-day and 10-day periods, there were very similar results, with statistically significant differences between the three treatments ($p<0,001$). The highest viability level was seen in the group of cells treated with MLT, followed by the Bio-Gide®-only group and by the Bio-Gide® + MLT group.

ELISA

As to the results of ELISA assays in concentrations of 1 mM of MLT in relation with BMP-2 secretion, there were no immunomarkers in the 72-hour, 7-day and 10-day periods in any of the assessed groups.

DISCUSSION

In the recent years, research studies have been performed of the possible effects of melatonin on bone formation. Besides, melatonin has been used locally in bone regeneration procedures[18,21,22]. Bearing in mind the importance of membranes in bone regeneration and of MLT in the initial

processes of new bone formation, this research study performed, in an innovative manner, an *in vitro* assessment of the effect on osteoblast cells derived from adding MLT to a resorbable membrane (Bio-Gide®). Cell proliferation and viability were assessed in pre-osteoblastic cells subject to the activity of melatonin, with or without a resorbable membrane (Bio-Gide®).

In this research study, the selection of the 1mM MLT concentrate was based on the experience of a previous study[21], that showed that the promising effects of MLT were seen mainly with low concentrations, especially, with 1mM concentrations. *In vitro* studies showed that in human osteoblasts, MLT was able to stimulate, in micromolar concentrations (mM), the proliferation and synthesis of Type I collagen, bone matrix proteins and bone markers (including alkaline phosphatase, osteopontin and osteocalcin)[18]. Proliferation and viability assessment periods chosen for this research (72 hours, 7 and 10 days) were based on the studies of Dalla-Costa et al. and Tera et al[21,23]. Bone metabolism is more intense between days 7 and 21, while the highest remodeling rate is seen after 7 days[23]. Higher cell proliferation is seen after 72 hours, and mineral nodule formation, after 10 days[21]. Thus, as the purpose of this study is to assess the effect of MLT on the initial events of new bone formation, we consider that the period chosen for the analyses of this study was adequate.

The results obtained in this study show that both in viability and cell proliferation analyses, MLT used in isolation showed the best results in all the three chosen periods (72 hours, 7 days and 10 days). In a similar research study[24], the molecular mechanism of melatonin was studied in the differentiation of mice MC3T3-E1 osteoblastic cells, where melatonin increased osteoblastic differentiation, compared with non-treated control groups. Such results confirm the findings of the present study, that show that melatonin stimulated cell events necessary for new bone formation.

In the present study, with the Bio-Gide® membrane tested in isolation, it was observed that, both in terms of proliferation and viability and, irrespective of the duration of the assessment, results were statistically lower than the results in the melatonin-only group. Osteoblasts prefer rugous surfaces where they can fix on[25]. But, there is a surface rugosity level that is ideal for increasing biocompatibility. Wang HL et al.[26] showed that a 30 to 50 nm porosity increases fixation and proliferation of undifferentiated mesenchymal cells, and that the increase or decrease in porosity reduces biocompatibility. Consequently, membrane porosity may affect biocompatibility, despite the fact that, in general, rugous surfaces support fixation and proliferation of osteoblast-like cells. In Rothamel et al.[27] the Bio-Gide membrane showed a more fibrous structure, when compared to the other membrane, with adjacent bone formation. These studies can justify the presence of cell proliferation and viability in the combined model and in the Bio-Gide®-only model, which may be ascribed to the characteristics of membrane surface. Interestingly, in this study, the Bio-Gide® + MLT association showed low cell viability and proliferation levels in the three periods under evaluation, with a higher cell proliferation level in the 72-hour period, while in the 7-day and 10-day intervals there was a decline in such proliferation. Schorn et al.[7] where biocompatibility in different membrane surfaces was assessed, concluded that membranes with altered surface structures bear high rates of cytotoxicity and that this might be the response to the low rate of viability shown in this group, where MLT was added to the membrane. In order to ratify this information, Liang et al.[3] showed that the use of enhanced membranes is not sufficient for obtaining successful results and treatments in terms of regeneration. They also showed that the combination of membranes and bone grafts may lead to better regenerative outcomes.

In Sam et al.[27] membrane development in third generation membranes was assessed. Apart from operating as barriers, such membranes also operate as means for releasing specific agents such as antibiotics and growth-factor drugs. According to all this information, MLT might be a candidate to be included in the list of new generation barriers. Yet, the results of this study did not show any advantage derived from such association. A plausible alternative for increasing the efficacy of the Bio-Gide® + MLT association, considering that both have had positive results when applied separately, would be to control the release patterns of such hormone, as per Liang et al.[3]. Another matter that needs to be discussed, as pointed out by Younho et al.[28], is the adequate concentration of hormones to be used. The mere fact that MLT led to good results with a 1 mM dosage when used in isolation[21] does not imply that this could be the ideal dose when used in association

with the membrane. And this is so if we bear in mind that its insufficient or excessive use may jeopardize results, especially because bone formation is comprised of a complex series of events involving growth factors and cytokines with time and dose-dependent activities.

BMPs are the most important inducers and stimulators of osteoblast differentiation and have a significant role in the process of bone formation[28]. According to Fan J. et al.[29] Bone Morphogenetic Protein 2 (BMP2) is the most powerful osteo-induction factor, extensively studied for treating multiple bone fractures and bone defects. ELISA test results for BMP2 did not show presence of immunomarkers in the secretion of such protein in the presence of MLT.

Dalla-Costa et al.[21] showed positive results with the secretion of Type I collagen and Osteoponin -key proteins in bone physiology- in the presence of MLT with a 1mM concentration. Another research study, by Tianyuan et al.[22], showed that, among the different BMPs, BMP-9 is one of the most effective factor for inducing osteogenesis in mesenchymal stem cells. Besides, under the stimulus of MLT, BMP-9 showed an increase in osteogenic differentiation. On the other hand, Younho et al[28] demonstrated that, when induced by BMP-4, MLT increased osteogenic differentiation, while in the case of BMP-2, when under the stimulating effects of MLT, there was only an increase in chondrogenic differentiation. In part, this might explain the absence of BMP-2 immunomarkers in the present study.

Biologic barriers have shown promising results in bone and tissue regeneration[30], yet, it is still necessary to do more research on the use of such membranes in association with substances such as MLT and on which are the best concentration and administration levels. Being an *in vitro* assessment, this study has the methodological limitations inherent with such procedures. For instance, the cells assessed in this study were immersed in a culture medium instead of being organized in a tissular structure, as it is in live organisms. This requires that future research is done, with animal models, so as to confirm the results of the present research study.

Our conclusion is that melatonin showed a stimulating impact on osteoblasts, but, when associated with a Bio-Gide®resorbable membrane, it did not show any beneficial impact on the cell events being assessed.

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DECLARATION OF CONFLICTING INTERESTS

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

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Tooth color in dental students from Buenos Aires University, Dental School, Argentina

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ABSTRACT

The aim of this study was to evaluate tooth color in dental students at the University of Buenos Aires, Argentina. The participants were 184 students (157 women and 27 men) aged 21 to 33 years, mean age 24.45 (SD 2.79) years, who were in the fourth year of their dental degree. They agreed to participate through an informed consent. Exclusion criteria were: having undergone a bleaching treatment within the previous six months, presence of total or partial peripheral restoration, pigmentations, fracture, carious and non-carious lesions or absence of the right upper central incisor (1.1). A dental prophylaxis procedure was performed on the buccal surface of each 1.1 tooth with a prophylaxis brush (TDV) mounted on a low-speed rotary instrument Kavo 2068 CHC (Germany) micromotor and a Kavo LUX K201(Germany) contra-angle. Shade was measured in the middle third of each 1.1 tooth, by the same observer, using a VITA Easyshade V spectrophotometer (Zahnfabrikn Bad Säckingen, Germany), which was calibrated before each determination according to manufacturer's instructions, in the same dental unit (Sino S2316), with natural illumination, in the same time slot, without using the dental unit lamp. The results were recorded in an ad-hoc form and rates and confidence interval were obtained. Shade prevalence percentages (95% CI) were: A1: 46.2 (38.83 - 53.68); followed by A2 and B2, both with 17.39 (12.21 - 23.66); A3: 6.52 (3.41-11.11); B1: 4.35 (1.9-8.39); D2: 2.72 (0.89 - 6.23); B3: 2.17 (0.60-5.47) and C2: 1.09 (0.13-3.87); D3, C3, A3.5 and A4: 0.54 (0.01-2.99). Shades D1 and C1 were not determined in any subject.

Within the conditions of this study, A1 was the most prevalent shade in central incisors, followed by A2 and B2.

Keywords: *tooth - color - spectrophotometry.*

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Color dentario en estudiantes de la Facultad de Odontología de la Universidad de Buenos Aires, Argentina

RESUMEN

El objetivo de este estudio fue evaluar la prevalencia de color dental en estudiantes de odontología de la Universidad de Buenos Aires (UBA). Los participantes fueron 184 estudiantes de cuarto año de la carrera de Odontología (157 mujeres y 27 hombres) de entre veintiuno y treinta tres años, con un promedio de edad de 24,45 (DE 2,79) años que aceptaron participar mediante consentimiento informado. Criterios de exclusión: quienes hayan recibido blanqueamiento en los últimos seis meses, tenían una restauración periférica total o parcial, pigmentaciones, fractura, lesiones cariosas y/o no cariosas en el incisivo central superior derecho (1.1) o éste estaba ausente. Se realizó profilaxis dental en la superficie bucal de cada 1.1 con un cepillo ad-hoc (TDV) montado en un micromotor de baja velocidad Kavo 2068 CHC (Alemania) y un contra-ángulo Kavo LUX K201 (Alemania). La medición del color se llevó a cabo en el tercio medio de cada 1.1, por el mismo observador, en la misma clínica dental, con iluminación natural en la misma franja horaria y sin utilizar la lámpara del equipo dental. Se utilizó un espectrofotómetro VITA Easyshade V (Zahnfabrikn Bad Säckingen, Alemania) que se calibró antes de cada determinación de acuerdo con las instrucciones del fabricante. Los resultados se registraron en una planilla ad-hoc y se obtuvieron tasas e intervalos de confianza. Prevalencia de colores % (IC 95%): A1: 46,2 (38,83 - 53,68), seguido de A2 y B2 ambos con 17,39 (12,21 - 23,66), A3: 6,52 (3,41-11,11), B1: 4,35 (1,9- 8,39), D2: 2,72 (0,89 - 6,23), B3: 2,17 (0,60-5,47) y C2: 1,09 (0,13-3,87); D3, C3, A3.5 y A4: 0.54 (0.01-2.99) D1 y C1 no se determinaron en ningún sujeto.

Dentro de las condiciones de este estudio A1 fue el color más prevalente en los incisivos centrales de estudiantes de odontología, seguido de A2 y B2.

Palabras clave: *diente - color - espectrofotometría.*

INTRODUCTION

Characterization and reproduction of tooth color is one of the main objectives of cosmetic and restorative dentistry. The increase in patients' aesthetic demands has resulted in the development of high-performance restorative materials such as ceramics and composites[1].

Color identification and communication has been a challenge in virtually every application area. Traditionally, the color of teeth has been described in terms of the parameters of the Munsell system, and its dimensions, hue, value and chroma. However, in order to facilitate the quantification of color differences, the CIE L*a*b* (CIELAB) chromatic model is currently employed, which enables description of all the colors perceived by the human eye, and is based on the standardization of sources of light and observers[2,3]. It was developed specifically for this purpose by the *Commission Internationale de L'eclairage* (International Commission on Illumination) in 1931[4] and was re-published in 1971[5]. The asterisks (*) that follow each letter are part of the name (L*, a* and b*), since they determine the chromatic coordinates L, a, and b. The three parameters in the model represent: the luminosity (value) of color (L*; L* = 0 indicates black and L* = 100 white), and while a* designates its position between red and green (negative values indicate green while, positive values indicate red), b* indicates its position between yellow and blue (negative values indicate blue and positive values indicate yellow)[2,4]. The CIELab color model is three-dimensional because color can only be adequately represented in a space[5]. Over the years, simplified, agile, more or less reliable and reproducible color measurement methods have been developed through the optimization of traditional dental color guides, and the recent introduction of digital instruments[6,7].

The most popular method for color determination is visual, which is based on elements called visual color guides (VCG) that employs standardized colors. One of the most frequently used VCG in dental practice and by laboratory technicians is the VITA Classical guide (VITA Zahnfabrik, Bad Säckingen, Germany)[1,8]. The visual color guides only achieve an approximation to tooth color, and their shades are the product of average values obtained in population studies[9]. Since the first VCG in 1956 (Vita's Lumin Vacuum), different representations associated with different brands of ceramic and composite materials have been marketed with their respective tables of equivalences, such as Vitapan Classical and Vita 3D Master (VITA), Chromascop (Ivoclar-Vivadent), Shofu Vintage Halo (Shofu) and Dentsply EsthetX (Dentsply)[10-15]. Ahn and Lee reported that one of the main drawbacks of VCGs is that the optical properties of the materials with which they are constructed are different from those of dental tissues; and some commercial guides are even manufactured with materials different from the restorative material whose color they are intended to reproduce[16]: These conditions are associated with significant error margins. Another limitation, described by Tung et al., is that evidence has been found from studies using spectrography that teeth present nearly 300 different possible shades (located in a cluster in the spatial distribution of color according to three-dimensional systems such as Munsell and CIELAB), while visual shade guides only have sixteen (Vitapan Classical) to twenty eight (Shofu Vintage Halo) shades[17].

The visual identification of color depends on many factors, some subjective, such as the perception of the observer; some related to the environment, such as lighting conditions, and some related to other properties of the teeth, such as translucency, surface smoothness, brightness, fluorescence and opalescence[18].

Color measurement devices have the potential to improve selection accuracy and reliability by removing the observer factor from equation, and in some cases even the effect of the lightning conditions[19]. Devices used for clinical tooth color determination include spectrophotometers, colorimeters, and digital camera systems[20,21].

Spectrophotometers (SP) are among the most accurate and useful instruments for color determination in dentistry[22], measuring the amount of light energy reflected by an object throughout the visible spectrum[6,23]. An SP contains a source of optical radiation (a set of ultraviolet (UV), infrared (IR) and visible light (VIS) radiation that are not ionizing but do cause thermal or photochemical effects, a means to scatter light, an optical measuring system, a detector and a mechanism to convert the received light into a signal that can be analyzed. The measurements obtained are often translated into dental shade guides or expressed in the CIE[24,25] system and converted into tabs of equivalent colors

with a coincidence in 93.3% of the cases[5]. An example of a spectrophotometer used in dentistry is the VITA Easyshade (Vita Zahnfabrik, Bad Säckingen, Germany) with different measurement modes such as single tooth mode, tooth area mode (cervical, middle and incisal shades), restoration shade check (includes clarity, chroma and hue comparison) and color tab mode[26].

Colorimeters are tools that determine the hue for a more objective measurement of color from the CIE system[24,27]. To do this, they quantify the tristimulus values, filter the light in red, green and blue areas of the visible spectrum, do not register spectral reflectance and can therefore be less accurate than spectrophotometers[4]. On the other hand, digital cameras use an additive model in which the red, green, and blue lights are added together in various ways to reproduce a wide range of colors. Digital cameras are perhaps the most basic approach to instrumental tooth color acquisition, and still require a certain degree of subjective human shade selection[28].

Studies in which the visual evaluation of color was compared to the colorimetric evaluation detected wide variations in the results[29]. On the other hand, there is evidence that the spectrophotometric evaluation of tooth color is more accurate than the visual evaluation[5,30-35].

Several authors have reported on the different factors that affect the color of teeth, such as age and sex[36,37]. Gonzalo-Diaz et al.[31] reported elderly subjects with darker and yellower teeth[31,38], and women with lighter teeth than men[31,39]. Karaman et al. found that the value was higher for central incisors than for lateral incisors and canines, in both men and women[38]. When males and females were compared with the CIE L*, a* and b* dimensions, significant differences were only detected in the a* dimension. In the general distribution (without considering age and sex), A2 (29.7%) and A1 (12.9%) were more frequently found in the central incisors, while B1, C4 and D2 (0.5%) were the least frequent. The most outstanding points in the L* a* b* distribution were: a) the lowest value of a* was found in the upper central incisor; b) the highest value of L* was found in the upper central incisor and, c) the highest value of b* was found in the central and lateral incisors[40]. To date, no publication has been found on the prevalence of tooth colors in population groups in Argentina.

The aim of this study was to evaluate tooth color in dental students from the University of Buenos Aires, Argentina.

MATERIALS AND METHODS

The participants were 184 students (157 women and 27 men), with ages ranging from 21 to 33 years, mean age 24.45 (SD 2.79) years (Fig. 1). They were all in the fourth year of a dentistry degree at the University of Buenos Aires and agreed to participate through informed consent approved by resolution number 030/2019 of the Ethics Committee of the Dental School of the above mentioned University. The exclusion criteria were: a) the presence of total or partial peripheral restorations in maxillary central incisors; carious and non-carious lesions, pigmentations caused by fluorosis, tetracyclines and hypoplasia; b) the absence of 1.1 and c) having received a teeth whitening treatment within the previous 6 months.

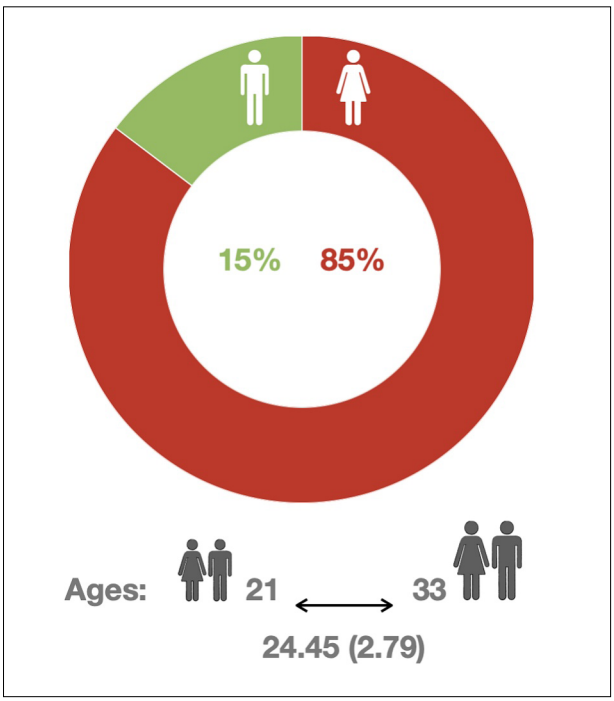

Fig. 1: Participants' gender and age distribution

Shade was measured in each subject in the middle third of the upper right central incisor (1.1), by the same observer, in the same dental chair (Sinol S2316), and time slot, with natural light and without using the dental chair light. Prior to determining color, the buccal aspect of the tooth to be examined was cleaned with a prophylaxis brush (TDV, Brazil) with low-speed rotary instruments Kavo 2068

CHC micromotor (Germany) and Kavo LUX K201 contra-angle (Germany). The VITA Easyshade V (VITA ES) spectrophotometer (Zahnfabrikn Bad Säckingen, Germany) was used, as proposed by Chu et al.[1] and according to the manufacturer's instructions, it was calibrated with the ad-hoc white tiles provided before taking each color sample. Probe protectors were used for each participant to avoid contamination and maintain biosafety standards. Color was determined by a single observer who examined each participant separately, without spandex, with the subject sitting on the dental chair in the most upright position, with the fiber-optic of the VITA ES positioned at 90º to the tooth surface. Two measurements were recorded in "single tooth" mode, and they were coincident with each other in all cases (Fig. 2). Rates in percentage and 95% confidence intervals were obtained for each VITA shade.

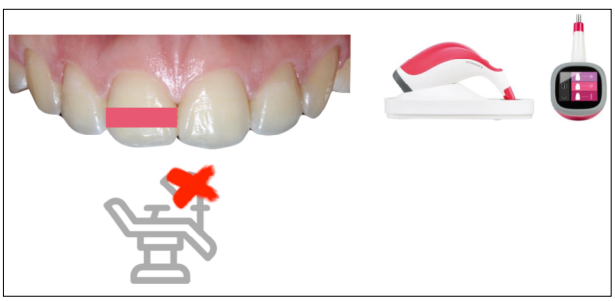

Fig. 2: Site and environmental conditions for color registration

Table 1. Frequency of shades in central incisors

Shades		Rate %	CI (95%)
A	A1	46.20	38.83 - 53.68
	A2	17.39	12.21 - 23.66
	A3	6.52	3.41 - 11.11
	A3.5	0.54	0.01 - 2.99
	A4	0.54	0.01 - 2.99
B	B1	4.35	1.90 - 8.39
	B2	17.39	12.21 - 23.66
	B3	2.17	0.60 - 5.47
C	C1	-	-
	C2	1.09	0.13 - 3.87
	C3	0.54	0.01 - 2.99
D	D1	-	-
	D2	2.72	0.89 - 6.23
	D3	0.54	0.01 - 2.99

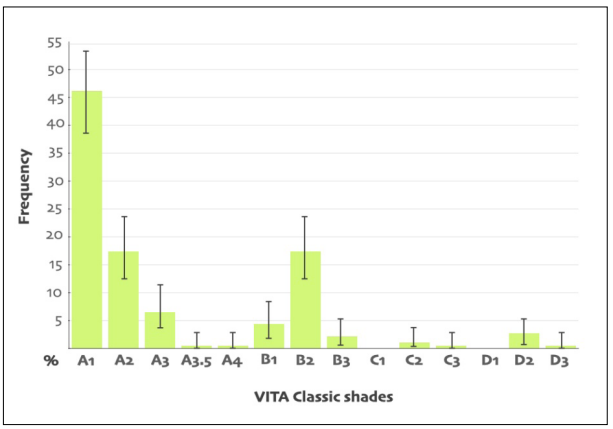

Fig.3: Frequency and Ci 95% of tooth color assessed with a spectrophotometer in fourth-year dental students from the University of Buenos Aires

RESULTS

The most frequently found color in central incisors of dental students from the University of Buenos Aires was A1 (46.2% CI 95%: 38.83 – 53.68). A2 and B2, each identified in 17.39% (CI 95%: 12.21 – 23.66) of the participants, shared the second place. Shades D1 and C1 were not found in any of the participants. Shade A3: 6.52% (CI 95%: 3.41-11.11) was more frequent than C2 1.09% (CI 95%: 0.13-3.87), D3, C3, A3.5 and A4: 0.54 (CI 95%:0.01-2.99), but shared the third place with B1: 4.35% (CI 95%:1.9-8.39), D2: 2.72% (CI 95%: 0.89 – 6.23) and B3: 2.17% (CI 95%: 0.60-5.47). See Table 1 and Fig. 3.

DISCUSSION

The aesthetic success of a restoration depends mainly on the reproduction of the natural shape of the tooth and its optical properties, such as color. This is more important when it involves an anterior tooth[15,35]. Different authors have assessed the prevalence of tooth colors in different populations (Fig. 4) and, in some cases, the effect of factors such as age and gender[34,35]. A2 and A1 were the most prevalent colors in studies on younger populations. In Turkey, Karaman et al. assessed tooth color of 202 subjects (89 men and 113 women) who attended the Elazig Oral Health Service, and whose ages ranged from 15 to 24 years, among whom the most frequent color was A2 (35.2%), followed by A1 (16.9%), while

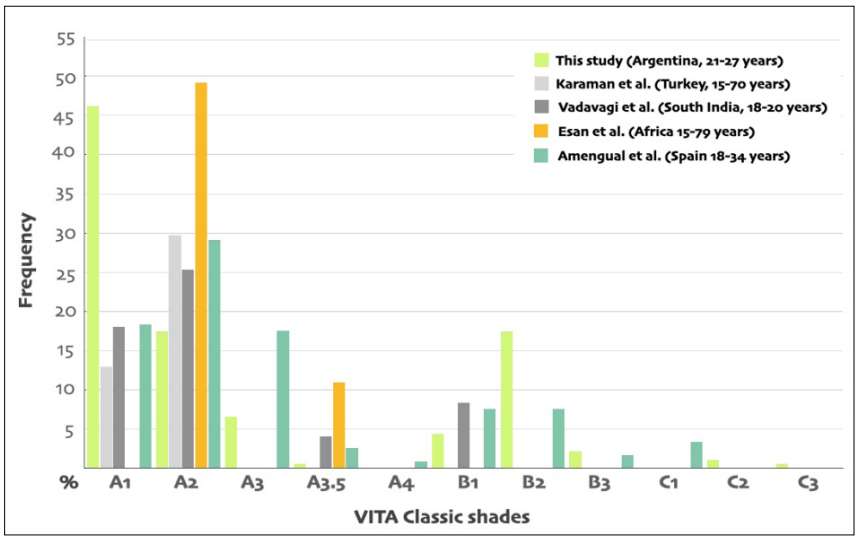

Fig. 4: Comparison of the frequencies of VITA Classic shades found in studies from different regions

B1, C4 and D2 (0.5%) were the least common[39]. In the Valencia community, in Spain, Amengual et al. evaluated, among other groups, residents aged 18 to 34 years and found that A2 (29.1%) was the most common color in both men and women, followed by A1 (18.3%) and A3 (17.5%), while C3, B3, B4 and D4 were not found in any subject[35].

In the present study, the most prevalent color was A1 (46.2%), followed by A2 and B2 (17.39%). The higher prevalence of lighter shades than in other studies may be due to three reasons: a) the high rate of women in the study population, b) the 21 to 33 year age range of the participants, and c) the fact that all participants were students of dentistry that may be a source of bias in terms of self-care or higher levels of buccal health information. This might be the case because both Vadavagi et al., in southern India[32], and Alrifai et al. in Lubli, Poland[33] worked on dental student populations, with ages between 18 and 20 years, and 19 and 32 years, respectively. In the first case, A2 was present in 34 % of subjects, A1 in 27.3 %, B1:15.3 %, B2: 8.7 %, C1: 8 % and A3.5: 6.7 %, and the authors observed that there were significant differences in prevalence of tooth color between sexes. Shade A1 was found in 27.3 % of females, while A2 was more often detected in males (42 %). In subjects with fair skin, the most prevalent color was A1 (52.3%), followed by A2 (29.7%)[32]. The study by Alrifai et al. reported that the most prevalent shade in central incisors was A2, followed by D3, C1 and A1. When participants were grouped by nationality, they found that the Polish population had lighter teeth shades than the Saudis and the Taiwanese, and when they were grouped according to gender, males seemed to have darker shades than females[33].

CONCLUSION

Within the limitations of this study, A1 was the most prevalent shade in central incisors of the dental students in evaluated Argentina, followed by A2 and B2, which shows a lighter color predominance compared to studies from other regions.

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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Root canal morphology of 1316 premolars from Brazilian individuals: an *in vivo* analysis using cone-beam computed tomography

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ABSTRACT

The aim of this study was to investigate the internal root canal anatomy of maxillary and mandibular premolars in a Brazilian subpopulation, in order to establish the prevalence of the different configurations proposed by Vertucci. Three hundred and ninety-eight cone-beam computed tomography scans were collected from a private imaging clinic database in Rio de Janeiro, including 217 maxillary and 226 mandibular scans. A total 1316 premolars (594 maxillary and 722 mandibular) were evaluated using an image viewer, and classified according to Vertucci. Two calibrated examiners determined the frequency of each morphological Type. A third examiner reviewed discordant cases. The Kappa test was applied to verify inter-rater agreement, and Fisher's Exact Test to verify gender-related differences. The most frequent root canal configurations of maxillary first and second premolars were Type IV (73.86%) and Type I (47.18%), respectively. Type I was the most prevalent in mandibular first and second premolars (80.59% and 95.86%, respectively). Only Types I and VIII presented a statistically significant difference between sexes. Type I was more frequent in females and Type VIII in males. A highly significant frequency of Type I was found in both mandibular first and second premolars, whereas the most frequent maxillary premolar root canal configuration was Type IV for first premolars and Type I for second premolars.

Keywords: *anatomy - cone-beam computed tomography - premolars - root canal.*

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Morfologia do canal radicular de 1.316 pré-molares de indivíduos brasileiros: análise *in vivo* usando tomografia computadorizada de feixe cônico

RESUMO

O presente estudo teve como objetivo investigar a anatomia interna de pré-molares superiores e inferiores em uma subpopulação brasileira para estabelecer a prevalência das diferentes configurações propostas por Vertucci. Trezentos e noventa e oito exames de tomografia computadorizada de feixe cônico foram coletados de um banco de dados de uma clínica privada de imagem no Rio de Janeiro, incluindo 217 exames maxilares e 226 mandibulares. Um total de 1.316 pré-molares (594 superiores e 722 inferiores) foram avaliados usando um visualizador de imagens e categorizados de acordo com a classificação de Vertucci. Dois examinadores calibrados determinaram a frequência de cada tipo morfológico. Um terceiro examinador revisou os casos discordantes. O teste Kappa foi aplicado para verificar a concordância entre os avaliadores e o Teste Exato de Fisher para verificar diferenças relacionadas ao gênero. A configuração do canal radicular mais frequente dos primeiros e segundos pré-molares superiores foi Tipo IV (73,86%) e Tipo I (47,18%), respectivamente. O tipo I foi o mais prevalente nos primeiros e segundos pré-molares inferiores (80,59% e 95,86%, respectivamente). Os tipos I e VIII foram os únicos que apresentaram diferença estatisticamente significativa entre os sexos. O tipo I foi mais frequente nas mulheres e o tipo VIII nos homens. Uma frequência altamente significativa do Tipo I foi encontrada em ambos os primeiros e segundos pré-molares inferiores, enquanto a configuração do canal radicular dos pré-molares superiores mais frequente foi o Tipo IV para os primeiros pré-molares e o Tipo I para os segundos pré-molares.

Palavras-chave: *anatomia - tomografia computadorizada de feixe cônico - pré-molares - canal radicular.*

INTRODUCTION

The root canal system (RCS) is complex and there is significant variability among dental groups, individuals, and different teeth in the same individual[1]. Lack of knowledge of root canal anatomy can lead to failure in endodontic treatment that may require maintenance or cause the onset of periradicular disease[2].

The literature is unanimous in stating that radiographic investigation is essential for root canal treatment. However, images obtained by digital or conventional radiography are restricted to two dimensions, so the anatomy of a three-dimensional region is analyzed in a two-dimensional image, with frequent overlapping of images. These limitations may lead to misinterpretation and consequently, misdiagnosis[3].

To compensate for this deficiency, computational methods were introduced in the field of medical and dental radiology, culminating in the advent of cone-beam computed tomography (CBCT) followed by micro-computed tomography (micro-CT). CBCT is a non-destructive method that enables three-dimensional and individual evaluation of a tooth, revealing peculiarities that are imperceptible in conventional radiographic images[1].

According to Martins et al.[4], premolars and molars have significant anatomical variability. The apparent simple external root canal configuration of single-rooted or two-rooted premolars may lead to the assumption of simple internal anatomy. However, research reveals the opposite: complex internal anatomy, with up to eight different canal configurations[5,6]. These teeth therefore pose a challenge, especially regarding the high occurrence of flattened canals, accessory canals, and isthmuses[1,6].

In addition, studies have reported that dental anatomy is genetically determined, varying according to ethnicity[2,6,7]. However, most of the published studies are based on Caucasian populations, and are therefore not applicable to Brazil, where the population is heterogeneous and miscegenated[8]. Studies of this kind in the Brazilian population could therefore contribute directly to knowledge of dental anatomy, and indirectly to the success of endodontic treatments, particularly for this group of teeth.

Based on the literature, the most probable hypothesis is that there would be extensive variability in the morphology of premolar root canals in the Brazilian population. Therefore, the aim of the present study was to analyze the internal root canal configurations of maxillary and mandibular premolars in a portion of the Brazilian population, establishing the frequency of the different morphological types proposed by Vertucci[5].

MATERIALS AND METHODS

A total 1,021 CBCT scans performed on male and female patients were analyzed. The scans were obtained from a private oral radiology clinic in Rio de Janeiro. All healthy or coronally restored maxillary and permanent mandibular premolars with fully formed root apices were included. Endodontically treated teeth, teeth with prosthetic restorations and intraradicular retainers, teeth with incomplete root formation, root resorption, root calcification, or images with artifacts that prevented correct evaluation were excluded. The CT scans were performed from January to March 2015 and acquired using the iCAT Classic unit (IMAGING SCIENCES, Hatfield, USA, 0.2mm voxel size, 120kv, 6-cm FOV (field of view), as suggested by other studies. All CBCT scans were performed with the minimum exposure required for adequate image quality. The ALARA (as low as reasonably achievable) protocol was strictly followed.

CBCT scanning indications included assessing bone volume for dental implant planning, diagnosis of dentoalveolar trauma, management of impacted teeth before orthodontic treatment, and treatment planning before nonsurgical and surgical endodontic treatment. This study was carried out after approval by the Research Ethics Committee of the Health Sciences Center of Estacio de Sá University (protocol number: 64659816.3.0000.5284). All patients provided written informed consent to participate. Following data protection of minors, the Institutional Research Ethics Committee does not allow the use of CBCT data from persons younger than 18 years, so only CBCT exams from individuals over 18 years of age were included.

After applying the exclusion criteria, a total 398 CT scans were analyzed using the Dental Slice software version 2.8, for Windows (2014, Bioparts Prototyping Biomedica, Brasília, DF, Brazil). The contrast and brightness of the images were modified when necessary to ensure optimal viewing. The images were evaluated from three planes: cross-sectional, axial and panoramic, using the space between the 1-mm cross-sections (Fig. 1a, 1b and

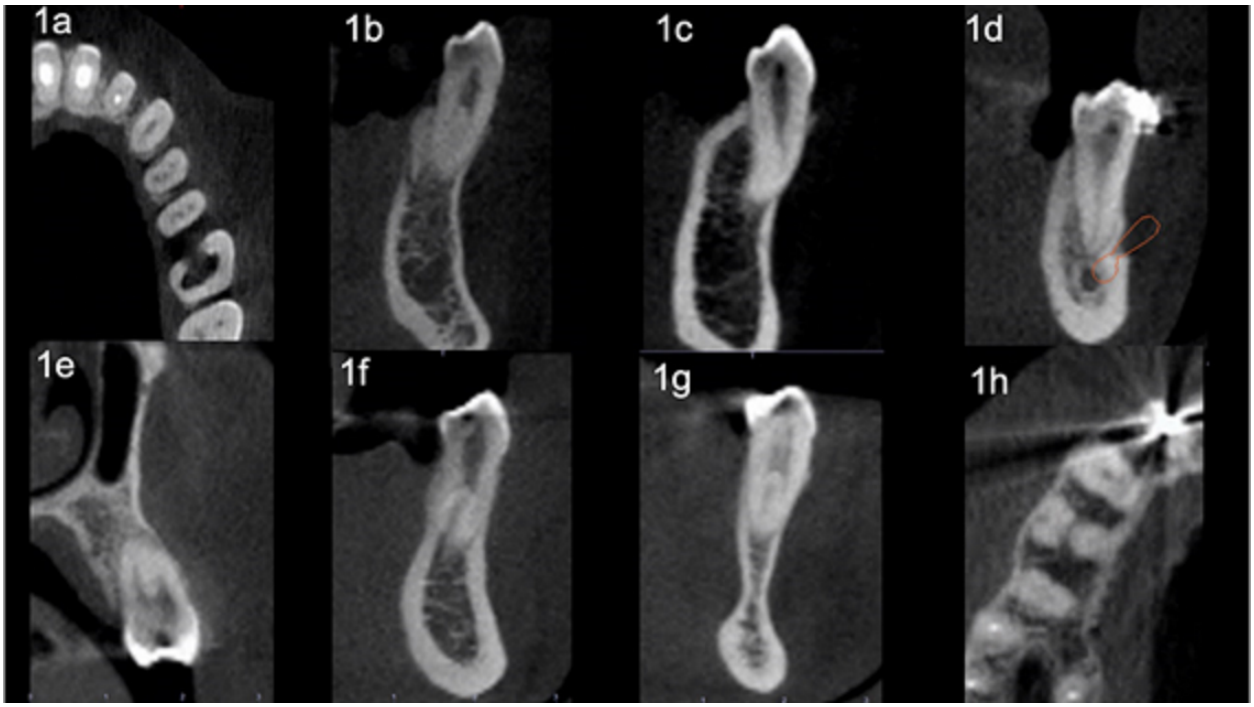

Fig. 1: Examples of different root canal configurations of maxillary and mandibular premolars. (1a) CBCT axial reconstructions showing different endodontic anatomy. (1b) CBCT sagittal reconstructions of a mandibular premolar showing Vertucci type V classification. (1c) CBCT sagittal reconstructions of a mandibular premolar showing Vertucci type V classification. (1d) CBCT sagittal reconstructions of a mandibular premolar showing Vertucci type I classification. (Red line showing the mandibular canal location). (1e) CBCT sagittal reconstructions of a maxillary premolar showing Vertucci type IV classification. (1f) CBCT sagittal reconstructions of a mandibular premolar showing Vertucci type V classification. (1g) CBCT sagittal reconstructions of a mandibular premolar showing Vertucci Type III classification. (1h) CBCT axial reconstructions of maxillary premolar showing Vertucci type VIII classification.

1c). They were then classified according to the eight morphological root canal configurations proposed by Vertucci[9].

The analysis was performed independently and randomly by two endodontists, one with a degree in dental radiology. The third examiner, also an endodontist, analyzed and decided the discordant cases.

Statistical analysis

All teeth were classified according to their internal anatomy, and the data were recorded on a spreadsheet. The first 300 teeth were used to assess inter-examiner reliability using the Kappa test, which revealed substantial agreement (0.679).

Data were then divided into four dental groups: maxillary first premolars, maxillary second premolars, mandibular first premolars, and mandibular second premolars, and the frequency of each root canal configuration was calculated for each group. Fisher's test was used to verify differences between sexes.

RESULTS

Descriptive data

The study included 398 CT scans, of which 217 were of the maxilla and 226 of the mandibles. The root canal configuration of 1316 premolars were analyzed. Of these, 310 images were of maxillary first premolars and 284 of maxillary second premolars; 407 images were of mandibular first premolars and 315 of mandibular second premolars. There were 501 pairs of contralateral teeth; of these, 443 had the same root canal configuration, and 58 had different root canal configurations.

Frequency of each root canal configuration

Table 1 summarizes the frequency of the root canal configuration of each group of premolars. The most frequent canal configuration was Type I (59%) (Fig. 1d), followed by Type IV (23%) (Fig. 1e), Type II (10%), Type V (6%) (Fig. 1b, 1c and 1f), Type III (2%) (Fig. 1g), and Type VIII (Fig. 1h). No teeth were classified as Types VI or VII (Table 1).

Table 1. Frequency distribution of root canal configurations according to Vertucci's classification (1984)

Tooth No	N	I (%)	II (%)	III (%)	IV (%)	V (%)	VI (%)	VII (%)	VIII (%)
14	156	5 (3)	27 (17)	-	119 (76)	1 (1)	-	-	4 (3)
24	154	6 (4)	34 (22)	1 (1)	110 (71)	-	-	-	3 (2)
15	140	59 (42)	37 (26)	6 (4)	35 (25)	3 (2)	-	-	-
25	144	75 (52)	31 (22)	2 (1)	35 (24)	1 (1)	-	-	-
34	196	159 (81)	-	6 (3)	-	30 (15)	-	-	1 (1)
44	211	169 (80)	1 (0)	4 (2)	-	36 (17)	-	-	1 (0)
35	162	155 (96)	1 (1)	1 (1)	-	5 (3)	-	-	-
45	153	147 (96)	1 (1)	-	-	4 (3)	-	-	1 (1)
Total	**1316**	**775 (59)**	**132 (10)**	**20 (2)**	**299 (23)**	**80 (6)**	**0**	**0**	10 (1)

Table 2. Frequency of distribution of the root canal configurations according to Vertucci's classification according to sex

		Morphological type						Total
		I	II	III	IV	V	VIII	
Sex	F	506	63	11	149	40	1	770
	M	269	69	9	150	40	9	546
Total		775	132	20	299	80	10	1316
p-value*		0.01	0.54	0.75	1.00	1.00	0.01	-

* P-value obtained by Fisher's exact test

Among the maxillary first premolars, the most frequent canal configuration was Type IV (76% were teeth No. 14 and 71% were teeth No. 24). The second most frequent canal configuration was Type II (17% were maxillary right premolars and 22% were mandibular left premolars) (Table 1).

The most frequent maxillary second premolar root configuration was Type I (42% were teeth No. 15 and 52% were teeth No. 25). The second most frequent maxillary right premolar root configuration was Type II (26%), while the most frequent maxillary left premolar root configuration was Type IV (24%) (Table 1).

The most frequent root canal configuration among the mandibular premolars was Type I (teeth No. 34 (81%), teeth No. 44 (80%), teeth No. 35 (96%), and teeth No. 45 (96%) (Table 1).

Upon completing the analyses, the Kappa test was repeated for all teeth evaluated (n = 1316), and the result was higher than the one obtained during the calibration phase (0.692), representing substantial agreement.

Table 2 shows the frequency of distribution of the root canal configurations according to sex. Only Type II and VIII root canal configurations presented a significant difference between sexes (p = 0.001).

DISCUSSION

The complexity of root canal treatment is directly associated with the number of root canals, bifurcations, anastomoses and isthmuses. Knowing and understanding these possible variations reduces the probability of leaving canals untreated, and increases the success rate of endodontic treatment[4,10,11].

Ethnicity predisposes to root canal variations[2,7], but it is not easy to classify the Brazilian population because of its significant heterogeneity[12]. Few studies in the current literature have analyzed the root canal anatomy from posterior teeth in the Brazilian population, and most of these only evaluated molars[8,12-14]. Studies of premolar anatomy in the Brazilian population are scarce compared to studies in other populations[2,15-17].

The study by Pécora et al.[18] evaluated the morphology of premolars in Brazilian individuals. However, the authors did not use Vertucci's classification[5], and only classified the number of root canals. One of the noteworthy aspects of the present study is that the sample was evaluated independently by two experienced endodontists, and a third examiner resolved conflicting cases. This methodology was the same as the one used in previous studies[2,19].

Nevertheless, in other studies[1, 20], only two examiners evaluated the images, and discussed any conflicting cases until they reached a consensus. There are also studies[4,21] in which only one examiner evaluated the images, increasing the probability of failure.

Although the percentages may vary, the most frequent root canal configuration of maxillary first premolars was Type IV (73.87%), which agrees with many previous studies[4,5,15,20,22,23]. A study in the Turkish population[24] found divergent results, with the most frequent root canal configuration being Type I (62.6%).

In the present study, the most frequent maxillary second premolar root canal configuration was Type I (47.18%), in agreement with other studies[1,4,9,15,23-25]. Among mandibular first and second premolars, the most frequent root canal configuration was Type I, which agrees with studies in other populations[2,4,10,19,21-26]. Similarly to many previous studies[2,4,9,15,19,21,23-26], the present study did not identify any root canals of Type VI and VII configuration. One of the limitations of this study is related to the use of CBCT, which cannot provide images as detailed as can micro-CT[15]. On the other hand, micro-CT cannot be used in in vivo studies. Nonetheless, CBCT has been validated in the literature as a reliable, non-invasive method for evaluating highly complex cases[3]. Further studies are needed to understand the morphology of root canal configuration in the Brazilian population.

CONCLUSION

It may be concluded that the morphology of the root canal system of premolars in the Brazilian population is as follows: (1) the most frequent root canal configurations found for the maxillary first and second premolars were Type IV and Type I, respectively; (2) a highly significant frequency of Type I configuration was found for the mandibular first and second premolars; and (3) morphology of the root canal system of premolars in the Brazilian population does not differ substantially from other populations (American, Turkish, Iranian, Chinese, Egyptian, Spanish, Arabic, and Portuguese), even though it is a heterogeneous population.

DECLARATION OF CONFLICTING INTERESTS

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

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Bulk-fill restorative composites under simulated carious and erosive conditions

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ABSTRACT

Acidic conditions can cause hydrolysis and accelerate degradation of resin composites (RCs). Since there are limited and controversial data on the effect of acids on bulk-fill RCs, this study assessed the surface roughness (SR) and flexural strength (FS) of these RCs under simulated carious and erosion conditions. Bars of Filtek Bulk Fill (FBF, 3M/ESPE), X-tra fil (XTF, Voco), Tetric N-Ceram Bulk Fill (TBF, Ivoclar/Vivadent), and Aura Bulk Fill (ABF, SDI) and a conventional RC [Filtek Z350XT (FZ, 3M/ESPE)] were allocated (n=15) to undergo caries or erosion conditions. The control group was kept in artificial saliva (AS). The bars were evaluated for SR change (final-baseline) and for three-point FS. Data were analyzed using ANOVA and Tukey's test. At the baseline ($p < 0.001$), the SR of RCs ranked as follows: (TBF = XTF) < FBF (none differed from FZ) < ABF. The interplay between RCs and conditions affected SR change ($p = 0.025$). While after storage in AS, there was no difference among RCs, following carious and erosive conditions, ABF showed higher SR change. For FS ($p < 0.001$), XTF > (FBF = FZ) > (TBF = FZ) > ABF, with no difference among control, carious and erosive conditions ($p = 0.148$). Depending on the restorative bulk-fill RCs, carious and erosive conditions roughen the surface but do not affect the FS of these materials.

Keywords: *composite resins - acids - dental caries - tooth erosion.*

Resinas compostas restauradoras bulk-fill sob condições simuladas de cárie e erosão

RESUMO

Condições acídicas podem causar hidrólise e acelerar a degradação de resinas compostas (RCs). Como há dados limitados e controversos sobre os efeitos de ácidos sobre RCs bulk-fill, este estudo avaliou a rugosidade de superfície (RS) e a resistência flexural (RF) dessas RCs sob condições simuladas de cárie e erosão. Barras de Filtek Bulk Fill (FBF, 3M/ESPE), X-tra fil (XTF, Voco), Tetric N-Ceram Bulk Fill (TBF, Ivoclar/Vivadent) e Aura Bulk Fill (ABF, SDI) e de uma RC convencional [Filtek Z350XT (FZ, 3M/ESPE)] foram alocadas (n=15) para estarem sob condições cariogênicas ou erosivas. O grupo controle foi mantido em saliva artificial saliva (SA). As barras foram avaliadas quanto à alteração de RS (final-inicial) e à RF de três pontos. Os dados foram analisados utilizando ANOVA e teste de Tukey. Inicialmente ($p < 0,001$) a RS das RCs foi a seguinte: (TBF = XTF) < FBF (nenhuma diferiu de FZ) < ABF. A interação entre as RCs e as condições acídicas influenciou a alteração de RS ($p = 0,025$). Após armazenamento na SA, não houve diferença entre as RCs, enquanto após condições cariogênicas e erosivas a ABF mostrou a maior alteração de RS. Para FS ($p < 0,001$), XTF > (FBF = FZ) > (TBF = FZ) >ABF, sem diferença entre controle e condições cariogênicas e erosivas ($p = 0,148$). Dependendo da RC bulk-fill restauradora, condições cariogênicas e erosivas aumentam a RS, mas não alteram a RF desses materiais.

Palavras-chave: *resinas compostas - ácidos - cárie dentária - erosão dentária.*

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INTRODUCTION

Resin composites are widely used due to their direct filling capability, minimally invasive nature, esthetics, and clinical performance[1]. A significant concern when placing a resin restoration is reducing polymerization shrinkage stress. One method to do so is to layer the resin composite incrementally[2]. However, since its efficiency in mitigating deleterious effects at the adhesive interface has been questioned[3], the bulk-filling technique has become more widely used following the development of materials with less shrinkage, polymerization stress and cusp deflection[4]. Such benefits have been attributed mainly to the increased translucency and the modification of the resin matrix, or photo-initiator dynamics[5].

Although previous meta-analyses have reported that bulk-fill restorations present survival rates and clinical performance similar to those of conventional composites[6,7] in contact with erosive drinks, there is evidence showing that physical and mechanical properties of bulk-fill resin composites are more negatively influenced than a conventional counterpart[8]. On the other hand, when the acid is of cariogenic origin, bulk-fill and conventional resin composites do not seem differ in degradation[9,10].

One reason for the acid-dependent behavior of bulk-fill resin composites may be the lower pH of erosive acids in comparison to cariogenic acids. This explanation is supported by the fact that hydrolysis can speed up under more acidic conditions, as pH affects reaction rates through catalysis[11,12]. Low pH solutions can act on the polymeric matrix of composites through catalysis of ester groups from dimethacrylate monomers present in their compositions (Bis-GMA, Bis-EMA, UDMA and TEGDMA)[13]. The hydrolysis of these ester groups can form alcohol and carboxylic acid molecules that may accelerate degradation of the resin composites due to a lowering of the pH within the resin matrix[13]. In addition, low pH solutions may also cause erosion of inorganic fillers and thereby their debonding[13].

Despite these possibilities, to the best of the authors' knowledge, no previous study has compared the effect of erosive and cariogenic acids on the physical and mechanical properties of bulk-fill resin composites. Thus, the aim of this study was to compare the surface roughness and the flexural strength of various bulk-fill restorative composites under simulated carious and erosive conditions.

MATERIALS AND METHODS

Experimental design and sample size calculation

This study followed a 5x3 factorial design, using five resin composites (one conventional: Filtek Z350 XT and four bulk-fill: Filtek Bulk Fill, X-tra fil, Tetric N-Ceram Bulk Fill and Aura Bulk fill, as shown in Table 1), and three storage conditions (carious, erosive, control), comprising 15 groups.

Sample size was calculated (G*Power 3.1.9.4, Heinrich-Heine Düsseldorf University, Düsseldorf, Germany) based on preliminary data collected from three samples per group, from which an effect size of 0.27 was obtained. At $\alpha = 0.05$ and a power of 0.80, a total sample size of 215 resin composite bars would be needed. Based on the 15 groups of this study, 15 specimens per group were required.

The dependent variables were: 1) average surface roughness (Ra, in μm), measured at baseline and after storage in the allocated conditions; and 2) three-point flexural strength (in MPa). Scanning electron microscopy (SEM) images were obtained to illustrate the changes that occurred.

Specimen preparation

For each resin composite (Table 1), 45 bars were prepared in a PTFE split mold (length: 12 mm; height and width: 2 mm). The mold was filled with the resin composite and covered with a polyester strip. The resin composite was made flush with the mold by use of glass slide and a 500-g axial load applied for 60 s. Specimens were light-cured for the time recommended by each manufacturer at three different locations along the top bar length with a LED curing unit (Radii-cal, Victoria, Australia, light power density: 950 mW/cm^2). A LED curing unit was used based on a previous paper that showed that it improved the mechanical properties of Filtek Bulk Fill[14]. After removal of the polyester strip, the bar was retrieved from the mold and stored (24 h, 37 °C, 100% relative humidity).

Storage of the bars under carious and erosive conditions

The bars of each resin composite were randomly allocated into three groups (n = 15), to be stored under carious, erosive or control conditions, as follows:

- *Acidic condition simulating caries:* bars were stored in demineralizing solution (pH 4.3)[15]. The solution contained 2.0 mM calcium, 2.0

Table 1. Characterization of the resin composites tested

Resin composite	Composition and filler loading	Shade	Manufacturer/ Batch number
Filtek Z350 XT *(conventional resin composite)*	Bis-GMA, UDMA, TEGDMA, Bis-EMA, Polyethylene glycol dimethacrylate, silane treated ceramic, silane treated silica, and silane treated zirconia 78.5% (by weight) / 58.5% (by volume)	A2	3M ESPE, St. Paul, MN, USA Batch number: 424453
Filtek Bulk Fill *(bulk-fill resin composite)*	Bis-GMA, Bis-EMA, UDMA, TEGDMA, polyacrilic resin, silica, zirconia, and zirconia/silica agglomerates 76.5% (by weight) / 58.4% (by volume)	A2	3M ESPE, St. Paul, MN, USA Batch number: 685666
X-tra Fil *(bulk-fill resin composite)*	Bis-GMA, UDMA, TEGDMA, barium aluminum silicate vitreous particles 86.0% (by weight) / 70.1% (by volume)	U	Voco Cuxhaven, Germany Batch number: 1514217
Tetric N-Ceram Bulk Fill *(bulk-fill resin composite)*	Bis-GMA, UDMA, Bis-EMA, mixed oxides, barium glass, isofillers, and ytterbium trifluoride 77.0% (by weight) / 55.0% (by volume)	IVA	Ivoclar Vivadent Schaan, Liechtenstein Batch number: 03089/03
Aura Bulk Fill *(bulk-fill resin composite)*	Bis-GMA, UDMA, silica, barium glass. 65.0% (by weight) / 81.0% (by volume)	BKF	SDI, Bayswater, Victoria, Australia Batch number: 150931

Bis-GMA, bisphenol A glycidyl methacrylate; UDMA, urethane dimethacrylate; TEGDMA, triethylene glycol dimethacrylate; Bis-EMA, ethoxylated bisphenol A glycol dimethacrylate.

mM phosphate, 0.03 ppm F, and 75 mM acetate buffer. Storage time was based on a report that resin-based materials soften after 14 days of 10 daily cariogenic challenges[16]. Since the decrease of saliva pH is below 5.5 for approximately 45 minutes, in order to simulate a total 14 days, the resin composite bars were stored individually in 1.0 mL of the demineralizing solution for 6,300 minutes (45 minutes for each one of the 10 cariogenic episodes over 14 days). During the storage time, the bars were kept in an oven at 37 °C. The demineralizing solution was renewed daily.

- *Acidic condition simulating erosion:* bars allocated to this storage condition were immersed in a 0.05 M citric acid solution (pH 2.3), commonly used in erosion models as a source of exogenous acid. To make the conditions of carious and erosive conditions equivalent, the bars in the erosion group were also exposed to 140 erosive episodes. Since salivary pH remains below the baseline value for 90 s after the intake of a citric-containing beverage[17], the bars were stored individually in 1.0 mL of the citric acid solution for 210 min (90 s for each one of the 10 erosive episodes over 14 days). Bars remained stored in an oven at 37 °C and erosive solution was renewed on a daily basis.
- *Control condition:* control group bars were stored in 1.0 mL of artificial saliva containing $MgCl_2$, NaCl, KCl, and $CaCl_2$ at 37 °C, for the same time used in the carious condition. As for the other two groups, the bars were stored in an oven at 37 °C and the immersion medium was renewed daily.

Measurement of baseline and final surface roughness

The top surface of each resin composite bar was evaluated using a surface roughness tester (Surftest SJ-210, Mitutoyo, Japan) at three different random locations, before and after storing the bars in their respective media. The cutoff value was set at 0.25 mm. Surface roughness was measured using the mean arithmetic deviation of the profile (Ra, in µm). The three values obtained at each time point were averaged and recorded as baseline and final Ra values, which were used to calculate the Ra change (ΔRa = final - baseline). Positive values indicate increase in surface roughness.

Flexural strength

After the storage period, each bar was positioned on a three-point bending apparatus with a span length of 20.0 mm. Each bar then underwent three-point bending test using a universal testing machine (Emic DL2000, São José dos Pinhais, Brazil) at a crosshead speed of 1 mm/min. The flexural strength was determined according to the following equation: σ=3PL/[(2wb)]^2, where "P" is the maximum load (in Newtons); "L" is the distance between the two supports (in mm); "w" is the bar width (mm) and "b" is the bar height (mm).

Scanning electron microscopy (SEM)

Bars from each group were sputter-coated and imaged at 1,000x- magnification using a scanning electron microscope (TM3030, Hitachi Ltd, Japan) to illustrate the surface micromorphology of each resin composite after being stored in carious or erosive conditions and artificial saliva.

Statistical analysis

Bulk-fill resin composites were compared for their baseline surface roughness using one-way analysis of variance (ANOVA) and Tukey's test. The interplay between the resin composites and storage conditions on the surface roughness change (ΔRa) and flexural strength was investigated using two-way ANOVA and Tukey's tests. IBM SPSS software (version 23, SPSS Inc., Chicago, IL, USA) was used for all statistical calculations. The significance level was set at 5%.

RESULTS

One-way ANOVA indicated that prior to storing the bars under different conditions, there were statistically significant differences among the resin composites ($p < 0.001$). Tetric N-Ceram and X-tra fil were significantly smoother than Filtek Bulk Fill and Aura Bulk Fill. Except for Aura Bulk Fill, which presented the highest surface roughness, none of the bulk-fill composites differed significantly from the conventional counterpart (Table 2).

Two-way ANOVA showed a significant interaction between the resin composites and storage conditions ($p = 0.025$) for surface roughness change (ΔRa). As found by Tukey's test, there was no statistically significant difference among resin composites when they were stored in artificial saliva (control). Under either carious or erosive conditions, the composite Aura Bulk Fill showed higher ΔRa than the other resin composites, which did not differ from each other (Table 2). Tukey's test also indicated that for the composites Filtek Bulk Fill, Tetric N-Ceram Bulk Fill and Filtek Z350 XT, the carious and erosive conditions did not pose increased roughness changes in relation to those caused by artificial saliva. For Aura Bulk Fill, however, both acidic conditions (carious and erosive) resulted in higher surface roughness change.

For the flexural strength data (Table 3), there was a statistically significant difference among resin composites ($p < 0.001$), with X-tra fil providing higher values than Filtek Bulk Fill, which presented higher flexural strength than Tetric N-Ceram Bulk Fill. The latter two bulk-fill resin composites did not differ from the conventional resin composite Filtek Z350 XT. Aura Bulk Fill had the lowest flexural strength values. The storage conditions did not significantly influence the flexural strength of the tested resin composites ($p = 0.148$).

The photomicrographs in Fig. 1 show a smooth surface and similar micromorphology for Filtek Z350 XT and Filtek Bulk Fill when comparing the bars stored in artificial saliva (1A and 1D), carious (1B and 1E) and erosive conditions (1C and 1F). X-tra fil (1G, 1H and 1I) presented detachment of some filler particles (line arrows), and bars stored under carious and erosive conditions (1H and 1I) had a rougher surface than the control group (artificial saliva). The Tetric N-Ceram Bulk Fill composite resin subjected to erosive condition (1L) presented filler particles exposed on the surface with irregularities (right-pointing double arrow). The Aura Bulk Fill composite resin displayed marked surface damage as a consequence of carious and erosive conditions (right-pointing thick arrow, 1N and 1O).

Table 2. Mean (SD) of baseline and increase in surface roughness (ΔRa = final – baseline) according to the resin composite and storage condition

Resin composite	Baseline Ra	ΔRa (roughness increase)		
		Artificial saliva	Cariogenic condition	Erosive condition
Filtek Z350 XT	0.129 AB (0.114)	0.008 Aa (0.013)	0.014 Aa (0.020)	0.013 Aa (0.009)
Filtek Bulk Fill	0.170 B (0.109)	0.012 Aa (0.008)	0.012 Aa (0.013)	0.017 Aa (0.010)
X-tra fil	0.088 A (0.098)	0.004 Aa (0.007)	0.016 Aa (0.020)	0.011 Aa (0.007)
Tetric N-Ceram Bulk Fill	0.083 A (0.068)	0.005 Aa (0.010)	0.008 Aa (0.006)	0.009 Aa (0.008)
Aura Bulk Fill	0.375 C (0.180)	0.007 Aa (0.011)	0.028 Bb (0.021)	0.032 Bb (0.022)
Grand mean	—	—	—	—

At the baseline, composite resins indicated by different uppercase letters differ from each other. For ΔRa, means followed by different uppercase letters indicate difference between resin composites (comparisons within each column), while lowercase letters indicate difference between storage conditions (comparisons within each row).

DISCUSSION

The main clinical benefits associated with bulk-fill resin composites are reduction in polymerization stress[18], shorter working time, and polymerization of increments up to 4-5-mm thick[19]. Notwithstanding these advantages over conventional resin composites, some bulk-fill composites degrade in the oral cavity as much as[9,10] or more[8] than conventional composites. This performance of bulk-fill composites seems to depend on the conditions to which they are exposed and/or the composition/brand of these restorative materials. Such speculations are supported by 1) studies that used acids associated with caries episodes, which did not affect physical and mechanical properties of some bulk-fill composites[9,10] and 2) a study that found that erosive beverages harmed the surface of bulk-fill composites[8]. However, to date, no research has been published on whether erosive and cariogenic acids, whose strengths are different, would affect bulk-fill resin composites differently. Thus, the current paper compares the effect of erosive and cariogenic acids on physical and mechanical properties of varying bulk-fill composites.

The findings of our study showed that both acidic solutions similarly increased the surface roughness of all composites, but the damage caused by cariogenic and erosive acids surpassed that caused by artificial saliva only for Aura Bulk Fill. This suggests that the behavior of bulk-fill composites may be more likely attributable to the differing chemistry of the monomeric resin formulations and filler characteristics (type, volume fraction, density and particle size and distribution)[20] as well as the filler-resin interface of the bulk-fill resin composite itself, than to the pH of the storage solution. Thus, even though the erosive solution had a substantially lower pH (2.3) than the cariogenic solution (4.3) and could potentially speed up hydrolysis and degradation of resin-based materials, such pH discrepancy did not cause detectable differences in the composite's surface roughness measured through the Ra parameter. However, as can be seem in Fig. 1 F, I, L and O, there was a trend toward the presence of increased irregularities, voids and cracks when the bulk-fill composites were under erosive conditions. Such qualitative evidence may be due not only to potentially accelerated hydrolysis by the low pH erosive solution, but also by erosion and debonding of inorganic fillers[13].

It is worth mentioning that among the resin composites tested in this study, Aura Bulk Fill had the lowest filler content by weight and thereby the highest resin matrix content. This may have accounted not

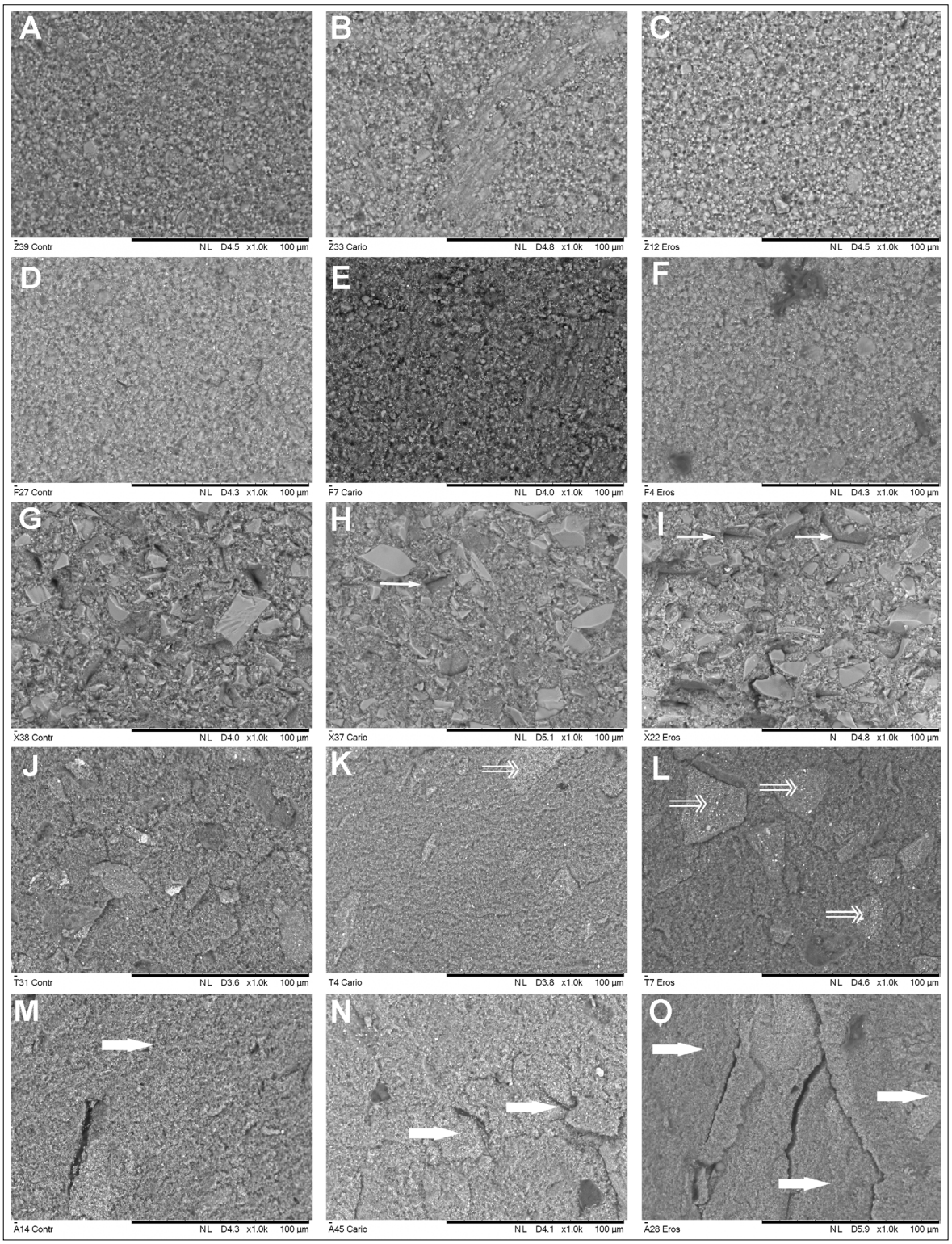

Fig. 1: Photomicrographs of restorative bulk-fill and conventional composites after storage in conditions of carious or erosive challenges or in artificial saliva.

A, B, and C) Filtek Z350 XT; D, E, and F) Filtek Bulk Fill; G, H, and I) X-tra fil; J, K, and L) Tetric N-Ceram Bulk Fill; M, N, and O) Aura Bulk Fill. The first column presents resin composites stored in artificial saliva (A, D, G, J, and M). The second column presents resin composites submitted to the in vitro caries model (B, E, H, K, and N). The third column presents resin composites submitted to the in vitro erosion model (C, F, I, L, and O). Note: The arrows indicate surface alterations: the line arrows (→) indicate a detachment of filler particles (H and I); the right-pointing double arrow (⇛) represents an exposure of filler particles (L); and the right-pointing thick arrow (⇨) indicates degradation associated with damage and irregular surface (N and O).

only for Aura Bulk Fill's higher susceptibility to the acidic solutions but also for its higher baseline surface roughness and lower flexural strength in comparison to the other materials tested. Indeed, when weight percentage of fillers decrease, the sorption increases[21,22], causing swelling and thereby softening and plasticization of composites[23] as well as compromising their mechanical properties[20]. This statement is supported by previous observations that low inorganic filler contents (<75 wt%) have been associated with low flexural strength[24] and Aura Bulk Fill was the only composite having low filler loading (65.0%) by weight. This is probably a result of the inclusion of pre-polymerized fillers[25].

In contrast to Aura Bulk Fill, the other resin composites (Filtek Z350 XT, Filtek Bulk Fill, X-tra fil and Tetric N-Ceram Bulk Fill) did not differ from each other regarding surface roughness changes, and were not affected by the storage conditions. This finding corroborates a previous paper that found that under biofilm accumulation and cariogenic challenges, a resin composite did not degrade faster[15], but disagrees with another study in which the lowest pH erosive solution caused the highest increase in surface roughness[26]. This may be ascribed to the fact that our solution had 10% of citric acid (0.05 M), whereas the other study used plain passion fruit juice containing 55% citric acid, in addition to other acids.

It is worth noting that although there is no clear information on the surface roughness threshold and the ideal morphological features of a resin composite to reduce biofilm accumulation, surface staining and wear, and to increase gloss retention, the smoother the surface, the better. In this regard, although Aura Bulk Fill became only 7% and 8% rougher after immersion in the carious and erosive acids, respectively, the roughness attained was the highest among the materials tested. Considering that the restoration surface should have a maximum roughness 0.50 μm if it is not to be detected by the patient[27], Aura Bulk Fill may not seem a good choice of restorative material as, on average, such threshold is exceeded when this composite is exposed to cariogenic and erosive acids. On the other hand, despite showing the lowest flexural strength values among the investigated materials, they are above the minimum 80 MPa stated in ISO standard 4049/2019[28].

The present findings on flexural strength agree with published data showing that the effects of lactic acid, a caries-associated acid[29] and citric acid solutions[30] did not differ from that caused by distilled or deionized water or artificial saliva[31]. Flexural strength was, however, composite-dependent, with the highest value measured for X-tra Fil, which contained the highest percentage of filler by weight. X-tra Fil, Filtek Bulk Fill and Tetric N-Ceram Bulk Fill presented flexural strength equivalent to the conventional composite.

Whether the current results hold up in clinical practice remains to be elucidated. It may be assumed that in the clinical scenario, while polishing procedures may reduce the resin matrix exposed, they may also cause filler particle dislodgement[32] and may aggravate sorption, swelling, softening and plasticization and compromise the mechanical properties of composites. On the other hand, such effects may be counteracted by saliva, which plays important roles in forming acquired pellicle, buffering, clearing, and diluting acids[33], which may reduce their effects on resin composites in the oral cavity.

Depending on the restorative bulk-fill composite, carious and erosive conditions roughen the surface, and therefore, not all materials would remain undetectable by patients. However, acidic conditions resembling caries and erosion do not affect the flexural strength of restorative bulk-fill composites, which still meet the minimal value recommended for load-bearing areas.

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DECLARATION OF CONFLICTING INTERESTS

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

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Fluoride and silver ion concentrations and pH in silver diamine fluoride solutions from Argentina

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ABSTRACT

The aim of this study was to measure the fluoride (F) and silver (Ag) ion concentration and the pH, over time, of 2 solutions of 38% silver diamine fluoride (SDF) produced in Argentina. The brand Fluorsilver® was established as Group 1 (G1) (Densell), and the brand FAgamin® (Tedequim) as Group 2 (G2), each with two different lots. The following were determined at time 0 (t0) and 30 days after opening (t30): a) fluoride concentration (w/v) by visible spectrophotometry b) Ag content (w/v) by atomic absorption spectrophotometry c) pH. Results: The data in the freshly opened bottles were for G1 lot1/lot2: a) 0.96/1, b) 8.3/7.8, c).11.5/11.3; G2 lot1/lot2: a) 11.5/9.9, b) 39/39, c) 7/6,9; and after 30 days, G1 lot1/lot2: a) 0.85/0.81, b) 7.2/8.2, c) 11.3/11.6; G2 lot1/lot2: a) 9.35/8.43, b) 38/38, c) 7.6/7.6. Conclusion In relation to the expected values (5.0-5.9% fluoride and 24.4-28.8% silver), the average concentration of fluoride and silver ions was lower for G1, but higher for G2. The pH was alkaline for G1 and neutral for G2. Over the 30 days, the content of fluoride and silver tended to decrease.

Keywords: *Silver diamine fluoride - stability - physical chemical properties.*

Concentraciones de fluoruro y plata y pH en soluciones de diamino fluoruro de plata producidos en Argentina

RESUMEN

El objetivo de este estudio fue medir las concentraciones de iones de fluoruro (F) y plata (Ag) y el pH, de 2 soluciones de diamino fluoruro de plata (SDF) al 38% producidas en Argentina. Se estableció como Grupo 1 (G1) la marca Fluorsilver® (Densell), y FAgamin® (Tedequim) como Grupo 2 (G2), cada uno con dos lotes diferentes. Se determinó: a) la concentración de fluoruro (p/v) por espectrofotometría visible, b) el contenido de Ag (p/v) por espectrofotometría de absorción atómica y c) el pH, y fue medido en un tiempo 0 (t0) y 30 días después de la apertura del frasco (t30). Resultado: En tiempo 0 para G1 lote1/lote2 fue: a) 0,96/1, b) 8,3/7,8 c).11,5/11,3 y G2 lote1/lote2: a) 11,5/9,9, b) 39/39, c) 7/6,9. A los 30 días G1 lote1/lote2: a) 0,85/0,81, b) 7,2/8,2, c) 11,3/11,6 y G2 lote1/lote2: a) 9,35/8,43, b) 38/38, c) 7,6/7,6. La concentración de iones de fluoruro y plata para G1 fue menor en relación a los valores esperados (5,0-5,9% de fluoruro y 24,4- 28.8% plata), sin embargo G2 obtuvo valores más altos. G1 muestra resultados de pH alcalino y G2 neutro. A lo largo de los 30 días, el contenido de fluoruro y plata tiende a disminuir.

Palabras clave: *diamino fluoruro de plata - estabilidad - propiedades físico-químicas.*

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INTRODUCTION

Several strategies are currently available for the prevention and control of the tooth decay process. Dentists must base their decisions on the available evidence, as well as on the characteristics and needs of the injury and the patient himself/herself. The treatment of caries lesions has changed over the years, following an increasingly conservative and less invasive trend in order to avoid the restorative cycle and preserve dental tissue[1-2].

Silver diamine fluoride (SDF) was originally formulated in Japan at 38% concentration (w/v) under the brand name Saforide®. This original formulation was presented in its composition $AgF(NH_3)_2$, with concentrations of 44,800 ppm F (µg/mL), 253,870 ppm Ag, and alkaline pH[3].

The only FDA-approved SDF product in the United States (Advantage Arrest™ Silver Diamine Fluoride 38%) is a colorless topical agent composed of 24.4-28.8% (w/v) silver and 5.0-5.9% fluoride, with pH 10[4]. It is mainly used for lesion control, being an efficient, affordable, safe product, with good results reported by several laboratory and clinical studies[3,5,6]. In addition to preventing caries, SDF stops the caries process efficiently[7].

SDF was introduced for the treatment of cavities in both primary and permanent teeth, although its use was not frequent. However, it is currently beingincreasingly adopted as a strategy[8]. SDF inhibits demineralization, promoting the remineralization of enamel and dentin. The SDF concentrations available on the market range from 12% to 38% (80,170 to 254,000 ppm Ag ion concentration), with alkaline pH[5,9-11]. The SDF mechanism is based on the combination of silver and fluoride in an alkaline solution, which results in a synergistic effect with the ability to arrest tooth decay, where in addition to inhibiting demineralization, it preserves the degradation of dentin collagen. SDF can react with calcium and phosphate ions to form fluorohydroxyapatite with reduced solubility, thus being one of the main factors in stopping carious lesions[12-14]. When SDF solution is applied to a dentin lesion, the fluoride ions interact with the free calcium ions in the hydroxyapatite, forming calcium fluoride (CaF2) and fluorapatite (Ca10 (PO4) 6OH2-xFx). Then, the silver ions interact with the free phosphate and form a layer of silver phosphate deposits (Ag3PO4) on the treated surface and the dentinal tubules. In addition, SDF fluoride strengthens the dental structure of acidic by-products of bacterial metabolism, decreasing its solubility, and silver interacts with the cell membrane and bacterial enzymes, inhibiting microorganism growth[13,15].

Topical application of SDF is considered a non-invasive treatment for caries lesions, being an effective, easy, low-cost, painless option for the patient. SDF has been used successfully for controlling carious lesions in children is because even when access to dental services is available, traditional restorative treatment can be complicated[9,13,15]. In addition, decayed dental tissues do not need to be removed before applying the SDF, which makes the process faster[14]. Another advantage of the SDF is that it can prevent and paralyze both coronary and root carious lesions, providing an effective option for socially vulnerable patients[9,13].

It is essential to assess the stability of the reagent in the SDF solutions. In 2012, the first study was published evaluating two Brazilian brands and one Japanese brand of SDF solution[16]. Other studies have continued to assess the fluoride concentration in commercial SDF solutions[3,11,17]. Because there is a lack of information about Argentine brands, the aim of this study was to measure the pH, fluoride (F) and silver (Ag) ion concentrations, and short-term stability of 2 solutions of 38% silver diamine fluoride (SDF) manufactured in Argentina.

MATERIALS AND METHODS

Sampling

The two brands of SDF solutions manufactured in Argentina, Fluorsilver® (G1) and FAgamin® (G2), were used in this study. Two different lots of each brand were purchased and coded. In the sequence, the analyses were performed after randomization using Microsoft Office Excel to enable blind assessment (Table 1).

Table 1. Brands analyzed

Group	Brand	Concentration (%)	Lot 1 Expiration Date	Lot 2 Expiration Date
1	Fluorsilver® (Densell)	38%	Sg 0398 (7/2022)	UE 0239 (05/2024)
2	FAgamin® (Tedequim)	38%	8247 (08/2023)	8245 (03/23)

Experimental design

The samples were analyzed by a private laboratory accredited by OAA (Argentine Accreditation Agency as Le 209 test laboratory, complying with IRAM-ISO/IEC17025 Standards.)

For the determinations, 10 ml of the solution were used for each batch. To determine the dimensional stability of the groups, the following variables were evaluated: Ag concentration (% w/v) using Atomic Absorption Spectrophotometry; Fluoride concentration (% w/v) using Visible Spectrophotometry, and pH using potentiometry (Table 2). All the equipment was calibrated with standard solutions before the analysis using a standard curve with a correlation coefficient of $r \geq 0.99$.

The SDF solutions were evaluated at baseline (day 0) and 30 days after they were opened (day 30).

The results were expressed in % w/v (1% w/v = 10,000ppm).

Determination of acidity, and fluoride and silver ion concentration

For the fluoride and silver concentration analyses, the samples were diluted (see Column 2 in Table 2) so that their concentrations corresponded to the ranges covered by their respective calibration curves (Lambert and Beer law compliance).

As a quality control of the results, the recovery of a solution of known silver concentration, added to the samples, was measured, complying with the recovery values found with the quality standards.

The pH was measured directly, without diluting.

Sample storage: After opening, the samples were stored for 30 days in their original packaging in the dark at room temperature.

RESULTS

Table 3 shows the results for F- and Ag concentrations, and pH, at 0 and 30 days.

Table 2. Methodology for determining F-, Ag and pH in the samples

Variables	Dilutions	Methods	Equipments
% Fluoride in HF	Group 1 1:5.000 Group 2 1:50.000	Visible Spectrophotometry	Ultraviolet-visible (UV-vis) spectrophotometer Brand: Hach Model: DR 2010 Serial number: 990800015058 Wavelengths: 580 nm
% Silver in AgNO3	Group 1 1:100.000 Group 2 1:500.000	Atomic Absorption Spectrophotometry	Atomic-Absorption Spectrophotometer Brand: Buck Model: VGP-210 Serial number: 333 Ionization method: Air-acetylene
pH	-	Potentiometry	pH meter Brand: SANXIN Model: SX 711 Serial number: 111071535

Table 3. Acidity, Fluoride and Silver concentrations in the different groups and times

Sample		Group 1: Fluorsilver ®		Group 2: FAgamin®		(expected)*
		lot 1	lot 2	lot 1	lot 2	
F- in HF	Day 0 (% w / v)	0.96	1	11.5	9.9	5.0-5.9%
	Day 30 (% w / v)	0.85	0.81	9.35	8.43	
Ag in $AgNO^3$	Day 0 (% w / v)	8.3	7.8	39	39	24.4-28.8%
	Day 30 (% w / v)	7.2	8.2	38	38	
pH	Day 0	11.5	11.3	7	6.9	pH neutro/alkaline
	Day 30	11.3	11.6	7.6	7.6	

DISCUSSION

There is undoubtedly renewed interest in the use of SDF solutions, especially since the COVID-19 pandemic triggered a search for procedures that generate the least amount of aerosols possible[18,19]. The advantages of using SDF solutions are that application time is fast, without the need for cavity preparation, and the product is effective in primary and permanent dentition, making it an important resource, especially for public dental healthcare[19-21].

For SDF solutions to be stable, their pH must be alkaline. However, fluoride bioavailability is higher at low pH values, so to compensate for this, fluoride concentrations are increased[3]. In this study, the pH remained basic in G1 and neutral in G2, in both periods evaluated. The values obtained in this study for both brands may compromise their therapeutic activity.

Several studies have evaluated fluoride concentration in products for personal and professional use, but only four studies in the literature deal with SDF[3,16-18]. Soares-Yoshikawa et al.[3] evaluated the concentration and pH of six 12% to 30% SDF formulations marketed in Brazil and their bioavailability with demineralized dentin, using ion-selective electrode (ISE) and pH strips. All the formulations had concentrations different from those reported by the manufacturers. In the analysis, the concentrations of fluoride and silver ions detected were different from the concentration values declared by the original *Saforide solution and those published by Advantage Arrest™. Several studies have shown that the concentrations of free F and Ag ions in SDF solutions change over time[16,17].

Patel et al.[18] analyzed one of the SDF brands manufactured and marketed in Argentina, FAgamin finding the values F- 120,760ppm/Ag 258,000, which are similar to the findings in the current study. Crystal et al.[17] studied the stability of F and Ag in SDF solutions and found that F concentrations increase slightly over time due to water evaporation. At 28 days, the pH of the product is stable, while the fluoride content tends to increase and the silver content tends to decrease.

Another important point is that manufacturers do not disclose all the ingredients in their SDF products, so the ingredients of different SDF brands may vary and influence the results, as reported by Mei et al.[13].

In the present study, measured and expected fluoride concentrations differed from the expected values in all samples, with smaller variations in Group 2. Fluoride concentrations were 20% of the expected value in Group 1, and double the expected value in Group 2. Fluoride concentrations decreased over time in both groups.

A concentration of 38% is sufficient to prevent caries progression. However, this requires guaranteed product quality[3]. The current study is the first to evaluate SDF formulations made in Argentina, which comprise only two brands.

Limitations to the study include the small number of samples and the short evaluation period. The results reinforce the knowledge that there is a need for greater rigor by regulatory institutions in order to prevent the marketing of products that are ineffective for managing dental caries. Future studies on a larger number of samples and tests that assess the anti-caries action, comparing the different brands, are suggested.

CONCLUSION

Over 30 days, the pH remained stable, while the fluoride and silver content tended to decrease. The F and Ag values found differed from those expected, and fluoride ion concentration changed over the 30-day period in both brands.

These findings point to a failure in quality control in these products, which could compromise their anti-caries effect.

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DECLARATION OF CONFLICT INTERESTS

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

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Longitudinal assessment of the impact of orthodontic treatment on adolescents' quality of life: a comparison between boys and girls using a condition specific questionnaire

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ABSTRACT

The aim of this study was to compare the impact of the first year of wearing of a fixed orthodontic appliance on the Oral Health-Related Quality of Life (OHRQoL) between boys and girls, by means of a condition-specific instrument. The study included 69 adolescents aged 10 to 18 years, who were undergoing orthodontic treatment with a fixed appliance. Of the 69 adolescents, 38 were girls (55.1%) and 31 were boys (44.9%). They answered the Brazilian version of the Impact of Fixed Appliance Measure (B-IFAM) questionnaire three months (T1) and one year (T2) after the fixed appliance was installed. This questionnaire contains 43 questions, distributed across nine domains. The higher the scores, the more negative the perception of the adolescent concerning the impact of the fixed appliance on his/her OHRQoL. Sociodemographic and clinical variables were also analyzed, and statistical analysis was performed. For the domains, the effect size (the magnitude of the difference between girls and boys) and the minimal clinically important difference were also calculated. The adjusted regression showed that there was a significantly greater increase in the overall B-IFAM score in girls than in boys, indicating a more negative perception of the OHRQoL over the study time [Coefficient=11.77 (3.47–20.60), p*=0.006]. From T1 to T2, there was a significantly greater increase in the scores (more negative perception of OHRQoL over time) in girls than in boys for the domains aesthetics (*p*=0.034) and physical impact (*p*=0.011). These differences were clinically significant. The effect size (the magnitude of the difference) was moderate. The impact of wearing a fixed appliance on the OHRQoL was more negative in girls than in boys during the first year of orthodontic treatment.*

Keywords: *adolescent - quality of life - orthodontic treatment - fixed appliance*

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Avaliação longitudinal do impacto do tratamento ortodôntico na qualidade de vida de adolescentes: comparação entre meninos e meninas utilizando um questionário condição especifica

RESUMO

O objetivo deste estudo foi comparar o impacto do primeiro ano de uso do aparelho fixo na qualidade de vida relacionada a saúde bucal (QVRSB) entre meninas e meninos, através de um instrumento condição específica. Sessenta e nove adolescentes entre 10 e 18 anos, em tratamento ortodôntico com aparelho fixo foram incluídos. Adolescentes responderam ao questionário Impact of fixed appliance measure (B-IFAM) no terceiro mês de uso do aparelho fixo (T1) e um ano após a colagem do aparelho fixo (T2). Este questionário possui 43 perguntas, distribuídas em nove domínios. Quanto maior os escores, mais negativa a percepção do adolescente com relação ao impacto do aparelho fixo na QVRSB. Variáveis sociodemográficas e clínicas também foram avaliadas. Análise estatística foi realizada. Para os domínios, tamanho de efeito (a magnitude da diferença entre meninas e meninos) e diferença mínima clinicamente importante também foram calculadas. Dos 69 adolescentes, 38 eram meninas (55,1%) e 31 eram meninos (44,9%). Na regressão ajustada, meninas apresentaram um aumento significativamente maior do escore total do B-IFAM do que meninos, indicando uma percepção mais negativa da QVRSB ao longo do tempo de acompanhamento [Coeficiente=11,77 (3,47–20,60), p*=0.006]. Meninas apresentavam um aumento significativamente maior dos escores de T1 para T2 (percepção mais negativa da QVRSB ao longo do tempo) em relação aos meninos para os domínios estética (*p*=0,034) e impacto físico (*p*=0,011). Estas diferenças também foram clinicamente significativas. O tamanho do efeito (a magnitude da diferença) foi moderado. Meninas demonstraram um impacto mais negativo do uso do aparelho fixo na QVRSB do que meninos nos 12 primeiros meses de tratamento ortodôntico.*

Palavras-Chave: *adolescente, qualidade de vida - tratamento ortodôntico - aparelho fixo.*

INTRODUCTION

There is increasing scientific interest in people's wellbeing, and research seeks to understand the relationship between multidimensional constructs such as quality of life or self-esteem, and health[1]. Oral health conditions have been found to exert major impact on quality of life, giving rise to the term oral health-related quality of life (OHRQoL)[2,3]. The literature has shown the negative impact of oral conditions such as malocclusion on the OHRQoL of young people, with strong repercussions on oral functions, and emotional and social wellbeing[4,5]. Orthodontic treatment with a fixed appliance seeks to correct tooth positions and skeletal discrepancies, providing more functionally and esthetically favorable occlusion, and, consequently, better quality of life[6].

Changes in OHRQoL have been observed during the orthodontic treatment of adolescents wearing fixed appliances. Deterioration in quality of life is common during the first months of wearing an orthodontic device, mainly due to the oral symptoms and functional limitations it causes[7]. However, interestingly, other studies have found a positive impact on adolescent emotional wellbeing, even during the initial stages of orthodontic therapy, thanks to their expectation of having their teeth corrected[7,8]. The longitudinal studies available in the literature evaluate the impact of orthodontic treatment on adolescent quality of life by means of generic questionnaires to evaluate a condition-specific outcome of wearing a fixed appliance. Condition-specific questionnaires are more sensitive and more responsive, and thus provide more reliable results for evaluating a construct as complex as OHRQoL[9].

A recent cross-sectional study used a condition-specific questionnaire on wearing a fixed appliance to compare the impact of orthodontic treatment between boys and girls in the sixth month of wearing the appliance. The impact on quality of life was more negative in girls than in boys. Girls expressed a more negative perception of the pain caused by the fixed device, difficulty in hygiene, and social impact, especially regarding interaction with their peers[10]. In the context of orthodontics, more in-depth evaluations of the differences between boys and girls are relevant, given that they may have different perceptions and expectations regarding health outcomes[11]. The aim of this longitudinal study is to compare boys and girls concerning the impact of the first year of orthodontic treatment with a fixed appliance on their OHRQoL, by means of a condition-specific instrument.

METHOD

Study design

This longitudinal study follows the guidelines set forth in *Strengthening the Reporting of Observational Studies in Epidemiology* (STROBE)[12].

Participants, study location, data collection period, and ethics

The sample consisted of 79 male and female adolescents, 10 to 18 years of age, who were in the third month of orthodontic treatment with a fixed appliance at the School of Dentistry of the Federal University of Minas Gerais (UFMG), located in Belo Horizonte, Brazil. Any adolescents with cognitive disorders reported by their parents/guardians or with craniofacial anomalies were excluded from the study. Participants were recruited from January 2017 to December 2018, and data were collected from January 2017 to February 2020. This study was approved by the UFMG Research Ethics Committee concerning research involving human beings (No. 62116216.2.0000.5149).

Study Variables

Dependent Variable: OHRQoL

The impact of wearing a fixed appliance on adolescent quality of life was evaluated with a condition-specific instrument – Impact of Fixed Appliances Measure (IFAM). This instrument was developed in England[13], translated to Brazilian Portuguese, cross-culturally adapted, and subsequently validated for use in the Brazilian population[14], resulting in a national version of the questionnaire, called B-IFAM.

The B-IFAM consists of 43 questions. The answer choices for each question follow the Likert scale of 1 to 5, with 1 = completely disagree, 2 = disagree, 3 = neither agree nor disagree, 4 = agree, 5 = completely agree. The overall B-IFAM score ranges from 43 to 215. Higher scores indicate more negative perception regarding the impact of wearing a fixed appliance on the respondent's quality of life. These 43 questions can be broken down into nine domains: aesthetics (5 questions), functional limitations (3 questions), dietary impact (6 questions), oral hygiene impact

(3 questions), maintenance (2 questions), physical impact (9 questions), social impact (5 questions), time constraints (5 questions), and travel/cost/inconveniences (5 questions). Each domain can also be scored individually. The B-IFAM was completed by the adolescents, without any help, with the exception of the questions in the two last domains, for which the parents/guardians helped them to answer the questions, as recommended by the authors. The adolescents answered the questionnaire to evaluate the impact of the wearing of a fixed appliance on their quality of life at two distinct times: three months (T1) and 12 months (T2) after beginning orthodontic treatment with a fixed appliance.

Independent variable: sex

Sex was the main independent variable, and participants were divided into two groups: female and male.

Confounding variables: demographic, socioeconomic, and clinical data

In addition to the sex variable, age and family income variables were collected. The family monthly income was evaluated according to the Brazilian minimum salary at the time of data collection, and established by adding together the monthly income of all the active family members. This variable was dichotomized by the median into adolescents whose families had a monthly income ≤2 minimum salaries and adolescents whose families had a monthly income >2 minimum salaries. The age variable was also dichotomized through the median in individuals who were ≤12 years old and individuals who were >12 years old.

During clinical data collection, the adolescents underwent clinical examinations, performed in a room with dental equipment, under artificial light, using a WHO (World Health Organization) probe, and a clinical mirror. During this examination, the orthodontic indication of the extraction of pre-molars (yes/no) and the severity of the malocclusion were evaluated through the Dental Aesthetic Index (DAI)[15]. The DAI is a cross-cultural index that enables the evaluation of 10 occlusal characteristics related to dental-facial anomalies, according to three components: dentition (number of absent incisors, canines, and pre-molars), crowding and/or spacing (crowding in the region of the incisors, spacing in the incisor region, diastema between the maxillary central incisors, greater irregularity in the maxillary anterior teeth, and greater irregularity in the mandibular anterior teeth), as well as occlusion (horizontal trespass, anterior crossbite, open bite, and anterior-posterior molar relationship). Scores for each occlusal characteristic were multiplied by a coefficient and added to the constant of 13 in order to obtain the total score of the DAI for each participant. Based on the DAI scores, the adolescent could be classified into one of four levels of severity of malocclusion: slight malocclusion (DAI≤25), defined malocclusion (DAI=26-30), severe malocclusion (DAI=31-35), and highly severe malocclusion (DAI≥36)[15]. In this study, the severity of the malocclusion was dichotomized into slight/defined malocclusion (DAI≤ 30) and severe/highly severe malocclusion (DAI≥31). The researcher responsible for data collection underwent a calibration process with another researcher who had experience and ability in the application of DAI. The calibration process took place in two stages, with the first stage being theoretical and the second, clinical. The theoretical stage consisted of discussing the criteria used in the DAI to classify the severity of the malocclusion and the need for orthodontic treatment. The clinical stage consisted of evaluating 15 plaster models and clinical exams of 15 adolescents who were not included in the main study. The evaluations were conducted separately by the two researchers in order to calculate inter-examiner agreement. After one week, the plaster models and the adolescents were re-evaluated by the researcher who collected the data in order to calculate intra-examiner agreement. The Kappa values were 0.80 and 0.90 for inter- and intra-examiner evaluations, respectively, being considered satisfactory values[16]. Finally, data on the type of fixed appliance worn (conventional, self-ligating, or aesthetic) were collected.

Pilot Study

A pilot study to evaluate the data collection strategy was conducted with 10 adolescents who were not included in the main study. No change in the data collection strategy was necessary.

Statistical analysis

Statistical analysis was performed using the Statistical Package for Social Science (SPSS, version 25.0, IBM Inc., Armonk, USA). First, the Kolmogorov-Smirnov test demonstrated that

the total B-IFAM scores in T1 and T2, as well as the difference between T1 and T2, had normal distribution. For the latter, the negative sign (-) indicated a more negative perception of the OHRQoL from T1 to T2. Participants with complete data were compared to individuals excluded because of the loss of data, using the Fisher test for categorical variables (sex, age, monthly household income, severity of malocclusion, and orthodontic tooth extraction), and the *t* test for numerical variables (overall B-IFAM score in T1).

Females and males were compared for the variables age, family monthly income, malocclusion severity, and indication of orthodontic extraction of pre-molars, as well as for the outcome variables overall score of B-IFAM in T1, T2, and the difference between T1 and T2. Fisher's test and Pearson's test were used for categorical variables and the *t* test was used for the numerical variables.

The crude and adjusted associations between sex and the variables difference in the overall B-IFAM score between T1 and T2 were tested with linear regression. Regression coefficients and 95 % confidence intervals (CI) were determined as association measures. The adjusted regression model included the control of the variables age, family monthly income, malocclusion severity, indication of orthodontic extraction, and the overall B-IFAM score in T1.

The domains of the B-IFAM were also compared between females and males, using the *t* test. The differences between girls and boys, the effect size (the magnitude of the difference), and the 95 % CI were determined. As a reference, effect size values close to 0.20 were small, values close to 0.50 were medium, and values close to 0.80 were large[17]. The minimal clinically important difference (MCID) was calculated by multiplying the standard deviation of the domain assessed for the entire sample (pooled standard deviation) by 0.5[18].

Finally, covariance was analyzed for comparisons of the domains of the B-IFAM between females and males, controlling for the type of fixed appliance.

RESULTS

Of the 79 adolescents that began the study, 10 were excluded due to missing data (they did not fill out the B-IFAM at T2, or some other information was not provided). No difference was found between the 69 adolescents who participated in the entire study and the 10 who were excluded, for the variables sex and age, monthly family income, malocclusion severity, indication of tooth extraction, and the overall score of B-IFAM in T1 (Table 1). Of the 69

Table 1. Comparison of adolescents in the study sample to those excluded because of missing data

	Study sample	Excluded	*p* value
Independent variables	**N (%)**	**N (%)**	
Sex			
Girls	38 (55.1)	03 (30.0)	0.183*
Boys	31 (44.9)	07 (70.0)	
Age			
≤12 years	44 (63.8)	05 (55.6)	0.720*
>12 years	25 (36.2)	04 (44.4)	
Family Monthly income			
≤2 minimum wages	44 (63.8)	05 (55.6)	0.720*
>2 minimum wages	25 (36.2)	04 (44.4)	
Malocclusion (DAI)			
Slight / Defined	41 (59.4)	04 (57.1)	0.999*
Severe / Very severe	28 (40.6)	03 (42.9)	
Tooth extraction			
Yes	09 (13.0)	00 (00.0)	0.594**
No	60 (87.0)	10 (100.0)	
Outcome measures	**Mean (SD)**	**Mean (SD)**	
Overall B-IFAM (T1)	97.26 (24.41)	96.50 (24.11)	0.952**
Overall B-IFAM (T2)	98.28 (22.57)	-	-
Overall B-IFAM (change T1-T2)	-1.01 (18.11)	-	-

DAI=Dental Aesthetic Index; SD=standard deviation
*Fisher's test, **Student's *t* test

adolescents who participated in the study, 38 were female (55.1%) and 31 were male (44.9%). Mean age was 12.32 years (±1.63). Fifty-five adolescents used a conventional fixed appliance, 13 used a self-ligating appliance, and one used an aesthetic device. Fig. 1 shows the flowchart of the study.

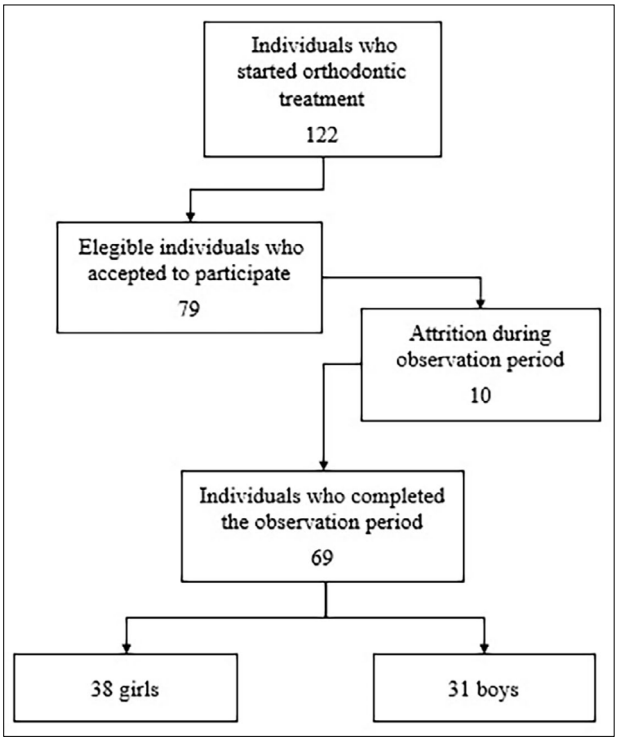

Fig. 1: Flowchart of the study

For the comparison between females and males, no significant difference was found for age, family monthly income, malocclusion severity, orthodontic tooth extraction, or the overall B-IFAM score in T1 (p>0.05). For the overall B-IFAM score in T2 (p=0.003), and for the difference of the overall B-IFAM score between T1 and T2 (p=0.039), girls had a more negative perception of the OHRQoL than boys (Table 2).

In the adjusted regression model, girls had a significantly greater increase in overall B-IFAM score than boys, indicating a more negative perception of the OHRQoL during the observation time (T1 to T2) (Coefficient = 11.77, 95 % CI = 3.47 – 20.60, p=0.006). In addition, a greater overall B-IFAM score in T1 was associated with a reduction in the overall B-IFAM score over time (coefficient = 15.62; 95 % CI = 7.55 – 23.69, p=0.001) (Table 3).

Comparison of the scores of the B-IFAM domains showed that females had a significantly greater increase in the score from T1 to T2 (more negative perception of the OHRQoL over time) than males for the aesthetic domain (p=0.034) and physical impact domain (p=0.011). As these were greater than the MCID, these differences were clinically significant. For these two domains, the effect size (the magnitude of the difference) was moderate (Table 4).

Table 2. Comparison of covariates and B-IFAM overall scores between female and male adolescents

	Girls	Boys	*p* value
Covariates	**N (%)**	**N (%)**	
Age			
≤12 years	26 (68.4)	18 (58.1)	0.453*
>12 years	12 (31.6)	13 (41.9)	
Family Monthly income			
≤2 minimum wages	27 (71.1)	17 (54.8)	0.211*
>2 minimum wages	11 (28.9)	1 (45.2)	
Malocclusion (DAI)			
Slight / Defined	25 (65.8)	16 (51.6)	0.325*
Severe / Very severe	13 (34.2)	15 (48.4)	
Tooth extraction			
Yes	04 (10.5)	05 (16.1)	0.721**
No	34 (89.5)	26 (83.9)	
Outcome measures	**Mean (SD)**	**Mean (SD)**	
Overall B-IFAM (T1)	99.09 (18.91)	93.54 (21.29)	0.241***
Overall B-IFAM (T2)	103.31 (21.31)	89.77 (22.07)	0.003***
Overall B-IFAM (change T1-T2)	-5.05 (18.10)	3.93 (17.12)	0.039***

DAI=Dental Aesthetic Index; SD=standard deviation
*Pearson's test, **Fisher's test, ***Student's *t* test

Table 3. Crude and adjusted associations between adolescents' sex and changes in the B-IFAM overall score

	Overall B-IFAM (change T1-T2)	Crude associations			Adjusted associations		
	Mean (SD)	Coefficient	95% CI	*p* value*	Coefficient	95% CI	*p* value*
Sex							
Girls	-5.05 (18.10)	Reference			Reference		
Boys	3.94 (17.12)	8.98	0.45 – 17.52	0.039	11.77	3.47 – 20.60	0.006
Age							
≤12 years	-1.27 (20.93)	Reference			Reference		
>12 years	-0.56 (11.97)	0.71	-8.40 – 9.83	0.876	-0.93	-9.76 – 7.90	0.834
Family Monthly income							
≤2 minimum wages	-1.61 (16.24)	Reference			Reference		
>2 minimum wages	0.04 (21.32)	1.65	-7.45 – 10.76	0.718	-1.79	-10.55 – 6.96	0.684
Malocclusion (DAI)							
Slight / Defined	-1.73 (16.39)	Reference			Reference		
Severe / Very severe	0.04 (20.63)	1.76	-7.15 – 10.68	0.694	-0.01	-8.26 – 8.25	0.999
Tooth extraction							
Yes	6.78 (18.41)	Reference			Reference		
No	-2.18 (17.92)	-8.96	-21.79 – 3.87	0.168	-10.76	-22.69 – 1.17	0.076
Overall B-IFAM (T1)							
Low impact (score ≤99)	-7.17 (14.41)	Reference			Reference		
High impact (score >99)	5.32 (19.49)	12.49	4.27 – 20.71	0.003	15.62	7.55 – 23.69	0.001

SD=standard deviation, CI=confidence interval
*Linear regression. Significant at $p < 0.05$

Table 4. Comparison of the B-IFAM domain scores and the minimal clinically important difference between female and male adolescents

	Number of questions (score range)	Girls Change T1-T2 Mean (SD)	Boys Change T1-T2 Mean (SD)	*p* value*	Difference between girls and boys Mean (95% CI)	Effect size 95% CI	MCID
Aesthetics	5 (5 – 25)	-0.63 (3.16)	1.00 (3.05)	0.034	-1.63 (-3.13 – -0.12)	0.50 (0.07 – 0.93)	1.60
Functional limitations	3 (3 – 15)	0.13 (2.50)	0.35 (2.87)	0.731	-0.22 (-1.51 – 1.06)	0.08 (-0.35 – 0.51)	1.32
Dietary impact	6 (6 – 30)	0.26 (5.36)	0.77 (5.97)	0.710	-0.51 (-3.23 – 2.21)	0.09 (-0.34 – 0.52)	2.80
Oral hygiene impact	3 (3 – 15)	-0.39 (3.14)	0.26 (3.36)	0.409	-0.65 (-2.21 – 0.91)	0.20 (-0.23 – 0.63)	1.61
Maintenance	2 (2 – 10)	-0.08 (2.04)	-0.01 (2.03)	0.873	-0.07 (-1.06 – 0.90)	0.03 (-0.40 – 0.46)	1.01
Physical impact	9 (9 – 45)	-2.89 (5.84)	0.94 (6.27)	0.011	-3.83 (-6.74 – -0.91)	0.60 (0.38 – 0.82)	3.14
Social impact	5 (5 – 25)	-0.42 (3.50)	0.58 (2.87)	0.205	-1.00 (-2.56 – 0.56)	0.30 (-0.13 – 0.73)	1.62
Time constraints	5 (5 – 25)	-1.08 (3.57)	0.09 (3.36)	0.167	-1.17 (-2.85 – 0.50)	0.33 (-0.10- 0.76)	1.75
Travel/cost/ inconveniences	5 (5 – 25)	0.05 (4.20)	-0.06 3.28)	0.900	0.11 (-1.72 – 1.96)	0.29 (-0.14 – 0.72)	1.89

SD=standard deviation, CI=confidence interval, MCID=minimally clinically important difference
*Student's *t* test. Significant at $p < 0.05$

Table 5. Analysis of covariance comparing the B-IFAM domain scores between female and male adolescents, controlling for type of fixed appliance worn

	Aesthetics	Functional limitations	Dietary impact	Oral hygiene impact	Maintenance	Physical impact	Social impact	Time constraints	Travel/cost/ inconve-niences
Sex									
Girls	-1.61 (0.75) *	-0.19 (0.65) *	-0.42 (1.36) *	-0.58 (0.77) *	-0.05 (0.49) *	-3.72 (1.45)	-0.97 (0.78) *	-1.16 (0.84) *	0.13 (0.93) *
Boys	0	0	0	0	0	0	0	0	0
p value	0.037	0.761	0.754	0.455	0.907	0.013	0.221	0.174	0.881
Type of fixed appliance									
Conventional	-0.39 (0.94) *	-0.59 (0.80) *	-1.95 (1.68) *	-1.70 (0.95) *	-0.49 (0.61) *	-2.57 (1.79) *	-0.70 (0.97) *	-0.16 (1.05) *	-0.54 (1.15) *
Self-lig/aesth	0	0	0	0	0	0	0	0	0
p value	0.674	0.464	0.251	0.080	0.419	0.156	0.469	0.873	0.637

Self-lig/aesth=self-ligating/aesthetic fixed appliance
*Coefficient (error)
p value=analysis of covariance. Significant at $p < 0.05$

The analysis of covariance showed that the increase in the score from T1 to T2 (more negative perception of the OHRQoL over time) in females than in males for the aesthetic domain (p=0.037) and physical impact domain (p=0.013) remained, regardless of the variable type of fixed appliance used (Table 5).

DISCUSSION

Significant changes were found in the OHRQoL of females over the first year of orthodontic treatment with a fixed appliance. After 12 months of wearing of the fixed appliance, girls had a more negative perception of the OHRQoL than boys. The literature shows favorable effects on the OHRQoL of adolescents, regardless of sex, after the first year of wearing a fixed appliance, showing improvement in their overall OHRQoL, as a result of the strong positive repercussions on their emotional and social wellbeing[19]. However, there are no longitudinal studies comparing the difference between sexes regarding the impact of the first year of wearing a fixed appliance on the OHRQoL. Studies that evaluated the impact of the need for orthodontic treatment and the OHRQoL of adolescents have shown that girls with occlusal disharmony have a more negative perception of their OHRQoL than boys in a similar condition. Females seem to have greater aesthetic concern and can feel more upset by dental disharmony and extremely exaggerated skeletal changes in the face[20]. Although the literature shows that girls seek orthodontic treatment more often to correct malocclusion[20,21], the present study found that girls, when compared to boys, have a more negative perception of the OHRQoL between the third month and the first year of treatment with the fixed appliance. This finding illustrates that the more negative perception of the quality of life before orthodontic treatment[20,22] seems to perpetuate itself throughout the 12 months after the fixed appliance has been installed. In the adjusted regression for the evaluated confounding variables, the girls continued to have a more negative perception of the OHRQoL throughout the orthodontic treatment with the fixed appliance, when compared to the boys. In this regard, a study conducted in 2014 evaluated the relationship between adolescents' OHRQoL and self-esteem during the first year of orthodontic treatment. It found that adolescents with better self-esteem at the beginning of the orthodontic treatment demonstrated minimal variability in the OHRQoL after the first year of wearing a fixed appliance[23]. The differences in self-esteem between adolescents of different sexes[24] may be useful to explain how orthodontic treatment impacts their quality of life[25]. Females had a more negative perception of the OHRQoL during the first year of orthodontic treatment in the aesthetic domain. Adolescents undergoing orthodontic treatment with a fixed appliance seem to need aesthetic approval from their peers and parents. They often compare their looks to those of their friends and people on the media, and feel that their own teeth should be similar to the dental and facial aesthetic standard imposed by others[22]. The results of the present study suggest that girls feel more uncomfortable and embarrassed, and avoid smiling when wearing a fixed appliance, because they might perceive the orthodontic device as ugly.

Pain and discomfort in the oral mucosa are common complaints during orthodontic therapy. In the present study, girls reported more complaints than boys regarding pain and sores (physical impact) during the first year wearing a fixed appliance. A previous cross-sectional study using the B-IFAM questionnaire on patients in the sixth month of wearing a fixed appliance, also found differences in the perception of pain between boys and girls. It reported that girls appeared to be more anxious about the possibility of pain caused by the activation of the fixed appliance[10]. The results of the present study showed that this fear of pain caused by the activation of the fixed appliance persists in the girls even after the first year wearing it. It is important to highlight that the adolescents answer the questionnaire shortly after the dental appointment to activate the fixed appliance, which may influence the perception of pain, given that three days after activation, its perceived tension often subsides[26].

It is important to emphasize that the statistically significant differences between girls and boys for the domains of aesthetics and physical impact appear to be clinically significant, as they were greater than the MCID. The MCID reflects the changes of a specific outcome during a specific intervention, which are expressive and significant for the patients who undergo that type of intervention. In other words, it would be the smallest difference in a given outcome considered to be beneficial and which would imply, in the absence of side effects and excessive costs, a change in the management of the patient[27]. In

studies that evaluate quality of life, a significant change in the OHRQoL is considered as one which results in a significant reduction in the symptoms or an improvement in function and wellbeing[28]. This concept helps resolve one of the greatest challenges of transforming scientific evidence into practice, which is the interpretation of research data in the light of clinical relevance[29]. Moreover, within our results, the MCID implications were confirmed by effect size for the two domains, which were moderate to high[30].

The results of studies on quality of life can be important to help the clinician to understand the physical, functional, and wellbeing consequences of orthodontic treatment. Based on the results of the present study, girls have a more negative perception of wearing a fixed appliance for reasons of aesthetics and probable pain and discomfort caused by the orthodontic device. Thus, early advice regarding the repercussions of orthodontic treatment should place greater emphasis on girls, to provide greater understanding of the possible complications of wearing a fixed appliance[10] to correct occlusal discrepancies, thereby improving the patient's quality of life at the end of the treatment[31]. Moreover, it is of utmost importance to clarify the effects of orthodontic therapy with a fixed appliance to the decision-makers, to enable improvement and better organization of the services providing orthodontic care[9].

CONCLUSION

The impact of wearing a fixed appliance on OHRQoL was more negative in female than male adolescents during the first 12 months of orthodontic treatment.

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DECLARATION OF CONFLICTING INTERESTS

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

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Students' perspective of the teaching-learning process of oral radiology before and during the COVID-19 pandemic

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ABSTRACT

The SARS-Cov-2 (COVID-19) pandemic changed the educational structure of dentistry courses and highlighted the importance of online tools. Understanding students' perception regarding these changes is essential to establishing future teaching-learning strategies to accommodate students' needs in higher education. The aim of this study was to assess students' perceptions of the Oral Radiology teaching-learning process before and during the COVID-19 pandemic. The sample consisted of students (n = 111) of the 2nd, 4th and 6th semesters of the dentistry course, who answered a questionnaire with 21 items: A) Students' demographic data (5 questions); B) Students' teaching-learning experiences during the pre-pandemic period (8 questions); and C) Students' teaching-learning experiences during the post-pandemic period (8 questions). Stuart-Maxwell tests revealed statistically significant differences between students' opinions before and during the pandemic when they were asked about the structure of the Oral Radiology module ($p = 0.008$); their previous experience with e-learning and teaching ($p < 0.001$); their thoughts about the importance of e-learning in Oral Radiology ($p < 0.05$); and the time they spent online for academic purposes ($p < 0.05$). Students seem to prefer on-campus activities (before COVID-19), but the pandemic increased their awareness of the importance of e-learning, the time they spent on online studies, and their knowledge of online educational tools.

Keywords: *COVID-19 - dentistry - education - radiology - students.*

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Perspectiva dos alunos sobre o processo de ensino-aprendizagem em radiologia odontológica antes e durante a pandemia de COVID-19

RESUMO

A pandemia de SARS-Cov-2 (COVID-19) mudou a estrutura educacional dos cursos de odontologia e destacou a importância das ferramentas online. Compreender a percepção dos alunos sobre as mudanças vivenciadas é essencial para estabelecer futuras estratégias de ensino-aprendizagem e acomodar as necessidades dos alunos no ensino superior. Este estudo teve como objetivo avaliar a percepção dos alunos sobre o processo de ensino-aprendizagem de Radiologia Odontológica antes e durante a pandemia de COVID-19. A amostra foi composta por alunos (n = 111) do 2º, 4º e 6º semestres do curso de odontologia que responderam a um questionário com 21 itens: A) Dados demográficos dos alunos (5 questões); B) Experiências de ensino-aprendizagem dos alunos no período pré-pandemia (8 questões); e C) Experiências de ensino-aprendizagem dos alunos no período pós-pandemia (8 questões). Os testes de Stuart-Maxwell revelaram diferenças estatisticamente significativas entre as opiniões dos alunos antes e durante as pandemias quando questionados sobre a estrutura do módulo de Radiologia Odontológica ($p = 0,008$); sua experiência anterior com ensino a distância ($p < 0,001$); seus pensamentos relacionados à importância da Radiologia Odontológica via e-learning ($p < 0,05$); e o tempo gasto online para fins acadêmicos ($p < 0,05$). Os alunos parecem preferir atividades no campus (antes do COVID-19), mas as pandemias aumentaram sua conscientização sobre a importância do e-learning, seu tempo dedicado aos estudos online e sua familiarização com ferramentas educacionais online.

Palavras-chave: *COVID-19 - odontologia - educação - radiologia - alunos.*

INTRODUCTION

Two years have passed since the onset of widespread infection by SARS-Cov-2 (COVID-19) worldwide[1]. In most countries, the teaching-learning process switched from a face-to-face relationship to a mainly virtual environment[2]. While some authors report general acceptance of online-based dental education[2], others report higher rates of dissatisfied students[3]. Given the practical nature of dental education, authors suggest the need for continuous research of students' perceptions regarding the current transitions in the teaching-learning process during COVID-19 times[4]. To achieve a more comprehensive perspective of students' perception, however, research should be country-specific, especially because of the global differences in the curricula and educational approaches. In 2021, a study across 34 European countries highlighted dental students' concerns regarding clinical experience and the skills needed to become dentists[3]. In the same year, a study on 779 Brazilian dentistry undergraduates corroborated students' dissatisfaction with the transition between traditional education and e-learning[5].

The situation of Brazilian dental education in COVID-19 times is particularly relevant because in 2020, the country was ranked second amongst the countries with the highest numbers of confirmed cases[6]. Moreover, Brazil has nearly 370,000 dentists[7] (estimated as 17% of the dentists in the world) and at least 374 dental schools offering 47,192 admission places[8] for students. The effects of COVID-19 on the educational process of Brazilian undergraduate dentistry students could eventually lead to potential harm of great magnitude given the number of professionals entering the market every year.

In specific fields of dentistry, computer-based classes are essential. Such is the case of Oral Radiology – a field that requires constant updates and training with state-of-the-art technology. Hence, students of Oral Radiology may accept transitions to e-learning more easily. A systematic literature review[9] found out that e-learning strategies used in the teaching-learning process of Oral Radiology seem to be as effective as other traditional approaches, such as lecture-based learning. Strategies used to bring online dentistry lessons closer to reality include case-based learning, problem-based learning, and research-based learning[2,10]. For image interpretation (an essential component of Oral Radiology), the available online tools could enable proper teaching and even enhance students' interaction with image analysis. On the other hand, practical training in radiographic techniques for image acquisition could be negatively impacted after the switch to the online environment[11]. In these cases, demonstrative instructions followed by laboratory practice with a restricted number of students (per group), and the use of facilities dedicated to oral radiology, including phantom heads, would be beneficial when teaching returns to being part online and part face-to-face[12]. Striving for the best conditions for students' educational development, however, depends on the available pedagogical solutions. Understanding the perspective of undergraduate dentistry students regarding the ever-changing educational scenarios experienced during the COVID-19 pandemics is fundamental to establishing more effective strategies for the teaching-learning interface in Oral Radiology. The aim of this study was to assess the perspective of students regarding the teaching-learning process of Oral Radiology before and during the pandemic.

MATERIAL AND METHODS

Study design and ethical aspects

This was an observational, survey-based, cross-sectional study with prospective data collection. It was approved by the Institutional Committee of Ethics in Research (protocol 42072720.9.0000.5374). The Declaration of Helsinki (DoH) 2013 was followed to ensure ethical standards in this medical research. EQUATOR (Enhancing the Quality and Transparency of Health Research) guidelines were followed and the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) checklist[13] for cross-sectional studies was used.

Sample and participants

The sample consisted of undergraduate dentistry students from a private institution in the southeast region of Brazil. The inclusion criteria for sample selection consisted of male and female students enrolled in the undergraduate course who had successfully concluded at least one semester of the discipline of Oral Radiology during the COVID-19 pandemic, age above 18 years. The exclusion criteria were students transferred from other institutions during the COVID-19 pandemic; students with a gap in their studies during the pandemic; and students

who failed the Oral Radiology course because of insufficient attendance rate.

Sample size calculation considered the total population of students (n = 171) enrolled at the undergraduate level (especially because the structure of the Oral Radiology discipline in the selected private institution is divided into the 1st, 2nd and 3rd semesters), with 95% confidence level and 5.53% confidence interval. Hence, the target sample size for the present study was estimated as 111 students.

Variables and measures

A self-administered questionnaire was tailored for the present survey. Following the scientific literature, the questionnaire was designed as closely as possible to the recommendations established for survey research[14]. The questionnaire consisted of 21 questions divided into three groups: A) General information about the students' demographic data (5 questions); B) Specific questions related to the students' teaching-learning experiences in the pre-pandemic period (8 questions); and C) Specific questions related to the students' teaching-learning experiences in the post-pandemic period (8 questions). Question structure was dichotomous (the question about whether they had had contact with e-learning tools before the pandemic) or multiple-choice (students should select a single answer). Questions in sections B and C were mirrored for the period pre- and trans-pandemic. This approach enabled the questions to be answered by the same students – so they could report on experiences regarding the Oral Radiology teaching-learning process at two different times (before/pre and during/trans pandemic) – creating a dependent association between answers (dependent variables between sections B and C) (Table 1). Because institutional activities were returning to face-to-face during the pandemic period, the questionnaire was provided on-campus and separately to students of the second, fourth, and sixth semesters of the undergraduate course. The main researcher supervised the application of the questionnaire in class. All the students were adults (age >18 years old) and expressed their consent to participate in the study within a signed informed consent applied before the questionnaire.

Data synthesis and analysis

Data were explored by means of descriptive statistics within absolute (n) and relative (%) frequencies. The descriptive approach was specifically pertinent to questions inherent to section A (General information about the participant demographic data). For the subsequent sections, comparisons between answers were performed between dependent groups of students. This means that students in the 4th and 6th semesters answered questions about their experiences with the teaching-learning process in Oral Radiology before and during the pandemic, while students in the 2nd semester only answered questions related to their experiences during the pandemic. In order to compare the experiences before and during the pandemic within each group of students (per semester), marginal homogeneity tests were applied (Stuart-Maxwell test). Statistical significance was set at 5% with a confidence interval of 95%.

RESULTS

General information about the students' demographic data (Questionnaire section A) showed that the questionnaire was answered by 31 (27.92%) male and 80 (72.08%) female students. Forty students (14 males and 26 females) were in the 2nd semester, while 42 (11 males and 31 females) and 29 (6 males and 23 females) were in the 4th and 6th semesters, respectively. Most of the students were 18 to 20 years old (75.67%), followed by students aged 21 to 24 years (21.62%), or over 25 years (2.71%). Most students did not know their internet speed at home (41.44%). Of the students that did know, most (25.22%) reported a speed between 110 and 150 Mb/s. Regarding the devices used to access e-learning content, notebooks (58.55%) and smartphones (27.02%) were the most prevalent. Desktops were only reported by students in older age categories (Table 2).

When asked about the quality of Oral Radiology teaching, most students of both the 4th and 6th semesters rated it as moderate or good before and during the pandemic. When students were asked about their performance in the Oral Radiology module, they tended to rate it as moderate or good. Particularly for students in the 6th semester, there was a predominance of "good" ratings (>41%) both before and during the pandemic. Students in the 4th semester considered the structure of the Oral Radiology module to be good before the pandemic, but moderate during the pandemic. Most students in the 6th semester rated the module structure as moderate at both times, and students that rated the module as good and very good (>24%) maintained their opinion. Nearly 80% of the

Table 1. Self-administered questionnaire provided to undergraduate students in the 2nd, 4th, and 6th semesters of the dentistry course, regarding their perspective of the Oral Radiology teaching-learning process before and after the COVID-19 pandemic.

A) Student's general information and demographic data		
A.1) What is your registered sex?	( ) Male ( ) Female	( ) Other ( ) Prefer not to say
B.2) How old are you?	( ) 18-20 ( ) 21-24	( ) 25-30 ( ) >30
B.3) In what academic year are you enrolled?	( ) 1st ( ) 2nd	( ) 3rd ( ) 4th
B.4) What is your internet speed at home?	( ) 10-50Mb ( ) 60-100Mb	( ) 110-150Mb ( ) >150Mb
B.5) What is your main device for e-learning?	( ) Desktop computer ( ) Notebook	( ) Smartphone ( ) Tablet
B) Students' teaching-learning experiences during the pre-pandemic period		
B.1) How do you rate the quality of Oral Radiology teaching before the pandemic?	( ) Very bad ( ) Bad ( ) Moderate ( ) Good ( ) Very good	
B.2) How do you rate your performance in Oral Radiology before the pandemic?	( ) Very bad ( ) Bad ( ) Moderate ( ) Good ( ) Very good	
B.3) How do you rate the structure of the Oral Radiology module before the pandemic?	( ) Very bad ( ) Bad ( ) Moderate ( ) Good ( ) Very good	
B.4) Had you experienced e-learning teaching before the pandemic?	( ) Yes ( ) No	
B.5) What were your thoughts related to Oral Radiology via e-learning before the pandemic?	( ) Not important ( ) Of little importance ( ) Moderate ( ) Important ( ) Very important	
B.6) How much time a day did you use to spend on the computer for non-academic purposes?	( ) <1h ( ) 1-3h ( ) 4-6h ( ) 7-10h ( ) >10h	
B.7) How much time a day did you use to spend on the computer for academic purposes?	( ) <1h ( ) 1-3h ( ) 4-6h ( ) 7-10h ( ) >10h	
B.8) How do you rate the assessment system of the Oral Radiology module before the pandemic?	( ) Inefficient ( ) Of little efficiency ( ) Moderate ( ) Efficient ( ) Very efficient	
C) Students' teaching-learning experiences during the trans-pandemic period		
C.1) How do you rate the quality of Oral Radiology teaching before the pandemic?	( ) Very bad ( ) Bad ( ) Moderate ( ) Good ( ) Very good	
C.2) How do you rate your performance in Oral Radiology before the pandemic?	( ) Very bad ( ) Bad ( ) Moderate ( ) Good ( ) Very good	
C.3) How do you rate the structure of the Oral Radiology module before the pandemic?	( ) Very bad ( ) Bad ( ) Moderate ( ) Good ( ) Very good	
C.4) Had you experienced e-learning teaching before the pandemic?	( ) Yes ( ) No	
C.5) What were your thoughts related to Oral Radiology via e-learning before the pandemic?	( ) Not important ( ) Of little importance ( ) Moderate ( ) Important ( ) Very important	
C.6) How much time a day did you use to spend on the computer for non-academic purposes?	( ) <1h ( ) 1-3h ( ) 4-6h ( ) 7-10h ( ) >10h	
C.7) How much time a day did you use to spend on the computer for academic purposes?	( ) <1h ( ) 1-3h ( ) 4-6h ( ) 7-10h ( ) >10h	
C.8) How do you rate the assessment system of the Oral Radiology module before the pandemic?	( ) Inefficient ( ) Of little efficiency ( ) Moderate ( ) Efficient ( ) Very efficient	

students in the 4th and 6th semesters had not had any contact with e-learning tools before the pandemic, becoming more familiar with them during the pandemic (Table 3).

Table 2. Descriptive assessment of the general information about the students' demographic data (questionnaire section A)

Students' personal data	2nd semester		4th semester		6th semester	
	N	%	N	%	N	%
Age (years)						
18-20	37	93%	34	81%	13	45%
21-24	3	8%	7	17%	14	48%
25-30	0	0%	0	0%	1	3%
>30	0	0%	1	2%	1	3%
Internet speed (Mb/s)						
10-50	1	3%	0	0%	0	0%
60-100	7	18%	3	7%	4	14%
110-150	11	28%	12	29%	5	17%
>150	9	23%	6	15%	6	21%
I do not know	12	30%	20	49%	14	48%
Device for e-learning						
Desktop	-	-	3	7%	1	3%
Notebook	18	46%	24	57%	23	79%
Smartphone	13	33%	13	31%	4	14%
Tablet	8	21%	2	5%	1	3%

N: absolute frequency; %: relative frequency; Mb/s: megabytes per second.

Table 3. Students' responses related to their experiences with the Oral Radiology teaching-learning process before and during COVID-19 pandemic (Part 1)

Teaching-learning process components	4th semester				6th semester			
	Before pandemic		During pandemic		Before pandemic		During pandemic	
	N	%	N	%	N	%	N	%
How do you rate the quality of Oral Radiology teaching?								
Very bad	1	2.4%	1	2.4%	0	0.0%	0	0.0%
Bad	4	9.5%	3	7.1%	1	3.4%	6	20.7%
Moderate	8	19.0%	18	42.9%	12	41.4%	10	34.5%
Good	21	50.0%	17	40.5%	12	41.4%	11	37.9%
Very good	8	19.0%	3	7.1%	4	13.8%	2	6.9%
How do you rate your performance in Oral Radiology?								
Very bad	0	0.0%	1	2.4%	0	0.0%	1	3.4%
Bad	4	9.5%	5	11.9%	2	6.9%	4	13.8%
Moderate	12	28.6%	17	40.5%	8	27.6%	9	31.0%
Good	23	54.8%	15	35.7%	14	48.3%	12	41.4%
Very good	3	7.1%	4	9.5%	5	17.2%	3	10.3%
How do you rate the structure of the Oral Radiology module?								
Very bad	1	2.4%	1	2.4%	4	13.8%	2	6.9%
Bad	3	7.1%	4	9.5%	7	24.1%	6	20.7%
Moderate	6	14.3%	18	42.9%	10	34.5%	13	44.8%
Good	28	66.7%	16	38.1%	7	24.1%	7	24.1%
Very good	4	9.5%	3	7.1%	1	3.4%	1	3.4%
Had you ever experienced e-learning?								
No	34	81.0%	3	7.1%	23	79.3%	0	0.0%
Yes	8	19.0%	39	92.9%	6	20.7%	29	100.0%

N: absolute frequency; %: relative frequency.

When asked about their opinion of e-learning for Oral Radiology, most students in the 4th and 6th semesters changed their opinion from moderate importance (before the pandemic) to important (during the pandemic), with a threefold increase in the importance of the module detected in both groups. The amount of time that students spent online for non-academic reasons remained the same before and during the pandemic. Most students (60-70%) reported a non-academic online time of 1-6 hours/day. In contrast, the time spent online for academic reasons was initially longer than for non-academic reasons, and increased during the pandemic. Among 4th semester students, those reporting online time from 7 to 10 hours doubled. Among students in the 6th semester, the number of students spending 7-10 hours online for academic reasons increased almost 5 times. Students' opinions regarding the assessment system of the module remained unchanged before and during the pandemic (Table 4).

Stuart-Maxwell tests revealed statistically significant differences between students' opinions before and during the pandemic when they were asked about the structure of the Oral Radiology module ($p = 0.008$, statistically significant for the students in the 4th semester only); their previous experience with e-learning ($p < 0.001$ for both groups of students); their thoughts related to the importance of online learning for Oral Radiology ($p < 0.001$ and $p = 0.03$ for students in the 4th and 6th semesters, respectively);

Table 4. Students' responses related to their experiences with the Oral Radiology teaching-learning process before and during COVID-19 pandemic (Part 2)

Teaching-learning process components	4th semester				6th semester			
	Before pandemics		During pandemics		Before pandemics		During pandemics	
	N	%	N	%	N	%	N	%
What are your thoughts related to e-learning for Oral Radiology?								
Not important	5	11.9%	1	2.4%	3	10.3%	0	-
Of little importance	5	11.9%	4	9.5%	4	13.8%	1	3.4%
Moderate	20	47.6%	4	9.5%	14	48.3%	4	13.8%
Important	9	21.4%	27	64.3%	6	20.7%	17	58.6%
Very important	3	7.1%	6	14.3%	2	6.9%	7	24.1%
How much time a day did you use to spend on the computer for non-academic purposes?								
< 1 hours	6	14.3%	7	16.7%	9	31.0%	5	17.2%
1-3 hours	20	47.6%	14	33.3%	8	27.6%	9	31.0%
4-6 hours	12	28.6%	15	35.7%	11	37.9%	11	37.9%
7-10 hours	2	4.8%	5	11.9%	1	3.4%	4	13.8%
> 10 hours	2	4.8%	1	2.4%	0	0.0%	0	0.0%
How much time a day did you use to spend on the computer for academic purposes?								
< 1 hours	9	21.4%	1	2.4%	11	37.9%	2	6.9%
1-3 hours	17	40.5%	12	28.6%	6	20.7%	3	10.3%
4-6 hours	9	21.4%	15	35.7%	9	31.0%	10	34.5%
7-10 hours	6	14.3%	13	31.0%	3	10.3%	13	44.8%
> 10 hours	1	2.4%	1	2.4%	0	0.0%	1	3.4%
How do you rate the assessment system of the Oral Radiology module?								
Inefficient	1	2.4%	3	7.1%	1	3.4%	2	6.9%
Of little efficiency	14	33.3%	16	38.1%	3	10.3%	6	20.7%
Moderate	24	57.1%	22	52.4%	17	58.6%	17	58.6%
Good	3	7.1%	1	2.4%	8	27.6%	3	10.3%
Very good	0	0.0%	0	0.0%	0	0.0%	1	3.4%

N: absolute frequency; %: relative frequency.

Table 5. Comparison of students' responses related to their experiences with the Oral Radiology teaching-learning process before and during the COVID-19 pandemic

Comparison of pre- and trans-pandemic perspectives	4th semester		6th semester	
	Before pandemic	During pandemic	Before pandemic	During pandemic
How do you rate the quality of Oral Radiology teaching?				
Difference (*p*)	0.053		0.257	
How do you rate your performance in Oral Radiology?				
Difference (*p*)	0.213		0.505	
How do you rate the structure of the Oral Radiology module?				
Difference (*p*)	**0.008**		0.832	
Had you ever experienced e-learning?				
Difference (*p*)	**<0.001**		**<0.001**	
What are your thoughts related to Oral Radiology via e-learning?				
Difference (*p*)	**<0.001**		**0.003**	
How much time a day did you use to spend on the computer for non-academic purposes?				
Difference (*p*)	0.188		0.185	
How much time a day did you use to spend on the computer for academic purposes?				
Difference (*p*)	**0.015**		**0.002**	
How do you rate the assessment system of the Oral Radiology module?				
Difference (*p*)	0.235		0.215	

Stuart-Maxwell test (significance set at 0.05).

and the time they spent online for academic purposes ($p = 0.015$ and $p = 0.002$ for students in the 4th and 6th semesters, respectively) (Table 5).

DISCUSSION

Research on students' perception of learning experiences serves as a quality control measure to understand the effects of the teaching process in practice. COVID-19 changed the way lectures were given, creating the need for immersion in the online environment. The concern of educational institutions regarding the quality of learning-teaching experiences increased worldwide, especially because the measures adopted as teaching solutions to pandemics were understood as temporary[15]. Hence, it is the objective of scientific studies to investigate and possibly predict whether the online solutions will continue to be used in the long term, in a post-pandemic future. The present study assessed the perception of undergraduate students regarding the experience with the Oral Radiology teaching-learning process before and during the pandemic.

The preliminary outcomes of this study show that most students use notebooks during the online lectures, while others use tablets or smartphones. These (recent) technologies are compatible with the age of the sample (over 75% of the students were 18 to 20 years old). Older students reported the use of desktop personal computers. Recent studies mention all these technologies as being useful during online activities[16]. More importantly, the student must be able to interact with the lecturer and the instructor using audio and video tools. From desktop personal computers to tablets, the equipment reported by the students enables access to the available technology to participate in online activities. As ¼ of the students had internet speed at home of 110-150 Mb/s, and almost 41% had access to internet even though they did not know their internet speed, it can be estimated that home facilities for online learning were available. Thus, it is necessary to enquire into students' perceptions of the teaching component.

Most of the ratings of the Oral Radiology teaching quality were moderate to good. The main difference was observed between students of the 4th and 6th

semesters. In the former, ratings were predominantly good before the pandemic and became moderate during it. In the latter, over 40% of the students rated the quality as good both before and during the pandemic. These differences may be explained by the previous academic experiences of senior students (6th semester) compared to students in earlier stages of the dentistry course. The senior students had already experienced practical activities on-campus when the pandemic began. In contrast, the 4th semester students were just beginning their clinical practice when they had to move to an online environment. This change may have interfered with the plans they had when they initially enrolled in the dentistry course. However, the educational structure worldwide is currently progressively moving back to "normal" and (following this rationale) students' perception of Oral Radiology teaching may become more positive over time. Some authors[15] report that students' perception of the effectiveness of face-to-face classes can influence their desire to return (or not) to on-campus activities. Consequently, if pre-pandemic ratings of students of the 4th semester were predominantly "good", a decrease to "moderate" is to be expected because the students certainly wish to return to on-campus activities. Secondarily, these outcomes corroborate the quality of traditional (face-to-face) teaching of Oral Radiology at the institution considered for this study.

Despite the students' interest in face-to-face activities interpreted between the lines of our outcomes, they seem to understand the importance of online teaching of Oral Radiology during the dentistry course. Their perception of the importance of online teaching increased up to three times during the pandemic ($p<0.05$). The rationale behind this phenomenon may be explained in two ways: I) students' own perception of the content of the module (which is relevant to supporting their studies during the dentistry course); and II) students' desire to continue their studies without a gap in time during the pandemic (hence, online tools are important to enable continuous academic training). It must be noted that online teaching of Oral Radiology is not only important because of the pandemic period. In 2012, some authors[17] demonstrated the significant role of blended teaching strategies as effective tools compared to traditional face-to-face (only) strategies. Blended teaching may figure as a strong approach in the post-pandemic period, especially because it can combine the advantages of online and face-to-face activities (e.g., online theory and face-to-face practice). The literature[18] advocates the possibility of fully implementing radiological content via e-learning methods and corroborates, once more, the importance of online teaching as part of an integrated training process in dentistry. More recently, a systematic literature review[19] indicated that most studies on the use of e-learning in undergraduate dental radiology curricula may lead to an enhancement of the learning process. However, case-specific strategies must be designed considering the facilities available at the higher education institutions.

The reduced clinical experience emerging from the transition from the face-to-face to online structures is of major concern to Oral Radiology. Some authors[20] have suggested a broad variety of digital resources for teaching, such as Zoom, Blackboard, and Google applications. Others[21] have suggested the implementation of online journal clubs and case discussions to trigger student engagement. The current technology available to students and instructors provides a scenario which is very different from the past[22], in which professionals were not fully familiar with digital resources, and the access to online solutions in radiology was scarcer. The present study shows that students' perception of the Oral Radiology teaching-learning process may change before and during the COVID-19 pandemic. Lessons learned during this period should be considered prior to strategic planning and preparation for the upcoming post-pandemic educational system – in which on-campus activities are returning.

Future research should be designed to assess student perception after the full return to face-to-face activities, and to understand potential changes in curricula as a result of the COVID-19 pandemic. Additionally, prospective research planning should take into account students' individual needs regarding their professional skills developed (or underdeveloped) during the dentistry course, which might lead to an increase in specialized postgraduate training[23]. Finally, sampling individuals from other semesters and additional higher education institutions (private and public) could improve the overview of students' perceptions and lead to a more comprehensive strategy for managing the Oral Radiology teaching-learning process in challenging situations such as pandemics.

In conclusion, students' perceptions of the Oral Radiology teaching-learning process during the

COVID-19 pandemic changed from the pre-pandemic to trans-pandemic periods. In general, students of the 2nd, 4th and 6th semesters had a predominantly positive perception of the Oral Radiology teaching-learning process. The COVID-19 pandemic led to stronger student interaction with e-learning tools and increased students' perception of the importance of online education in times of social distancing.

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

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Adaptations in dental public health services during the COVID-19 pandemic in municipalities of Southern Brazil: a grounded theory and collaborative research

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ABSTRACT

The aim of this study was to analyze oral health actions in four municipalities in Brazil during the COVID-19 pandemic according to a theoretical framework model on oral healthcare management. It was a qualitative study carried out in two stages. A theoretical-empirical model on the significance of oral healthcare management was developed, following the Grounded Theory method. Fourteen dentists and five healthcare managers participated, through open interview. Subsequently, collaborative research was performed, and the model was applied to analyze the documents produced to address the pandemic by each of four municipalities in Santa Catarina State. The model provided a framework for analyzing actions for coping with the pandemic regarding oral health services. Actions were identified in all dimensions of the model: reduction in supply of dental care due to restricted access to elective services; search for biosafety care standards; dissemination of standardized science-based guidelines; attempt to maintain comprehensive dental assistance through re-adaptation of specialized services and collective actions; and relocation of oral health professionals to assist in other sectors. The oral health care management framework can serve as a reference for redesigning oral health actions and services in other municipalities during the COVID-19 pandemic, in a broader perspective.

Keywords: *oral health - health services administration - primary health care.*

Adaptations in dental public health services during the COVID-19 pandemic in municipalities of Southern Brazil: a grounded theory and collaborative research

RESUMO

Analisar as ações de saúde bucal em quatro municípios brasileiros durante a pandemia de COVID-19, segundo um modelo de referencial teórico sobre gestão da atenção à saúde bucal. Estudo qualitativo realizado em dois momentos. Foi desenvolvido um modelo teórico-empírico sobre o significado da gestão do cuidado em saúde bucal, seguindo o método da Teoria Fundamentada nos Dados. Participaram 14 dentistas e cinco gestores de saúde, por meio de entrevista aberta. Posteriormente, no segundo momento, foi realizada uma pesquisa colaborativa, e o modelo foi aplicado para analisar os documentos produzidos em cada município para o enfrentamento local da pandemia, em quatro municípios do Estado de Santa Catarina, sul do Brasil. O modelo forneceu uma estrutura para analisar as ações de enfrentamento da pandemia nos serviços de saúde bucal. Foram identificadas ações em todas as dimensões do modelo: redução da oferta de atendimento odontológico devido à restrição de acesso aos serviços eletivos; a busca por padrões de assistência à biossegurança; disseminação de diretrizes padronizadas e com base científica; a tentativa de manter a assistência odontológica integral por meio da readaptação de serviços especializados e ações coletivas; e realocação de profissionais de saúde bucal para atendimento em outros setores. O referencial de gestão da atenção à saúde bucal pode servir de referência para redesenhar as ações e serviços de saúde bucal em outros municípios em período de pandemia de COVID-19, em uma perspectiva mais ampla.

Palavras-chave: *saúde bucal - administração de serviços de saúde - atenção primária à saúde.*

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INTRODUCTION

The organization and provision of oral health services, dental education courses and the industry in this field have suffered a direct impact related to the COVID-19 pandemic[1-3]. In oral healthcare environments, the Sars-Cov-2 virus can be transmitted directly through inhalation or exposure of mucous membranes to infected droplets, or indirectly via contaminated surfaces[4]. Direct or indirect transmission, and the fact that a considerable number of asymptomatic individuals can spread the virus, mean that aerosols are generated in environments where dental procedures are performed. The high risk of disease transmission in these locations is already known[5]. The possibility of cross-infection between oral health professionals and patients therefore requires the adoption of strict infection control protocols[1,6]. With regard to this issue, academic texts (articles, guidelines) and government regulations have been published, compiling recommendations for biosafety in oral healthcare services[5,7-11] in order to guide healthcare managers and professionals working in public and private oral health services, mostly aiming to address the pandemic and maintain the continuity of oral health services. In Brazil, technical note 04/2020 of the National Health Surveillance Agency[9] was published and updated, providing guidelines for dentists and other healthcare professionals in relation to preventive measures and infection control in oral healthcare services.

The reorganization of so many actions and services in the oral healthcare system involves challenges to public administration[12-16]. At the beginning of the pandemic, there was a drastic reduction in access to oral healthcare services due to suspension of elective care and the restriction of healthcare procedures to emergency care[17]. In this context, healthcare management must be viewed as a reference for the availability and provision of health technologies according to demand, considering individual, family, professional, organizational, systemic, and societal dimensions[18]. Thus, the effectiveness of healthcare management requires a system that acts on situations that increase people's risk or vulnerability to diseases and illnesses. In oral health, efforts have been made to ensure a broader approach to oral health problems, seeking to overcome the hegemonic model of care, which has failed to establish comprehensive care for the population[19-21]. It is therefore relevant to investigate the possibilities of public services to provide proper oral healthcare management and the limitations involved. It is equally important to evaluate the losses suffered by the population, oral healthcare professionals, and the system, when such public services are inadequate. Few studies have been published on coping with the COVID-19 pandemic from the perspective of oral healthcare management in Brazilian patients. The objective of this study was to analyze the actions carried out in the scope of oral health care during the COVID-19 pandemic in the municipal context, based on a theoretical-empirical model on the significance of oral healthcare management.

MATERIALS AND METHODS

This was a cross-sectional study with a qualitative approach, carried out in two stages. The first stage dealt with the development of a theoretical-empirical model on the significance of oral healthcare management, based on the Grounded Theory method (GTM), in the Straussian perspective[22]. In the second stage, collaborative research[23-25] was carried out in partnership with stakeholders in the conduction phases (data collection and analysis) and dissemination of results[24], in which the model on oral healthcare management was applied with documentary analysis of the records produced and released regarding local actions taken to address the COVID-19 pandemic.

The theoretical model was developed through GTM in the context of Primary Healthcare (PHC) services in three municipalities in the state of Santa Catarina (Florianópolis, Biguaçu and São José) which have oral health teams at this level of care. The municipalities and participants were selected intentionally and for convenience. Health professionals with experience and knowledge in oral health care management were invited to participate in the composition of sample groups. The first sample group consisted of 14 professionals from the PHC oral health team (dentists, oral health technicians, and oral health assistants), and the second group consisted of five municipal health managers, totaling 19 participants.

In the second stage (Collaborative research), oral healthcare managers from four municipalities in Santa Catarina (Joinville, Lages, Rio do Sul, and Araquari) were selected intentionally and for convenience. They were contacted and invited to

participate in the study. Four managers joined, one from each municipality, all women, with extensive experience in oral health management. Table 1 presents information on each municipality.

To develop the theoretical model, data were collected through semi-structured interviews conducted by a single researcher with individual participants from November 2018 to April 2019, at a time and place chosen by the participant. The interviews were digitally recorded, and stored in MP3 format for subsequent transcription in Word, Microsoft® software.

To collect data on the actions taken, each manager listed the relevant documents from their municipalities, available in the public domain, which had been prepared from March to August 2020 to address the COVID-19 pandemic, including decrees, technical notes, and resolutions, among others. As inclusion criteria, texts that addressed any aspect related to the reorganization and re-adaptation of oral healthcare actions and services, including management and professional performance, were considered.

Following the GTM framework, interview data were collected and analyzed concurrently. Structured systematic analysis[22] was performed using the coding strategy (open, axial, and selective). In the open coding, a detailed analysis of the textual material was initiated, selecting information representative of the characteristics of the content, followed by grouping codes according to similarity. In the axial coding, conceptual categories were identified by compiling codes of the same property and dimension. Selectively, codes that did not respond to the objective of the study were disregarded. In the next phase, relationships were established among the categories, considering the components of the Strauss and Corbin paradigmatic model[26]: context, cause, intervening conditions, action strategies and consequence. Based on the orderly integration of the categories and referencing the scientific literature, the theoretical-empirical model on the significance of oral healthcare management was developed.

In the second stage, the model served as a reference for document analysis. The text of the documents from each municipality was analyzed individually by its respective collaborating manager, who was also in charge of filling out the data in a spreadsheet. A virtual meeting was held to standardize the analysis strategies with all participating researchers. A Microsoft Excel® spreadsheet was prepared, containing the categories of the theoretical model in the rows, distributed among each of the components, and the municipalities in the columns, resulting in cells for filling in the excerpts of the documents. Then, one of the authors (the study coordinator) proceeded to the Content Analysis stage. The excerpts were coded[27] and grouped by similarity for each municipality. At the end, all the information was gathered in a final column, discarding repeated or very similar codes, combining data from all four municipalities. For confirmation, adjustments, and final consensus, a second virtual meeting was held, at which the data analysis stage ended.

The study followed the guidelines and provisions of Resolution No. 466/12. The project was referred to the Human Research Ethics Committee of the Federal University of Santa Catarina (UFSC), and submitted to evaluation by the municipal entities involved. It was approved under number 1.789.874

Table 1. Characteristics of municipalities participating in Stages 1 and 2 regarding population, dentists in PHC, availability of oral health services and oral health coverage. 2020

	Stage 1			Stage 2			
	FLN	BIG	SJO	ARA	RSL	LAG	JVL
Estimated Population 2020	508,826	69,486	242,927	39,524	71,061	157,544	597,658
Dentists in PHC	76	19	50	8	21	38	118
Dentists in specialized and emergency services	27	16	18	0	17	24	37
Health centers under municipal management with oral health service	53	13	27	6	17	21	45
% Oral health coverage (Aug 2020)	30	53	24	41	66	85	27

PHC: Primary Health Care; FLN: Florianópolis City; BIG: Biguaçu City; SJO: São José City; ARA: Araquari City; RSL: Rio do Sul City; LAG: Lages City; JVL: Joinville City.
Source: Research data.

(CAAE 59833916.1.0000.0121). Participants provided informed written consent, which was kept by the authors.

RESULTS

As a result of the first stage of the study, the theoretical-empirical model on oral healthcare management was composed of ten analytical categories, divided into five dimensions (Table 2). The model points to the meaning of oral health care

Table 2. Oral healthcare management in coping with the pandemic of COVID-19: examples of Codes according to the Dimension and Category of the Analytical Model. Santa Catarina, 2020.

Dimension	Category	Code Examples
Context	Observing BPHS principles	State / government actions to address the pandemic. Compromise of comprehensive health care. Suspension of actions and services due to the risk of contamination. Limitation of access to healthcare services. Offer restriction for urgent and emergency actions only.
Cause	Inserting oral health in primary care and the role of public managers	Search for technical and scientific information to minimize the impact of the pandemic in relation to the health of professionals and users. Integration of oral healthcare with other areas in PHC. Creation of protocols with service flows. Verification of the peripheral role of dentistry when analyzing the Technical Notes and guidelines. Lack of integration of oral healthcare management at the federal, state, or municipal level. Provision of the necessary resources related to the biosafety of healthcare. The need to adapt the offices and physical spaces of healthcare clinics. Development of strategies for training PHC oral healthcare professionals.
Intervening Conditions	Promoting interdisciplinarity	There was a greater integration of oral healthcare professionals (OHP) with the rest of the healthcare team. OHP trained to support and perform screening in healthcare clinics. OHP contribute to monitoring suspected or confirmed cases of COVID-19. OHP actively participate in admittance and guidance of users. Meetings with managers to reorganize the work process. Participation of dentists in the COVID-19 screening center.
	Integrating teaching and service	Absence of teaching-service integration in smaller municipalities. Internships in healthcare clinics suspended without expected return. The interactions between professors, tutors, and students became virtual through use of communication and information technologies.
Action Strategies	Listening to the user	The meetings of the municipal health council became virtual. Institution of an emergency council composed of representatives of the executive, the legislature, and civil society.
	Ensuring access to oral healthcare	Elective dental care suspended or restricted. Decrease in the offer of vacancies to ensure less flow of patients at healthcare clinics. Care restricted to pregnant women and emergency care for patients with chronic illnesses. The municipality follows the guidelines of the Ministry of Health (MH) and the State Health Secretariat (SHS). Reduction in the number of oral healthcare professionals through dismissal of those belonging to a risk group. Healthcare clinics extend hours of service, assigning work shifts to the teams. Emergency Care Unit with dental care 24 hours a day and seven days a week.
	Monitoring oral health indicators	Oral health indicators continue to be monitored using existing information systems. MH and SHS stated in a note that the transfer of funds to oral healthcare services (PHC, RDPL and DSC) will not be affected due to the reduction / suspension of services. Creation of a commission to adapt and improve the electronic production data management system.

Consequences	Organizing the work process	Professionals from risk groups, symptomatic, or those who tested positive for COVID-19 were removed by granting vacation, leave, or work from home. Changes in biosafety issues required adjustments to the offices, patient screening, alignment with the reception team and use of electronic tools. Creation of protocols for the organization of oral healthcare. The number and time of consultations were modified considering the structure and quantity of equipment and the time required for aerosol sedimentation and disinfection of the environment. Assistance was concentrated to make up for the lack of dental equipment and PPE for all professionals.
	Integrating health promotion, education, prevention, assistance, and rehabilitation actions	Educational materials were developed to be shared on social networks. Guidance on dental care and the importance of oral hygiene during the pandemic period. The actions of promotion, education and prevention for oral health care were impaired due to the halt in the activities of the Health at School Program. Delivery of toothbrushes, toothpaste, and folders with oral hygiene guidelines to the students at the municipal schools where classes were suspended. Home visits were restricted to the active search for pregnant women. Educational actions with the group of pregnant women were maintained through virtual means.
	Performing actions in the field of dentistry	The professionals carried out distance training on the topic of Minimal Intervention in Dentistry. Urgent care remains with minimally invasive procedures. Aerosol-generating procedures are scheduled for specific days. The attendance at the DSCs was initially suspended and the resumption was gradual, with a reduction in the number of consultations. Return of the most needed referrals to the RDPL. The biopsy service continued throughout the period. Care for hospital-level surgeries for special patients was maintained.

RDPL=Regional Dental Prosthesis Laboratory; PPE=Personal Protective Equipment; MH=Ministry of Health; DSC=Dental Specialty Center; PHC=Primary Health Care; OH=Oral Health; OHP=Oral Health Professionals; SHS=State Health Secretariat; ECU=Emergency Care Unit.
Source: Data produced by the study from document sources.

management as the guarantee of comprehensive care for the population, presenting as action strategies the following: guaranteed access, monitoring of indicators and listening to users. Interdisciplinarity and teaching-service integration are conditions that intervene in the qualification of healthcare. Although they present a significance that is consistent with the care model recommended by the Brazilian Public Health System (BPHS), in practical terms, actions remained focused on a traditional, individual, clinical-curative approach. In this case, the participation of professionals and users in decision-making is limited. The imbalance between the availability of professionals and the high demand for oral health care prevents its management to be fully exercised in practice. Even though oral health professionals are aware of the importance of actions to promote oral health, and thereby quality of life and disease prevention, from a collective perspective, they assign minimum space to them in their routine. Table 2 also summarizes the findings of the main codes that represent actions in the field of oral healthcare undertaken to address the COVID-19 pandemic. The results are reported below to explain the integration of the analytical categories of the model with the content resulting from the study documents.

The context of oral healthcare management: observing BPHS principles

The model presented identifies the observance of the fundamental doctrinal and organizational principles of BPHS, while pointing out certain difficulties faced in putting them into practice, reflecting a conflict between intentionality and realities in the organization of oral healthcare. It recognizes the guarantee of comprehensive oral healthcare, with continuity in actions and services, scope (promotional, preventive, curative, and rehabilitation), both individual and collective, as well as coordination among the levels of healthcare. It also highlights interdisciplinarity, healthcare provision, bonding, humanization, health surveillance, and intersectionality as necessary strategies for oral healthcare management.

This background is also supported by the content of the documents analyzed, as they seek to respond to the impacts of the pandemic based on these fundamental principles. Recognition of these principles is maintained, with emphasis on the suspension of elective dental care at the primary, secondary, and tertiary levels and collective actions for oral health, as well as the limitation to emergency care as events that compromise users' access and comprehensive care.

The documents reveal the local efforts necessary to adapt and reorganize oral healthcare services, so that they may contribute to coping with the pandemic, maintain urgent care, and gradually return to healthcare in the safest way, according to each new epidemiological situation.

The insertion of oral health care in primary care and the role of managers

Oral healthcare management is shaped by the actions and services coordinated by the PHC. The profile of the oral health manager and the mechanisms for financing oral health via financial incentives are factors that directly influence oral healthcare management. Also noteworthy are political interventions, incomplete health teams, lack of physical infrastructure, targeting preventive actions solely to teams working in the Family Health Strategy (FHS), and focusing on the production of clinical procedures to the detriment of oral health promotion actions.

In the context of the pandemic, new challenges have arisen in the management of oral health care. Technical and scientific information was sought to minimize unwanted effects on the health of users and professionals. An adaptation of the physical structures of the clinics in the Health Center (HC) was developed, as well as greater integration and support of the oral health teams with the medical/nursing teams, who began to perform other duties in the PHC. Managers and health professionals organized themselves to develop biosafety protocols, provide guidance on the flow of care, and provide training for health professionals.

Data analysis revealed different experiences according to the management and financing capacity of the four municipalities. Biosafety materials were purchased for professionals working on the front lines of COVID-19 to a lesser extent for small municipalities. In addition, there was a mismatch of information in the contents of the municipal documents, which proved to be disaggregated in relation to the official technical notes at federal and state levels.

The promotion of interdisciplinarity and the integration of teaching and healthcare service as intervening conditions

Interdisciplinarity was identified through the insertion of students and interaction/collaboration among the members of oral healthcare teams with other health professionals. The context of the pandemic meant that oral health teams were assigned to assume active roles, through the implementation of collaborative practices and integration among teams and professionals from other areas. Strategies were adopted to reorganize the work process, such as meetings between oral health professionals and managers; training to collaborate in the reception, screening, and monitoring of suspected or confirmed COVID-19 patients, and providing care at screening centers.

The documents showed that teaching-service integration was absent from smaller municipalities. Municipalities that provide internships for undergraduate dental students reported that these activities had been suspended. Strategies were adopted using Health Information and Communication Technologies (HICT) to circumvent the suspension of activities and avoid educational setbacks. Activities were reorganized for remote access to maintain permanent education and professional training.

Action strategies for oral healthcare management: ensuring access, monitoring indicators, and listening to the user

The theoretical model considered oral health management beyond the clinical-care activities performed by dentists in PHC. However, it revealed that the workload and the production indicators based exclusively on quantity of dental procedures do not allow proper management of oral healthcare. Another situation with negative influence is the disproportion between oral health teams and the population in the area covered by oral healthcare clinics, as well as incomplete oral health teams (i.e., no oral health assistant). Considering the care aspect, the pandemic significantly reduced users' access to oral health services. For the protection of users and professionals, at first, elective dental care

was suspended or restricted according to guidelines from responsible entities.

The municipalities followed the guidelines from the Ministry of Health and State Health Secretariat, maintaining only emergency dental care if the potential risk in the region was severe or extreme, according to the potential risk assessment matrix for COVID-19. Urgencies and emergencies were maintained in the HC and emergency rooms. Consultations with the Dental Specialty Centers (DSC) were initially suspended and then resumed partially and gradually, according to the oral health management guidelines of each municipality. Decisions on the return of activities were based upon the possibilities of making necessary adjustments to the work process to ensure biosafety for users and professionals.

Care and procedures offered were restricted due to concern regarding aerosol-generating procedures, and to the reduction in the number of oral health professionals available as some of them were on leave because they belonged to the groups most vulnerable to COVID-19. The aim of the initial suspension of elective care was to prevent the spread of the virus. Measures were provided to adjust and adapt to biosafety standards.

Adequate supplies were purchased to address the pandemic, and there was a redistribution of oral healthcare teams in the HC, adapting processes, work shifts, and workplaces. Exclusive areas were assigned for the flow of users with symptoms of the disease. These measures prevented crowding of users in waiting rooms and reduced contact between COVID-19 symptomatic and asymptomatic patients.

Communication channels with the community were opened using HICT. Service teams were organized and trained to provide information and perform pre-clinical care by phone, video calls, and text messages. In this way, guidelines on COVID-19, health in general (including oral health) and psychological guidelines for health professionals were implemented.

Oral health indicators continue to be entered into the information systems for monitoring. The availability of financial resources (state and federal) would not be affected by the reduction/suspension of services, in either primary or secondary care (DSC and Prosthetic Laboratories).

The social isolation imposed by the COVID-19 pandemic hindered personal contact between managers and users. Health councils began to meet on virtual platforms available to the community. Emergency committees (composed of community representatives, city councils, health departments, public and private institutions, and local businesses, among others) were set up to organize contingency plans and guidelines on appropriate conduct to guarantee the health and safety of users and healthcare professionals.

The consequence of oral healthcare management in primary health care: organization of the work process through the integration of health promotion, education, prevention, assistance, and rehabilitation actions, with a focus on dentistry

The organization of the work process of oral health professionals as a consequence of the management of oral health care was identified. The work process seeks to integrate actions of health promotion, education, prevention, assistance, and rehabilitation. In this regard, the organization of the agenda is important because it is the means by which healthcare professionals define how much time they allocate to each activity, with the focus remaining on clinical actions.

Dental care was reorganized in response to the pandemic. There was a change in routines to include careful anamnesis with focus on COVID-19, previously screened urgent and emergency care, application of minimally invasive dentistry techniques, and reinforcement of strict biosafety protocols. The “advanced access” model (i.e., resolving the user’s main complaint at the time of the first consultation) was adopted in the HC, to seek greater resolution and reduce patient flow to a minimum.

Specialized care was suspended and/or reduced, with losses in the referral and counter-referral processes in municipalities where there is no regulation system.

The reduction of clinical work due to restrictions on clinical visits led to dentists becoming involved in actions going beyond clinical activities. For example, they developed and distributed to the public health education pamphlets about oral hygiene habits and their relationship with the spread of the new coronavirus. They also posted information on the topic on social networks and other media.

Some, though not all municipalities sought ways to benefit families who participated in the Health at

School Program by including oral hygiene products in the basic food baskets delivered to them. In addition, community health agents conducted a campaign through video lectures providing guidelines for prenatal care.

DISCUSSION

This study analyzed a series of actions in the fight against the COVID-19 pandemic by promoting oral healthcare in four municipalities of Santa Catarina State, using as a reference the dimensions of the theoretical-empirical model on oral healthcare system management. The framework of the model enabled the study of the contents of the documents guiding oral healthcare manager decision-making for organizing actions and services. All the dimensions of the model identified actions to combat the pandemic, finding that the actions either ratified, amplified, or contradicted the model initially studied for theoretical development, compromising, in the latter case, oral healthcare management.

The literature has called attention to the fact that the crisis caused by the pandemic[28,29] brings with it an opportunity to rethink public management practices and the work of oral health professionals. Efforts must be made to be more responsive to the needs of the population, prioritize groups with a greater burden (or risk of development) of oral diseases, recover the preventive approach, and discuss such obstacles in the oral healthcare system[2,6,30,31].

Limiting the offer of public healthcare services to emergency care is particularly damaging to the most vulnerable populations, who are also at greater risk of contracting COVID-19, thus exacerbating inequity[32]. The literature regards this situation as an opportunity to redirect dental practices to the preventive approach[6], rethink the future of dentistry, and to discuss the failures of the healthcare system[31]. The relevance of studies such as the current one is that they show the possibility of coordination between theoretical references and the implementation of public policies, as well as the way in which their actions result in benefits for the population.

Several studies have reported reduced access to dental care upon restricting the supply of care only to urgent cases[33-36]. The crisis caused by the abrupt need to suspend oral healthcare of elective nature[6,9,37] accentuated the overvaluation attributed to individual clinical dental procedures. As an immediate response, priority strategies were adopted to make dental care feasible, presented in both governmental[5] and multilateral documents[38] and in scientific literature[10]. Such measures have proven to be effective in controlling the transmission of the virus and reducing occupational risk for public service professionals involved in dental care[11].

In parallel, an amplification of oral healthcare management was identified by incorporating interdisciplinary actions and services. This integration among professionals has been reported as an important strategy in the consolidation of oral healthcare[30] given that these professionals are not usually present in actions of this nature. In particular, dentists can and should contribute to the workforce in a global health crisis[31], considering that they are trained in dealing with stressful situations, generally possess communicative skills, are familiar with infection control procedures, routinely use protective equipment, are immunized against the main infectious diseases, and tend to be familiar with protocols of medical emergencies[12].

Although studies show that there is persistent difficulty in coordinating and integrating oral healthcare management in the different sectors and levels of the healthcare system[14,30], in order to define strategies to address COVID-19 it was necessary to promote such discussions, including oral healthcare issues, with managers. This aspect was identified as enhancing oral healthcare management from a proactive position of the oral health manager in solving the problems arising from the pandemic. However, it is noteworthy that in Brazil, during the first months of the pandemic, oral health and the particularities of dental care were not included in government publications. This made it difficult to obtain targeted, standardized, science-based guidelines, and to make them widely known among managers and professionals. In a way, this was overcome when the Ministry of Health published the "Guide for dental care in the context of COVID-19"[14].

The guarantee of comprehensive healthcare depends on the degree of implementation and functionality of healthcare systems themselves[15]. Even before the pandemic, this was already a challenge for oral health care[14] due to an insufficient workforce and the requirement of managers for minimal clinical production in a scenario of high patient demand. In this regard, other studies also show that the high demand from patients interferes with the continuity

of care, as well as its effectiveness, quality of care, and the coordination among different levels of healthcare[15,27]. With the pandemic, the situation was aggravated by the limitation or impossibility of conducting collective actions, typically by educational and preventive health promoters, and of maintaining full dental services in PHC and specialized services.

The adoption of "advanced access" was mentioned as a model to reorganize the provision of oral health care. Although there is almost no scientific production on the subject in the field of dentistry, this strategy seeks to balance demand and service capacity, with greater control over users' waiting time. The literature points it out as a promising scheduling model for primary care because it provides the possibility of offering consultations in sufficient quantity for that day, not restricting future consultations, and ensuring continuity of care[22]. The feasibility and effects of its adoption in a pandemic period still need more research.

Care management involves interventions in the field of health promotion, disease prevention, treatment, and rehabilitation[14]. When considering care as a technology for organizing dental work processes, it is necessary to search for ways to achieve comprehensiveness in a broad perspective, and therefore to reflect on the care technologies that are established in public policy actions for oral health[15]. Outstanding among these identified care technologies is the use of teledentistry, especially as an alternative tool for contacting patients via telemonitoring and tele-guidance. The literature has positively reported the use of strategic teledentistry in public oral health services. Particularly, in the new pandemic scenario, it has gained strength as one of the central strategies for the reorganization of oral health care practices remotely[30-32].

This study had some limitations. Initially, the intentional selection of participating municipalities restricts the ability to generalize the results. Despite recognizing the different coping capacities of each municipality, due to the availability of institutional and financial resources, it was not an objective to compare local government actions. Furthermore, the realities presented do not necessarily represent other municipalities in the same South Brazilian state. It was the first opportunity in which the theoretical-empirical model on oral health care management was used to analyze oral health actions and services, not having undergone a previous validation process. The collection of data by means of documents refers only to the events recorded by those municipalities, without allowing value judgment or inference of difficulties or facilities experienced by management, professionals, or users.

Thus, it is suggested that studies should be conducted to gain more in-depth theoretical constructs on oral healthcare management, thereby improving models of this kind. The relevance of continuing to study the implications of decision-making by local management in relation to public oral health actions and services in coping with the pandemic is emphasized. Particularly noteworthy are responses regarding access and comprehensive care, the care of the most vulnerable populations, the psycho-emotional burden of professionals, biosafety practices, and the incorporation of less invasive and more effective dental technologies and clinical procedures. Additionally, the worsening of epidemiological indicators must be put into context, highlighting the importance of ongoing maximum alert status of oral health services and academia.

It is hoped that this study contributes to improvements within the scope of oral healthcare management at local level. Only with the evolution of scientific and technological knowledge and its application in natural settings will it be possible to implement safer and more viable ways of providing oral healthcare to the population in the context of the COVID-19 pandemic.

CONCLUSION

This study identified actions to tackle the COVID-19 pandemic in all dimensions of the oral healthcare management model while following BPHC principles, the role of oral health management, access, comprehensiveness, interdisciplinarity, teaching-service integration, popular participation, monitoring of indicators and actions specific to oral healthcare clinics. These actions ratified, amplified, or compromised the management of oral health care. In the four municipalities studied, there was a reduction in the supply of services due to the restriction in access to elective dental care and preventive actions; search for guarantee of biosafety assistance standards; dissemination of standardized and science-based guidelines for professionals and the population; attempt to maintain the comprehensiveness of care through the readaptation

of specialized services and collective actions; and relocation of oral health professionals to assist in other sectors and activities. Oral healthcare management can serve as a reference to redesign oral health actions and services at the local level during the COVID-19 pandemic, in an expanded perspective.

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

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