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Comparison of osseointegration in areas grafted with deproteinized bovine bone and native bone. A preclinical study

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ABSTRACT

The aim of this study was to evaluate the osseointegration of implants placed in rat tibia sites grafted with Deproteinized Bovine Bone (DBB) and Native Bone (NB). Twenty-eight rats were divided into two groups according to the type of substrate in which the implants were to be placed: NB – implants placed in native bone; DBB – implants placed in areas grafted with DBB. In the DBB group, the bone defect was made and filled with the bone substitute 60 days before placing the implant. The animals were euthanized 15 or 45 days after implant placement. Osseointegration was assessed by the removal torque, volume of mineralized tissues around the implants (BV/TV), bone-implant contact (%BIC), and bone between threads (%BBT). The implants placed in NB presented higher removal torque (8.00 ± 1.26 Ncm vs. 2.33 ± 0.41 Ncm at 15 days and 22.00 ± 2.44 Ncm vs. 4.00 ± 1.41 Ncm at 45 days), higher %BV/TV (47.92 ± 1.54% vs. 33.33 ± 4.77% at 15 days and 70.06 ± 0.91% vs. 39.89 ± 5.90% at 45 days), higher %BIC (39.68 ± 5.02% vs. 9.12 ± 5.56% at 15 days and 83.23 ± 4.42% vs. 18.81 ± 7.21% at 45 days), and higher %BBT (34.33 ± 5.42% vs. 13.24 ± 8.72% at 15 days and 82.33 ± 3.13% vs. 22.26 ± 8.27% at 45 days) than the implants placed in DBB grafted areas. The degree of osseointegration was lower in implants placed in the area grafted with DBB than in NB in rat tibias.

Keywords: *bone substitutes - dental implants - osseointegration.*

Comparação de osseointegração em áreas enxertadas com osso bovino desproteinizado e osso nativo. Estudo Pré-clínico

RESUMO

O objetivo deste estudo foi avaliar a osseointegração de implantes instalados em sítios enxertados com Osso Bovino Desproteinizado (DBB) e Osso Nativo (NB). Vinte e oito ratos foram alocados em dois grupos de acordo com o tipo de substrato onde os implantes foram colocados: NB - Implantes colocados em osso nativo; DBB - Implantes instalados em áreas enxertadas com DBB. No grupo DBB, o defeito ósseo foi confeccionado e preenchido com o substituto ósseo 60 dias antes da instalação do implante. Os animais foram sacrificados após 15 e 45 dias da colocação do implante. A osseointegração foi avaliada pelo torque de remoção, volume de tecidos mineralizados ao redor dos implantes (%BV/TV), contato direto do osso com o implante (%BIC), e área de osso entre roscas dos implantes (%BBT). Os implantes instalados em NB tiveram um maior torque de remoção (8.00 ± 1.26 Ncm vs. 2.33 ± 0.41 Ncm aos 15 dias e 22.00 ± 2.44 Ncm vs. 4.00 ± 1.41 Ncm aos 45 dias), um maior %BV/TV (47.92 ± 1.54% vs. 33.33 ± 4.77% aos 15 dias e 70.06 ± 0.91% vs. 39.89 ± 5.90% aos 45 dias), um maior %BIC (39.68 ± 5.02% vs. 9.12 ± 5.56% aos 15 dias e 83.23 ± 4.42% vs. 18.81 ± 7.21% aos 45 dias), e um maior %BBT (34.33 ± 5.42% vs. 13.24 ± 8.72% aos 15 dias e 82.33 ± 3.13% vs. 22.26 ± 8.27% aos 45 dias) que os implantes colocados nas áreas enxertadas com DBB. Implantes instalados em áreas enxertadas com DBB apresentaram menor osseointegração que os implantes instalados no osso nativo em tíbias de ratos.

Palavras-chave: *implantes dentais - osseointegração - substitutos ósseos.*

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INTRODUCTION

The osseointegration process is the basis for the treatment of edentulism with dental implants. Osseointegration consists of bone tissue formation in direct contact with implant surfaces[1], which enables the implants to withstand occlusal chewing forces predictably for long-term periods[2,3]. All types of edentulism have been treated successfully with implant-supported prostheses[3,4], however, the presence of bone tissue in good quantity and quality for implant placement is not always observed[5,6]. Guided bone regeneration (GBR) techniques have therefore often been used to increase the availability of bone tissue for implant placement[6,7].

Although autogenous bone graft is considered the gold standard bone substitute biomaterial[8,9], autograft can cause side effects related to donor site morbidity, and has limitations due to the limited availability and high resorption rates of this kind of graft[10]. This has promoted the use of alternative bone substitutes, especially deproteinized bovine bone (DBB), in guided bone regeneration techniques[7,11].

DBB is an osteoconductive bone substitute that is efficient for treating bone defects with high success rates and predictability in humans[12,13]. Preclinical studies have shown that DBB presents low rates of resorption, which benefits the maintenance of the volume of the grafted area[14-16]. However, this property is related to the reduction of bone formation observed in areas grafted with DBB[14,17]. The impact of this reduced bone formation in the grafted area on the osseointegration process has been little explored. The objective of this preclinical study was to compare the osseointegration of implants placed in areas of native bone and DBB grafted area in rat tibias.

MATERIALS AND METHODS

This study was submitted and approved by the Animal Ethics Committee of our institution (CEUA: 26/2016). Twenty-eight rats (Rattus novergicus, Hotzman variation), 12 weeks old, weighing 250–300 g, were used. The animals were kept in an environment with controlled temperature (21 ± 1ºC), humidity (65-70%), and light cycles (12 hours). They were offered water and food *ad libitum*. This study was conducted according to the ARRIVE protocol for preclinical studies.

Groups and study design

The animals were randomly assigned to 2 groups of 14 animals each, according to the type of substrate where the implants were to be placed: NB Group –implants were placed in native bone; DBB Group: implants were placed in areas previously grafted with Deproteinized Bovine Bone (Bio-Oss®, Geistlich AG, Wolhusen, Switzerland – Small granules 0.25-1mm). The bone defect was performed and grafted with the DBB 60 days before implant placement. At baseline, the implants were placed directly in the native bone (NB) or in the areas grafted with DBB. After 15 or 45 days, the animals were euthanized by anesthetic overdose (Fig. 1).

Surgical procedure – Bone defect and grafting procedures

The animals in the DBB group were anesthetized by a combination of Ketamine (Agener União Ltda, Sao Paulo, SP, Brazil) at 0.08 ml / 100g body mass with Xylazine (Rompum, Bayer SA, Sao Paulo, SP, Brazil) at 0.04 ml / 100g body mass.

An incision was made in planes over the tibial tuberosity. The bone tissue was submitted to osteotomy by means of a counter-mounted spherical drill with the aid of a 1200 rpm electric motor (BLM 600 - Driller, São Paulo, SP, Brazil) under abundant irrigation with sterile saline solution. The final measurements of the defects formed were 4mm in length and width, and 1.5mm in depth, and they were subsequently filled with DBB. The tissue was sutured by planes internally with 5.0 resorbable thread (Vicryl Ethicon, Johnson & Johnson, São Jose dos

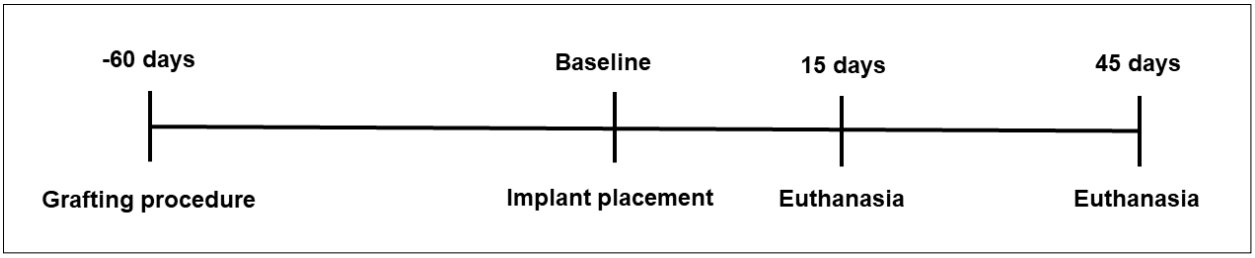

Fig. 1: Flowchart of the study design.

Campos, Brazil) and externally with 4.0 silk thread (Ethicon, Johnson & Johnson, São Jose dos Campos, Brazil). The animals received a single intramuscular dose of streptomycin-associated penicillin at 0.1 ml / kg (Multibiotic Small, Vitalfarma, São Sebastião do Paraíso, MG, Brazil) and 0.1 ml / kg ketoprofen (Ketoflex; Mundo Animal, São Paulo, Brazil).

Surgical procedure – Implant Placement

After 60 days, the animals in both groups were subjected to implant placement in the NB and DBB areas. An incision similar to the first procedure was made over the tibial tuberosity in right and left tibias. The grafted region was prepared for implant placement by applying a progressive sequence of drills (spear drill; 2.0 mm spiral drill - Neodent®; Curitiba, PR, Brazil) to accommodate a machined surface implant 4 mm high and 2.2 in diameter (Neodent®; Curitiba, PR, Brazil). All drilling was performed with the aid of an electric motor, adjusted to 1200 rpm, under abundant irrigation with sterile saline solution. The implant was installed with the aid of a digital key (1.2mm hexagonal digital key - Neodent, Curitiba, PR, Brazil). The tissue suture and the postoperative drug protocol were similar to those used in the first surgery. The animals in the native bone group were only subjected to surgery for implant placement with the same surgical and post-surgical protocols. The right tibia was used for microtomographic and histomorphometric analysis, while the left tibia was used for biomechanical analysis.

Biomechanical Evaluation

After euthanasia, the left tibias were stabilized in a small vise. A hexagon wrench was attached to both the implant and torque wrench (Tohnichi, model ATG24CN-S, Tokyo, Japan) and a counterclockwise movement was performed to unscrew the implant. The maximum torque required to move the implant was noted as the removal torque value (Ncm).

Microtomographic evaluation

The right tibias were fixed in 4% paraformaldehyde for 48 hours and stored in 70º alcohol. These samples were scanned by micro CT scan (Skyscan, Aatselaar, Belgium) with the following parameters: Camera pixels: 12.45; x-ray tube power: 65 kVP, x-ray intensity: 385 µA, integration time: 300 ms, filter: Al-1 mm and voxel size: 18 µm3. The images were reconstructed, spatially repositioned and analyzed by specific software (NRecon, Data Viewer, CTAnalyser, Aatselaar, Belgium). The region of interest (ROI) was defined as a 0.5 mm circular region around the entire diameter of the implant. This ROI was defined as Total Volume (0.5mm margin around implants - 4.5mm x 3.2mm). The threshold used in the analysis was 25-90 shades of gray, and the volume values of mineralized tissue around the implants (BV/TV) were obtained as a percentage[18]. A trained examiner blinded to the experimental groups performed this analysis.

Histomorphometric evaluation

After scanning, the right tibias were dehydrated in a staggered ethanol solution (60 - 100%) and embedded in light-curable resin (Technovit 7200 VLC, Kultzer Heraus GmbH & CO, Wehrheim, Germany). The blocks containing the implant and bone tissue were cut at a central point using a disposable system (Exakt Apparatebeau, Hamburg, Germany). The final sections were approximately 45 µm thick. They were stained with Stevenel's blue associated with acid fuchsin and analyzed under an optical microscope (DIASTAR - Leica Reichert & Jung products, Wetzlar, Germany) at 100X magnification. Histomorphometric evaluation was performed using image analysis software (Image J, San Rafael, CA, USA). The percentages of bone-implant contact (% BIC) and bone area between implant turns (% BBT) were evaluated separately in the first three threads. These analyses were performed by a blind, trained examiner.

Statistical analysis

GraphPad Prism 6 software (San Diego, CA, USA) was used for the statistical analysis. The data generated by the histometric, microtomographic and biomechanical analyses were numerical, so they were submitted to the Shapiro-Wilk Normality test to evaluate whether they were distributed according to the central distribution theorem. All data distributed according to the normallity. Then, the parametric unpaired t-test were used for the inferential analysis. All tests in this study were applied with a significance level of 95%. The sample size calculation was referenced to % BIC data from a previous study that evaluated the effect of an implant surface osseointegration in grafted areas in a similar experimental model and assessment as performed in

this study[19]. Considering that the smallest difference between the means in the groups where there were statistically significant differences was 19.29% with standard deviation difference between these groups 6.59%, it was found that a sample of 7 animals per group / period was sufficient for application of statistical tests with type α error set at 0.05 and β power of 0.90.

RESULTS

All animals survived after the surgical procedures and were healthy throughout the experimental period.

Removal torque analysis

Removal torque increased in the longer evaluation times in both groups ($p<0.05$). The implants placed in DBB-grafted areas presented lower removal torque values than implants placed in native bone at both evaluation times (8.00 ± 1.26 Ncm vs. 2.33 ± 0.41 Ncm at 15 days and 22.00 ± 2.44 Ncm vs. 4.00 ± 1.41 Ncm at 45 days) ($p <0.05$) (Table 1).

Table 1. Mean and standard deviation of implant removal torque data in all experimental groups and periods

Groups / Period	15 days	45 days
DBB	2.33 ± 0.41[b]	4.00 ± 1.41[a]
NB	8.00 ± 1.26[***b]	22.00 ± 2.44[***a]

*** p <0.05- Higher value of implant removal counter torque compared to the DBB group – unpaired t-test. Different letters indicate different statistical levels within each group – unpaired t-test

Micro Ct analysis

The BV/TV around the implants was higher at 45 days than at 15 days for both groups. Implants placed in NB areas presented higher BV/TV values than implants placed in DBB grafted areas at both times (47.92 ± 1.54% vs. 33.33 ± 4.77% at 15 days and 70.06 ± 0.91% vs. 39.89 ± 5.90% at 45 days) ($p <0.01$) (Table 2).

Table 2. Mean and standard deviation of BV / VT data around implants in all groups and experimental periods

Groups / Period	15 days	45 days
DBB	33.33 ± 4.77[b]	39.89 ± 5.90[a]
NB	47.92 ± 1.54[***b]	70.06 ± 0.91[***a]

*** p <0.01- Higher BV / TV value compared to the DBB Group- unpaired t-test. Different superscript letters indicate different statistical levels within each group – unpaired t-test

%BIC and %BBT analysis

Histometric analysis showed that the degree of osseointegration improved at 45 days compared to 15 days for both groups. However, the implants placed in DBB grafted areas presented lower %BIC (39.68 ± 5.02% vs. 9.12 ± 5.56% at 15 days and 83.23 ± 4.42% vs. 18.81 ± 7.21% at 45 days), and %BBT (34.33 ± 5.42% vs. 13.24 ± 8.72% at 15 days and 82.33 ± 3.13% vs. 22.26 ± 8.27% at 45 days) values than implants installed in NB at both evaluation times ($p <0.001$) (Table 3). Fig. 2 shows representative histomorphology images.

Table 3. Mean and standard deviation of %BIC and %BBT data in all groups and experimental periods

Parameters	Groups / Period	15 days	45 days
%BIC	DBB	9.12 ± 5.56[b]	18.81 ± 7.21[a]
	NB	39.68 ± 5.02[***b]	83.23 ± 4.42[***a]
%BBT	DBB	13.24 ± 8.72[b]	22.26 ± 8.27[a]
	NB	34.33 ± 5.42[***b]	82.33 ± 3.13[***a]

*** p <0.05- Higher %BIC and %BBT value than the DBB group - unpaired t-test. Different superscript letters indicate different statistical levels within each group – unpaired t-test

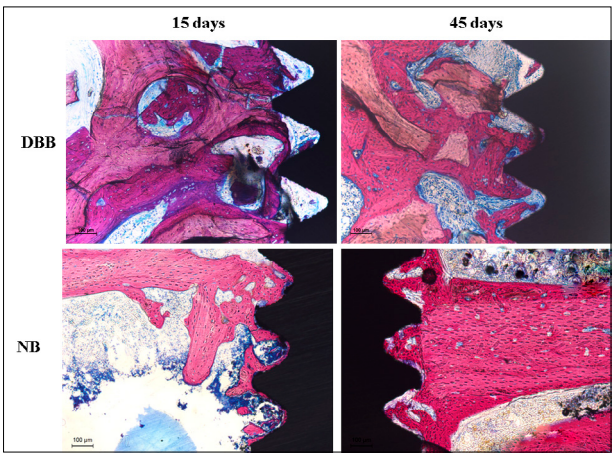

Fig. 2: Representative images of the non-decalcified sections showing a better pattern of the osseointegration of implants placed in NB than of implants placed in DBB.

DISCUSSION

In some clinical conditions, limited bone availability for direct implants warrants grafting procedures[7]. Due to the limitations of autogenous bone grafts[10], the use of osteoconductive bone substitutes has become more commonplace[11]. Placing implants in

areas grafted with osteoconductive biomaterials can improve implant success rates[20]. Although previous systematic reviews have shown that DBB induces sufficient bone formation for implant installation with high success rates[21,22], some clinical studies have shown that implants placed in areas grafted with DBB presented relatively lower success rates[13,20] than implants placed in native bone[23]. In fact, it is likely that the lower formation of bone tissue associated with the presence of biomaterial particles that are included in the matrix, but remain non-vital, may influence the resistance of these grafted areas to microbial challenges, as well as reducing the osseointegration process. In general, this study demonstrated that implants placed in areas grafted with DBB presented worse parameters than implants placed in the native bone area in all the analyses performed to evaluate the osseointegration process.

The implants placed in areas grafted with DBB had lower removal torque values than implants placed in native bone area. When dental implants are placed in grafted areas, it is recommended clinically to lock the apical portion of the implants in native bone in order to ensure good primary stability and avoid having a large portion of the implant remaining within the grafted area[24]. In the experimental model used in this study, two thirds of the implant were within the grafted area[25]. Thus, the experimental model used may explain the poor result of secondary stability achieved by the implants placed in the DBB group.

Moreover, the smaller amount of mineralized tissues around the implants placed in the grafted areas may also have had a negative influence on the biomechanical parameters of the DBB group in this study. The BV/TV data recorded in this study contradict other studies that have reported that areas grafted with DBB have good properties for maintaining volume and filling bone defects[15,26]. However, it should be noted that in this study, the comparison was performed with the native bone of the tibia, which has cortical morphology, certainly influencing the BV/TB results for the NB group. It is also likely that a good part of the tissue repaired in the grafted areas with DBB in its coronal portion is soft tissue. Since a membrane was not used to cover the defects, the more coronal DBB particles may have been involved by fibrous connective tissue, a finding that has been described previously in a preclinical study evaluating the healing of post-extraction sockets filled with DBB in dogs[15].

Another interesting finding in this study was that the data from the histometric analysis (%BIC and %BBT) were also lower in implants placed in areas grafted with DBB than in implants placed in a native bone area. These results agree with histological findings from another preclinical study that demonstrated that implants placed in edentulous canine jaws previously grafted with DBB presented higher osseointegration with the native lingual bone crest than with the buccal bone crest contained the grafted area[26]. In addition, implants placed in mini-pig maxillary sinuses that were grafted with autogenous bone also showed a higher degree of osseointegration than implants placed in maxillary sinuses grafted with DBB (42.9% vs. 13.9%)[27]. The data from the current and the abovementioned studies showed that the reduced bone formation in areas grafted with DBB has a negative influence on the osseointegration process in these areas.

Despite the findings of the current study, the limitations regarding the use of autogenous bone graft and in healing critical defects commonly present in the oral cavity do not contraindicate the use of DBB as a bone substitute material. It is worth mentioning that DBB has been applied with great success in different clinical situations, such as in the maintenance of post-extraction dental sockets[28], maxillary sinus lifting[29], and in augmentation of vertical and horizontal bone tissue in edentulous edges[30]. However, the waiting time for implants placement in the grafted areas and the application of prosthetic loads should be performed later in grafted areas when the implants could not be placed immediately in a good amount of native bone[20]. In addition, supportive therapy in these regions should be performed more frequently than with implants that have been installed in areas of native bone[31]. Finally, the search for associations of growth factors that can improve the pattern of bone tissue formation in areas grafted with DBB should be investigated.

The current study has some drawbacks that should be considered for the interpretation of our findings. It used implants with untreated surfaces, which are rarely used in daily clinical practice. The pattern of osseointegration in grafted areas is better when treated surfaces are used[19]. Another important limitation is that these results are more applicable in situations of previously grafted and healed alveolar

ridges, and these findings are not applicable to clinical situations where the implants are placed immediately. On the other hand, it is possible to infer that the prosthetic loading protocols for implants placed in grafted areas with DBB may be delayed compared to implants placed in native bone, according to the findings of this study.

To conclude, implants placed in areas grafted with DBB presented a lower degree of osseointegration than implants placed in native bone in a rat tibia model.

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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Determination of microhardness of bulk-fill resins at different depths

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ABSTRACT

The aim of this study was to determine Vickers microhardness (HV) in bulk fill resins at different depths. Test specimens were prepared with different bulk fill resins: Filtek Bulk-Fill (3M ESPE) [FBF], Surefill SDR flow (Dentsply) [SDR], Fill-UP (COLTENE) [FU] and Surefill (Dentsply) [SF]. Semi-cylindrical test specimens were prepared in a mold 6 mm in diameter and 4 mm thick (n=5). A 1000 mW/cm2 light curing unit was applied (Coltolux LED - Coltene) for 20 seconds. HV was determined with three indentations (Vickers Future Tech FM300, 300 g, 8 s) at four depths: 1, 2, 3 and 4 mm from the top surface to the interior. Data were recorded immediately (t0) and 24 hours later (t24). Results were analyzed with two-way ANOVA (p<0.05), and multiple comparisons were performed using Tukey's test. Mean and SD of HV at t0 for each mm were: [FBF] t0: 49.23(4.65) / 48.32(3.36) / 44.38(2.06) / 40.59(2.58); [FBF] t24: 61.37(3.47) / 62.63(3.03) / 57.27(5.22) / 56.37(5.88); [SDR] t0: 27.81(3.13) / 28.07(2.4) / 27.24(2.94) / 25.71(3.0); [SDR] t24: 35.11(2.16) / 35.17(1.96) / 35.53(1.81) / 33.18(2.08); [FU] t0: 41.43(1.41) / 39.87(0.88) / 38.11(1.81) / 39.09(1.92); [FU] t24: 49.27(1.54) / 48.77(1.77) / 48.65(1.88) / 46.76(4.93); [SF] t0: 71.35(7.09) / 67.39(9.76) / 68.95(6.21) / 64.1(8.35); [SF] t24: 76.06(6.61) / 75.31(9.37) / 75.2(11.57) / 69.81(12.14).

ANOVA showed significant effect of material, depth and recording time (p<0.05), and Tukey's test showed that recording sites (depths) differed significantly, giving four homogeneous groups.

Under the conditions of this study, it can be concluded that microhardness of bulk-fill resins can be affected by depth and post-curing time.

Keywords: *hardness - composite resins - hardness test.*

Determinación de microdureza de resinas bulk-fill en diferentes profundidades

RESUMEN

El objetivo del presente estudio fue determinar la microdureza Vickers (HV) en resinas bulk-fill a diferentes profundidades. Se confeccionaron probetas semicilíndricas de 6 mm de diámetro y 4 mm de profundidad con diferentes composites de aplicación en bloque (Bulk-fill): Filtek Bulk-Fill (3M ESPE) [FBF], Surefill SDR flow (Dentsply) [SDR], Fill-UP (COLTENE) [FU] y Surefill (Dentsply) [SF]. Se polimerizaron con Coltolux LED (Coltene) con 1000 mW/cm^2 durante 20s. La HV se determinó realizando 3 indentaciones con 300 g durante 8 s a 1,2 3 y 4 mm desde la superficie de la probeta hacia el interior inmediatamente después de curada y a las 24 h. Se utilizó un microdurómetro Vickers Future Tech FM300. Los resultados se analizaron estadísticamente mediante ANOVA de dos vías y Prueba de Tukey.

La media y DS de HV fueron: [FBF] t0: 49,23(4,65) / 48,32(3,36) / 44,38(2,06) / 40,59(2,58); [FBF] t24: 61,37(3,47) / 62,63(3,03) / 57,27(5,22) / 56,37(5,88); [SDR] t0: 27,81(3,13) / 28,07(2,4) / 27,24(2,94) / 25,71(3,0); [SDR] t24: 35,11(2,16) / 35,17(1,96) / 35,53(1,81) / 33,18(2,08); [FU] t0: 41,43(1,41) / 39,87(0,88) / 38,11(1,81) / 39,09(1,92); [FU] t24: 49,27(1,54) / 48,77(1,77) / 48,65(1,88) / 46,76(4,93); [SF] t0: 71,35(7,09) / 67,39(9,76) / 68,95(6,21) / 64,1(8,35); [SF] t24: 76,06(6,61) / 75,31(9,37) / 75,2(11,57) / 69,81(12,14).

La evaluación con análisis de varianza mostró el efecto significativo de las variables material, profundidad y momento del registro (p<0,05) y la prueba de Tukey mostró que los sitios de registro (profundidad) fueron estadísticamente significativos, dando cuatro grupos homogéneos.

Bajo las condiciones de este estudio podemos concluir que la microdureza de las resinas de inserción en bloque se ve afectada por el nivel de profundidad y el tiempo pos curado.

Palabras clave: *dureza - resinas compuestas - pruebas de dureza.*

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INTRODUCTION

Light-curing composites are the most frequently used materials in dental practice, with a wide range of applications. In recent years, a new kind of composite resins has been developed, known as bulk-fill composites because they can be placed in a single increment, thereby simplifying and shortening the restoration procedure. They are presented commercially according to consistency as high- or low-viscosity, and according to polymerization activation as self-curing, light-curing or dual-curing. These materials polymerize adequately when applied in layers 4 or 5 mm thick, according to brand. Some manufacturers explain that the greater curing depth of these materials is due to the addition of a more sensitive photoinitiator system and greater translucence of the material[1]. At the same time, they generate less shrinkage stress, which may vary according to composition, whether by modification of monomers or the filler content, or by addition of stress mitigators or polymerization modulators[2,3].

Increasing the thickness of the layer of material would imply an increase in polymerization shrinkage. This needs to be considered in the development of these materials in order to compensate for it by modifying the formulations, e.g., by increasing the ceramic filler load or the molecular weight of the monomers[4]. These modifications imply an increase in the modulus of elasticity of the material, which minimizes the possibility of dissipating tensions generated during polymerization[5].

Flowable bulk-fill composites have a greater content of organic matrix, which may lead to greater polymerization shrinkage and low mechanical properties, which conditions its application in occlusal areas. Bulk-fill composite manufacturers therefore indicate that they must be covered with a layer of conventional composite[3,6]. Some authors confirm a reduction in shrinkage stress in bulk-fill composites with low percentage of filler despite the increase in thickness of the layer of material[2,4]. These materials with low percentage of ceramic filler, such as SDR Flow, minimize shrinkage stress because they contain a chemical component that acts as polymerization modulator, with the aim of slowing polymerization speed to reduce shrinkage stress in spite of being polymerized with curing units in continuous, high-intensity mode[2].

The aim of this new kind of restorative composites, which is to shorten operation times by increasing the thickness of each layer, may hinder the penetration of curing light, reducing the degree of conversion of monomers to polymers[7]. The degree of conversion of a composite depends not only on its composition, but also on factors related to photoactivation, including the curing unit used, the type of photoactivation selected and the quantity of energy applied[1]. Another factor to consider with relation to degree of conversion is the possibility of composite resins undergoing elution in the oral cavity, with special interest in the release of monomers, due to their potential cytotoxicity[8]. It has been shown that monomer release is inversely proportional to the degree of conversion of monomers into polymers, which is related to exposure time to light, among other factors. Nevertheless, arbitrarily increasing polymerization time with the aim of preventing lack of curing may damage not only the pulp, but also adjacent tissues due to increase in temperature[9-11].

Previous studies have shown that degree of conversion can be measured directly or indirectly. Czasch et al.[12] and Leprince et al.[13] recommend evaluating the degree of conversion directly, while other authors recommend measuring microhardness as an indirect method for determining degree of conversion[14-16], since there are publications that have reported a good correlation between degree of conversion and microhardness[17-19]. Another method for evaluating degree of curing according to thickness of the material is by evaluating hardness at the surface exposed to the light (top) and the opposite surface (bottom), considering polymerization to be adequate when the ratio between them is 80% or higher.

The aim of this study was to determine Vickers microhardness (HV) in bulk-fill resins at different depths.

MATERIALS AND METHODS

Four bulk-fill composites were used for this study: 1) Filtek Bulk-Fill (3M ESPE), 2) Surefill SDR flow (Dentsply), 3) Fill-UP (COLTENE), and 4) Surefil (Dentsply) (Table 1).

Semi-cylindrical test specimens were prepared in a mold 6 mm in diameter and 4 mm deep (n=5). The flat surface was dismountable to allow microhardness to be determined in the depth of the specimen (Fig. 1). Specimens were cured with a Coltolux LED unit (Coltene) at intensity 1000 mW/cm^2 for 20 s.

Table 1. Information on the materials used

MATERIAL	MANUFACTURER	DESCRIPTION	BATCH
Filtek Bulk-Fill **[FBF]**	3M	Light-cured composite with filler loading 42% by volume	Batch N711074
Surefill SDR flow **[SDR]**	Dentsply	Flowable light-cured composite with filler loading 44% by volume	Batch 1508283
Fill-UP **[FU]**	Coltene	Dual-cured flowable composite with filler loading 49% by volume	Batch H28295
Surefil **[SF]**	Dentsply	Light-cured packable composite with filler loading 62% by volume	Batch 131024

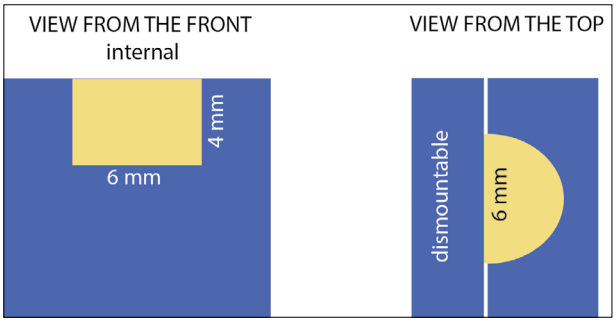

Fig. 1: Diagram of test specimens.

An extra-fine indelible marker was used to draw a vertical mark on each specimen to divide it in half and separate the indentations made immediately after light curing (t0) on one side from those made at 24 hours (t24) on the other side (Fig. 2).

Hardness was measured with a Vickers Future Tech FM300 microhardness tester by indenting with 300 g for 8 seconds at depths of 1, 2, 3 and 4 mm. Fig. 3 shows an example of the indentations made.

Measurements were recorded and analyzed statistically by ANOVA for repeated measures ad

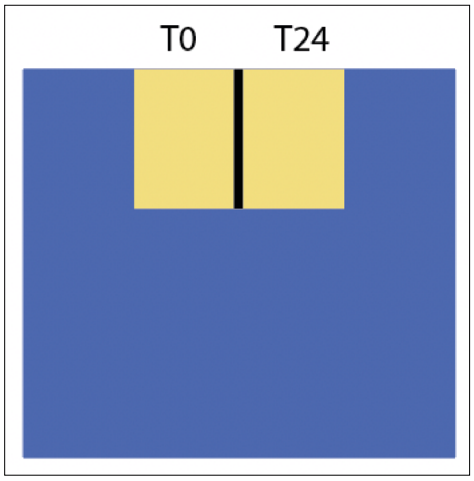

Fig. 2: Diagram showing division of the test specimen for indentations at T0 and T24.

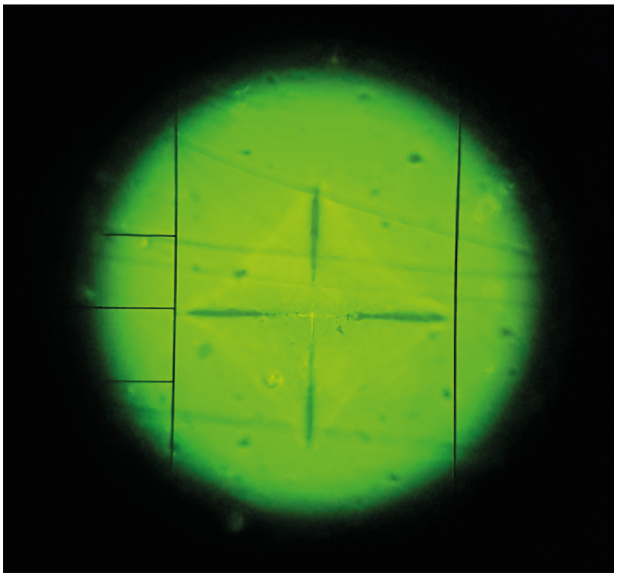

Fig. 3: Photomicrograph of an indentation made with the hardness tester.

Tukey's test. Two-way ANOVA was used to analyze the time variable.

RESULTS

Table 2 shows means and standard deviations of the values recorded.

Table 3 shows the value of the ratio of the hardness measured at 4 and 1 mm depths, according to the formula *hardness at 4 mm / hardness at 1 mm.*

Analysis of variance showed a significant effect of depth and depth-time interaction ($p<0.05$) when microhardness was measured immediately after polymerization (T0) (Table 4). Tukey's test described the presence of 3 subsets: 1) Surefill SDR flow, 2) Fill-UP and Filtek Bulk-Fill, and 3) Surefil.

At 24 hours (T24), a statistically significant difference was found for depth and not for depth/material interaction (Table 5). Tukey's test showed four subsets, with all materials differing significantly from each other.

Table 2: Mean and standard deviation found for each material immediately after light-curing (0) and 24 hours later (24)

Depth	FBF0	FBF24	SDR0	SDR24	FU0	FU24	SF0	SF24
1 mm	48.9 (3.9)	60.3 (4.7)	28.0 (3.6)	34.8 (2.9)	41.9 (1.6)	49.2 (1.1)	73.3 (6.6)	74.7 (9.1)
2 mm	48.3 (3.4)	60.9 (4.2)	27.6 (2.3)	35.2 (1.9)	40.0 (0.7)	48.0 (2.2)	63.1 (12.8)	73.4 (9.8)
3 mm	44.0 (2.5)	57.5 (5.9)	27.0 (3.4)	35.6 (2.1)	38.2 (2.2)	49.8 (1.5)	67.5 (6.6)	74.2 (13.4)
4 mm	41.5 (2.5)	55.1 (8.7)	25.5 (3.4)	33.3 (2.4)	39.2 (1.9)	45.9 (5.0)	62.6 (9.2)	66.8 (11.9)

Table 3: Ratio between hardness values at 4 and 1 mm depths for each material at 24 hours

	4 mm / 1 mm ratio
FBF24	0.92 (92%)
SDR24	0.95 (95%)
FU24	0.95 (95%)
SF24	0.92 (92%)

Table 4: Analysis of variance of data recorded immediately after polymerization

Effect	Value	F	DF of the hypothesis	DF of the error	Sig.
Depth	0.801	17.425	3	13	<0.001
Depth * material	1.164	3.169	9	45	0.005

Table 5: Analysis of variance of data recorded 24 hours after polymerization

Effect	Value	F	DF of the hypothesis	DF of the error	Sig.
Depth	0.489	4.145	3	13	0.029
Depth * material	0.368	0.700	9	45	0.706

Table 6: Post-hoc analysis. Tukey's test (microhardness at 4 mm)

Hardness (4 mm)				
Material	N	Subset		
		1	2	3
Surefill SDR flow -2-	10	29.6500		
Fill-UP -3-	10		42.86	
Filtek Bulk-Fill -1-	10		49.05	
Surefil -4-	8			66.00

Taking as a reference the values detected at 4 mm depth, analysis of variance showed the significant effect of the variables time and material (p<001), with no significant difference in the interaction between these two variables (p=0.706). Tukey's test described the presence of 3 subsets: 1) Surefill SDR flow, 2) Filtek Bulk-Fill and Fill-UP, and 3) Surefil (Table 6).

DISCUSSION

The flowable bulk-fill resins used in this study had lower microhardness values than those of regular consistency, in agreement with previous studies[3,11,16,20], possibly due to their low ceramic filler content. In addition, SDR Flow resin is light-curing, while Fill Up resin is dual-curing, which suggests that it may be harder than SDR Flow as a result of the sum of the two forms of activation. It is also important to consider the post-cure factor, since microhardness values measured immediately after curing the composites differed significantly from those measured 24 hours later[11,21,22].

Composite resin microhardness is also affected by the thickness of the layer[20]. It was concluded in that study that resin hardness in the area farthest from the curing unit (bottom) differed significantly from hardness at the top in specimens 4 or 5 mm thick. Lower microhardness values at 4 mm thickness agree with results of other studies[23].

Regarding the evaluation of microhardness in depth, some studies have determined top and bottom hardness of specimens of different thicknesses of light-cured composite resin to define its curing depth. Kim et al.[20] evaluated Vickers microhardness only at top and bottom of different specimens 2, 3 and 4 mm thick, using a load of 200 grams with a 10-second dwell time, finding that hardness decreases with increasing depth, though the decrease is less in bulk-fill composites. They conclude that there is statistically significant difference in microhardness according to type and thickness of the material, and the interaction between them, in agreement with the results found in the current study, even though a different measuring method was used. Another variable considered in the literature is the uniformity of polymerization throughout the thickness of the material, e.g., the study by Fronza et al.[16] showing that degree of conversion is not uniform in specimens thicker than 4mm. In that study, only SDR and FBF showed uniform

polymerization throughout the restoration. It is therefore necessary to evaluate microhardness not only at the surface, but also at different depths. Our study took measurements at different depths in each specimen to minimize the factors that could influence results. This methodology was also used by Comba et al.[24], who evaluated Vickers microhardness not only by means of the bottom/top ratio, but also at each millimeter in depth in specimens 6 mm thick. Considering surface microhardness values as reference points, the regression analysis showed that SDR had a significant difference at 2 mm depth, and X-tra Base and Filtek Bulk Fill showed a significant difference at 3 mm depth, with values lower than those recommended by the manufacturer. They also found that SDR had the lowest microhardness values, attributable to its low percentage of ceramic filler. According to the authors, other materials such as Filtek Bulk Fill, showed a low percentage in filler content by volume, but higher microhardness values, which may also be attributed to other factors unrelated to filler content, but strictly associated to the composition of the matrix.

Although the results showed statistically significant differences at different depths, analysis of the general behavior shows that the level of polymerization was acceptable at the depths suggested by the manufacturers, considering that the ratio between hardness measured at depths of 4 and 1 mm was greater than 80% for all materials. A bottom/top hardness ratio higher than 80% is usually used as a minimum clinically acceptable threshold for degree of conversion. Although our study did not directly evaluate top and bottom microhardness, but measured it instead at each millimeter of depth, the hardness ratio between mm 1 and mm 4 was 80% or more for the bulk-fill composites used. This means that the study materials can be adequately placed and cured in thicknesses of 4 mm, with statistically significant differences at the depths evaluated. These results agree with Kim et al.[20] and Rizzante et al.[3], who concluded that the bottom/top ratio was higher than 80% down to depths of 4.0 and 4.5 mm in all Bulk-Fill composites.

It would be advisable to conduct further studies to evaluate the degree of cytotoxicity of this type of bulk-fill resins in order to securer a more complete evaluation of their characteristics.

CONCLUSIONS

Under the conditions in this study, it can be concluded that the microhardness of bulk-fill resins is affected by the material evaluated, depth, and post-curing time.

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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Impact of the COVID-19 pandemic on the timing of dental care in elderly peruvians

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ABSTRACT

In Peru, there is low dental service use among older adults, in addition to a social gradient for use. Furthermore, the COVID-19 pandemic triggered complex scenarios characterized by inadequate access to health services, with greater impact on vulnerable population groups such as the elderly. The aim of this study was to determine the impact of the COVID-19 pandemic on the time since last dental care visit among elderly Peruvians. It was a cross-sectional study conducted on a population of 5247 respondents in 2019, and 5066 respondents in 2020. The study considered the answers of people who responded to the question on the last time they had received dental care, extracting a total 4045 subjects for 2019 and 3943 for 2020. The dependent variable was time since last dental care measured in years, while the variables on health, geographic and sociodemographic characteristics were grouped within three dimensions. A descriptive bivariate and multivariate analysis was applied by means of multiple linear regression to analyze the behavior of the variables. Time since the last dental care visit was 7.93 years (SD=8.03) in 2019 and 7.94 years (SD=7.28) in 2020. A hierarchical multiple linear regression analysis was performed, where no variable in the 2019 model was significant; while for 2020, health, geographic, and sociodemographic characteristics variables were significant. In the analysis where the 'year' variable becomes independent, only model 4, which considers all variables, was valid (p=0.018). The variables 'area of residence' and 'wealth index' were also significant. To conclude, the 2020 pandemic year for COVID-19 had no impact on the time since last dental care visit among elderly Peruvians, though factors such as area of residence and wealth index were found to be associated with the time since last dental care visit.

Keywords: *delivery of health care - dental care for aged - COVID-19 - health services accessibility - cross-sectional studies.*

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Impacto de la pandemia de COVID-19 en el tiempo de la atención odontológica en adultos mayores peruanos

RESUMEN

En Perú, los adultos mayores presentan un bajo uso de los servicios odontológicos, además de percibirse una gradiente social para su utilización. Adicionalmente, la pandemia por COVID-19 desencadenó escenarios complejos, caracterizados por un inadecuado acceso a los servicios de salud, que afectó con mayor énfasis a grupos poblacionales vulnerables, como las personas en edades extremas. El objetivo de este estudio fue determinar el impacto de la pandemia por COVID-19 en el tiempo de atención odontológica en adultos mayores peruanos. Estudio transversal con población de 5247 encuestados para el 2019 y 5066 para el 2020. Se consideraron los registros de quienes respondieron a la pregunta sobre el tiempo desde su última atención odontológica, extrayéndose del 2019 un total de 4045 y para 2020, 3943 sujetos. La variable dependiente consistió en el tiempo desde la última atención odontológica medido en años; mientras que dentro de tres dimensiones, se agruparon a las variables sobre características de salud, geográficas y sociodemográficas. Se aplicó un análisis descriptivo, bivariado y multivariado, donde el tiempo desde la última atención odontológica en el año 2019 fue de 7.93 años (DE=8.03) y en el 2020 fue de 7.94 años (DE=7.28). Se realizó un análisis de regresión lineal múltiple jerárquica, donde ninguna variable del modelo 2019 fue significativa; mientras que para 2020, las variables características de salud, geográficas y sociodemográficas si lo fueron. Al análisis, donde el año pasa a ser independiente, sólo el modelo 4, que considera todas las variables fue válido (p=0.018); asimismo, el área de residencia e índice de riqueza presentaron significancia estadística. Para concluir, en el año 2020 la pandemia por COVID-19 no impactó en el tiempo de atención odontológica en adultos mayores peruanos, sin embargo, factores como área de residencia e índice de riqueza si presentaron asociación con el tiempo desde la última atención.

Palabras clave: *prestación de atención de salud - cuidado dental para ancianos - COVID-19 - accesibilidad a los servicios de salud - estudios transversales.*

INTRODUCTION

Most governments in the world adopted strategies to contain the spread of COVID-19, such as mandatory social distancing and lockdown. Health systems readjusted the provision of care to ensure hospital access for cases of COVID-19 with severe symptoms, even though this decision would result in a considerable reduction in the use of healthcare services, especially by people with pre-existing chronic pathologies, either because of fear of exposure to SARS- COV-2 or to avoid overloading the system[1-3]. Some developing countries responded weakly to the health emergency, which led to more complex scenarios characterized by inadequate access to medical care, with greater impact on the most vulnerable population groups such as the elderly[4,5].

The situation of dental care was uncertain, and health authorities recommended limiting it to urgent or emergency cases, with the intention of reducing the risk of cross-infection by aerosols[6]. This increased the unemployment of dentists during the first months of the pandemic. However, dental services were resumed a few months later, subject to strict compliance with new biosafety protocols and with increased cost of dental procedures. This exacerbated one of the main barriers to oral health care: cost, and worsened dental services, which were already a public health problem prior to COVID-19[7]. It is important to mention that among the most affected vulnerable populations during this period were older adults, a group that is more likely to be economically disadvantaged and have limited or no dental insurance, and people who belong to ethnic or racial minorities and are subject to a more complex health situation[8,9]. In Peru, there is low dental service use among the elderly. There is also a social gradient, with people with lower purchasing power and higher age making lower use of dental services[10,11]. There is a clear need to generate more evidence to make visible and update the situation faced by these individuals in relation to access to dental care, especially during a health emergency. Thus, the aim of this study was to determine the impact of the COVID-19 pandemic on the time since last dental care visit among elderly Peruvians.

MATERIALS AND METHODS

This cross-sectional study considered the databases of the Demographic and Family Health Survey (ENDES) for the years 2019 and 2020, made available by the National Institute of Statistics and Informatics of Peru (INEI). This survey is applied every year, through home interviews, to a sample of two-stage stratified conglomerates representative at national and regional levels, and according to urban and rural areas. It also includes information on access to dental care for adults over 60 years of age. For 2019, a sample size of 36,760 households was determined, amounting to 5,247 respondents, while for 2020, the sample size was 37,390 households and 5066 respondents. It is assumed that the decrease in participants between years could be linked to the COVID-19 pandemic. This study only considered the responses of those who answered the question about the time since their last dental care visit, which included 4045 subjects (77.09%) for the year 2019, and 3943 (77.83%) for 2020. The total sample for both years was 7988 (77.46%)[12].

Time in years since the last dental care visit was defined as the dependent variable, and the years (2019 and 2020) as independent variables, considering that the COVID-19 pandemic took place in Peru during 2020. Other covariates were added to the study, which were grouped following a 3-dimenseional structure: health characteristics, geographic characteristics and sociodemographic characteristics. Health characteristics considered health insurance coverage and place providing dental care (care provided by the Ministry of Health, Social Security [EsSalud], the Armed Forces and Police [FF.AA./PNP] or the private sector). Geographic characteristics considered area of residence (urban or rural); natural region (metropolitan Lima [Peru's capital city], the rest of the coast, highlands and jungle); place of residence (capital, city, town or countryside); and altitude (a dichotomous variable, defined as less or more than 2500 meters above mean sea level [MAMSL]). The sociodemographic dimension was composed of the wealth quintile, defined as the particularities and availability to each household of certain consumer goods and services, to which the methodology of the Demographic and Health Surveys Program is subsequently applied, based on which a score is assigned to each household (and that same value is assigned to each of its residents) to rank them from poorest to richest[12,13]. Respondents were also grouped according to age (60 to 74 or 75 to 97 years) and sex. It is important

to highlight that previous research has analyzed the behavior of the mentioned covariables.[13,10,14]

The databases were obtained from the INEI's official website (http://iinei.inei.gob.pe/microdatos/) through various modules, which were then unified into a single database to be analyzed using the SPSS® v. 25.0 program (IBM, NY, US). This was done using the complex samples module, since it is a national survey with possible representative estimates. It should be noted that for the statistical analysis, a descriptive analysis was performed for the qualitative variables through the evaluation of the absolute and relative frequencies; then, the average and standard deviation of the variable time since last dental care visit was calculated. The Kolmogorov-Smirnov test was used to evaluate whether the result variable had normal distribution according to the other variables. Nonparametric Mann-Whitney U tests were applied for dichotomous variables and Kruskal-Wallis for polytomous variables. A hierarchical multiple linear regression was prepared with the aim of constructing models between the independent variables and the time since the last dental care visit, where each dimension were evaluated according to the year of care separately and then as a whole; highlighting that prior to this process, a logarithmic transformation was applied to the result variable, with the aim of giving it the characteristic of normality. For this research, a confidence level of 95% was considered, and a value of $p<0.05$ as an indicator of statistical significance in all the applied tests. For the analysis, SPSS® v. 25.0 (IBM, NY, US) was used.

Since this is a database with a secondary, anonymous source of information, the approval of an ethics committee was not necessary. Moreover, the information was freely available, provided by the INEI.

RESULTS

Time since the last dental care visit was 7.93 years (SD=8.03) in 2019 and 7.94 years (SD=7.28) in 2020, being statistically significant by bivariate analysis (p=0.021). However, statistical significance could be attributed to the use of non-parametric tests, as well as to the sample size used. In 2019, time since the last dental care visit differed significantly according to health insurance type, natural region, altitude and age ($p<0.05$); while in 2020, there was no difference according to the covariates ($p>0.05$) (Table 1).

A hierarchical multiple linear regression analysis was performed, analyzing the models in separate years and as a whole. When the analysis was done by separate years, the models were not significant for any variable in 2019 ($p>0.05$). For 2020, models 1, 2 and 3, which consider health, geographic and sociodemographic characteristics, were significant (Table 2). When analyzed as a whole, and year is an independent variable, only model 4, which considers year, health, geographic and sociodemographic characteristics, is valid (p=0.018) with an R2% of 1.10 and constant equal to - 792.728; with the significant variables being area of residence with an unstandardized regression coefficient (b) of -1.428 and a 95% confidence interval (95%CI) between -2.633- -0.223 (p=0.020), and wealth index with b= -0.493 and 95%CI= -0.852- -0.133 (p=0.007) (Table 3).

DISCUSSION

During the COVID-19 pandemic, health systems worldwide faced a critical scenario, a situation that was exacerbated in vulnerable populations, who were limited either because of their condition of risk for the disease or when seeking health services[15]. Moreover, the vulnerability that has characterized older adults in all contexts is indisputable, especially because they have limited oral health, little knowledge regarding the importance of dental health, and difficulty in moving around, with those living in shelters or confined to their homes being at greater risk[16]. The current study found no difference in time since the last dental care visit for the years 2019 and 2020 among elderly Peruvians; however, the bivariate statistical analysis does show significant differences.

Regarding the situation in Peru, Azañedo et al. reported that dental care for older adults was scarce. Only a quarter of this age group had received dental care, and most of them came from urban areas and received care at private health facilities, or from the coastal region of Peru[11]. It should also be considered that there is a gradient associated to receiving oral health care according to socioeconomic level, where older adults with lower purchasing power have a low probability of receiving dental services, as reported by Carbajal-Rodriguez et al[10]. Similarly, Azañedo et al. reported that at national level, the use of dental health services decreased with age, which could be due to complications related to aging, such as difficulties in mobility, functional

Table 1. Health, geographic and sociodemographic characteristics of elderly Peruvians in 2019 and 2020

Variables	n	%	Time since last dental care visit									
			2019					2020				
			n	%	X	SD	p	n	%	X	SD	p
Year of the COVID-19 Pandemic	7988	100.00	4045	50.64	7.93	8.03		3943	49.36	7.94	7.28	0.021*
Health characteristics												
Place providing dental care												
Ministry of Health	1561	26.32	1110	27.54	7.72	8.27	0.097**	451	23.72	8.73	8.84	0.578*
Social Security (EsSalud)	477	8.04	324	8.04	7.11	6.69		153	8.05	7.56	6.42	
Armed Forces and Police	47	0.79	25	0.62	10.56	13.56		22	1.16	10.77	13.54	
Private sector	3846	64.85	2571	63.80	8.10	8.01		1275	67.07	8.17	8.04	
Health Insurance												
Without insurance	1108	13.87	591	14.61	8.78	8.46	0.003*	517	13.11	8.04	7.37	0.638*
With insurance	6880	86.13	3454	85.39	7.79	7.94		3426	86.89	7.91	7.27	
Geographic characteristics												
Area of residence												
Urban	4951	61.98	2455	60.69	7.77	7.57	0.608*	2496	63.30	7.74	6.86	0.422*
Rural	3037	38.02	1590	39.31	8.18	8.68		1447	36.70	8.26	7.95	
Natural region of residence												
Lima metropolitan area	1073	13.43	518	12.81	7.64	7.37	0.020**	555	14.08	7.73	6.76	0.358**
Rest of the coast	2294	28.72	1131	27.96	8.05	7.98		1163	29.50	7.98	7.04	
Highlands	3254	40.74	1752	43.31	7.55	7.60		1502	38.09	7.60	6.83	
Jungle	1367	17.11	644	15.92	9.01	9.53		723	18.34	8.69	8.76	
Place of residence												
Capital	751	39.74	509	40.05	7.30	6.76	0.560**	242	39.10	7.65	7.24	0.765**
City	460	24.34	305	24.00	7.55	7.06		155	25.04	8.66	9.17	
Town	342	18.10	235	18.49	8.20	9.22		107	17.29	8.96	9.34	
Countryside	337	17.83	222	17.47	8.69	9.48		115	18.58	8.57	9.02	
Altitude												
Less than 2500 MAMSL	5220	65.35	2561	63.31	8.33	8.46	0.001*	2659	67.44	8.15	7.59	0.057*
Over 2500 MAMSL	2768	34.65	1484	36.69	7.24	7.17		1284	32.56	7.48	6.58	
Sociodemographic characteristics												
Wealth Quintile												
1st quintile	458	17.53	233	17.09	8.57	10.69	0.220**	225	18.01	8.98	9.02	0.482**
2nd quintile	452	17.30	237	17.39	7.81	7.24		215	17.21	7.94	7.07	
3rd quintile	542	20.75	291	21.35	8.20	7.80		251	20.10	8.31	7.63	
4th quintile	530	20.29	272	19.96	7.93	7.64		258	20.66	7.64	7.50	
5th quintile	630	24.12	330	24.21	6.54	5.43		300	24.02	7.45	5.92	
Age												
60-74	6021	75.38	2934	72.53	7.68	7.63	0.003*	3087	78.29	7.90	7.19	0.980*
75-97	1967	24.62	1111	27.47	8.62	8.95		856	21.71	8.03	7.61	
Sex												
Male	3746	46.90	1833	45.32	8.09	8.60	0.642*	1913	48.52	7.88	7.17	0.999*
Female	4242	53.10	2212	54.68	7.80	7.52		2030	51.48	7.98	7.38	

*Mann Whitney U Test. **Kruskal Wallis Test.

Table 2. Hierarchical multiple regression models for health, geographic and sociodemographic characteristics for the time since last dental care visit in the study sample (n=32 111)

Variables	Determination Coefficient % (R2%)	Change of R2%	p-value Change of R2%	Constant	Non-standardized Regression Coefficient	Standardized Regression Coefficient	95% Confidence Interval	p-value	p-value Model
2019 Model 1									
Health characteristics	0.20	0.20	0.231	9.512					0.231
Place of dental care					0.002	,000	-0311 - 0.315	0.990	
Health Insurance					-0.968	-,048	-2.085 - 0.148	0.089	
Model 2									
Health characteristics									
Place of dental care					0.035	0.006	-0.284 - 0.353	0.832	
Health Insurance					-0.895	-0.044	-2.017 - 0.228	0.118	
Geographic characteristics	0.60	0.40	0.337	1.475					0.279
Area of residence					-0.578	-0.031	-1.923 - 0.768	0.400	
Natural region of residency					0.166	0.022	-0.286 - 0.618	0.472	
Place of residence					0.422	0.064	-0.027 - 0.872	0.065	
Altitude					-0.445	-0.025	-1.567 - 0.677	0.437	
Model 3									
Health characteristics									
Place of dental care					0.100	0.018	-0.226 - 0.427	0.546	
Health Insurance					-0.884	-0.044	-2.006 - 0.239	0.123	
Geographic characteristics									
Area of residence					-1.106	-0.059	-2.592 - 0.379	0.144	
Natural region of residency	1.10	0.50	0.096	2.159	0.051	0.007	-0.425 - 0.526	0.834	0.129
Place of residence					0.337	0.051	-0.121 - 0.795	0.149	
Altitude					-0.569	-0.032	-1.695 - 0.558	0.322	
Sociodemographic characteristics									
Wealth Quintile					-0.375	-0.071	-0.800 - 0.051	0.084	
Age					0.884	0.049	-0.102 - 1.870	0.079	
Sex					0.279	0.018	-0.554 - 1.111	0.512	
2020 Model 1									
Health characteristics	1.40	1.10	0.012	7.597					0.012
Place of dental care					-0.641	-0.101	-1.143 - -0.138	0.013	
Health Insurance					1.306	0.052	-0.678 - 3.290	0.197	
Model 2									
Health characteristics									
Place of dental care					-0.630	-0.099	-1.133 - -0.128	0.014	
Health Insurance					1.245	0.050	-0.737 - 3.227	0.218	
Geographic characteristics	2.50	1.50	0.162	8.634					0.018
Area of residence					-0.838	-0.044	-2.727 - 1.050	0.384	
Natural region of residency					-0.666	-0.087	-1.317 - -0.014	0.045	
Place of residence					0.615	0.088	-0.066 - 1.295	0.077	
Altitude					0.291	0.015	-1.354 - 1.937	0.728	
Model 3									
Health characteristics									
Place of dental care					-0.529	-0.083	-1.038 - -0.019	0.042	
Health Insurance					1.072	0.043	-0.917 - 3.062	0.290	
Geographic characteristics									
Area of residence					-1.772	-0.093	-3.850 - 0.305	0.094	
Natural region of residency	3.50	2.00	0.102	12.042	-0.954	-0.124	-1.651 - -0.256	0.007	0.011
Place of residence					0.398	0.057	-0.307 - 1.103	0.268	
Altitude					-0.043	-0.002	-1.708 - 1.621	0.959	
Sociodemographic characteristics									
Wealth Quintile					-0.767	-0.135	-1.437 - -0.098	0.025	
Age					0.810	0.040	-0.778 - 2.398	0.317	
Sex					0.526	0.033	-0.740 - 1.792	0.415	

Table 3. Hierarchical multiple regression models for year, health, geographic and sociodemographic characteristics for the time since last dental care visit in the study sample (n=32 111).

Variables	Determination Coefficient % (R2%)	Change of R2%	p-value Change of R2%	Constant	Non-standardized Regression Coefficient	Standardized Regression Coefficient	Confidence Interval 95%	p-value	p-value Model
Model 1	0.00	0.00	0.438	-578.721					0.438
Year of the COVID-19 Pandemic					0.290	0.018	-0.443 - 1.024	0.438	
Model 2	0.20	0.10	0.295	-666.743					0.385
Year of the COVID-19 Pandemic					0.335	0.021	-0.402 - 1.071	0.373	
Health characteristics									
Place of dental care					-0.197	-0.034	-0.464 - 0.070	0.148	
Health Insurance					-0.368	-0.017	-1.348 - 0.611	0.461	
Model 3	0.50	0.40	0.149	-660.893					0.200
Year of the COVID-19 Pandemic					0.332	0.020	-0.405 - 1.069	0.377	
Health characteristics									
Place of dental care					-0.179	-0.030	-0.448 - 0.091	0.193	
Health Insurance					-0.353	-0.016	-1.335 - 0.628	0.480	
Geographic characteristics									
Area of residence					-0.775	-0.041	-1.869 - 0.318	0.165	
Natural region of residence					-0.117	-0.015	-0.489 - 0.255	0.538	
Place of residence					0.482	0.071	0.106 - 0.858	0.012	
Altitude					-0.211	-0.012	-1.137 - 0.715	0.655	
Model 4	1.10	0.60	0.008	-792.728					0.018
Year of the COVID-19 Pandemic					0.398	0.024	-0.338 - 1.134	0.289	
Health characteristics									
Place of dental care					-0.100	-0.017	-0.374 - 0.175	0.478	
Health Insurance					-0.371	-0.017	-1.351 - 0.610	0.459	
Geographic characteristics									
Area of residence					-1.428	-0.076	-2.633 - -0.223	0.020	
Natural region of residence					-0.284	-0.037	-0.676 - 0.109	0.157	
Place of residence					0.362	0.054	-0.023 - 0.747	0.065	
Altitude					-0.401	-0.022	-1.332 - 0.531	0.399	
Sociodemographic characteristics									
Wealth Quintile					-0.493	-0.091	-0.852 - -0.133	0.007	
Age					0.838	0.045	-0.002 - 1.678	0.051	
Sex					0.391	0.025	-0.304 - 1.087	0.270	

dependence and possible compromise of social skills for communication[17]. That is why, with the aim of contributing to improving the comprehensive health and quality of life of people aged 60 years and older, the Peruvian Ministry of Health designed the "Peru Smiles" plan, whose implementation period is from 2019 to 2022, to provide oral rehabilitation through complete dental prostheses for older adult beneficiaries[18]. Despite these efforts, the health authority has not yet presented official information on the progress of the plan.

The onset of the COVID-19 pandemic evidenced the complex situation of older adults as a result of their greater risk of developing complex conditions or dying if they contracted the infection, possibly associated with pre-existing comorbidities. The disease also became a potential threat to the physical and psychological wellbeing of individuals over 60 years of age, due to the sequelae following the development of the infection such as decreased immunity, fragility, and cognitive impairment; resulting in a decrease in the levels of quality of life[19, 20]. Within this context, health services were

restructured to prioritize care for cases of COVID-19, in addition to trying to contain the spread of the disease. Regarding dental care, Brian et al. mention that access to oral health services is even more limited for those populations at high risk of infection with COVID-19, ratifying previous provisions that suggested delaying non-urgent or emergency care as much as possible[21]. Some authors projected that dental care for older adults would decrease by up to 80%, resulting in deterioration of their oral health. This was subsequently observed when dental activities were resumed during the pandemic[8,22], possibly because efforts were no longer directed to prevention strategies. The suspension of dental practices nationwide during the first three months of the pandemic due to the health emergency resulted in a significant reduction in people seeking dental services and a consequent delay in treatment. Subsequently, thanks to the establishment of Measures for Citizenship towards a New Coexistence[23], in May 2020, oral health services were reactivated, adapting their biosafety protocols, identifying risk factors and strictly following the provisions defined in Health Directive No. 100-MINSA-2020-DGIESP[24].

Regardless of the years evaluated, the current study shows that residents in rural areas presented significant differences in time since last dental care visit, observing a decrease in the period to access the services, compared to those who came from urban territories. Likewise, some studies have shown that the area of residence is associated with the emergence of inequities in access to dental services[10,11]. On the contrary, some reports have determined that living in urban areas is favorably linked to the practice of habits beneficial to oral health, such as optimal toothbrushing in a sample of Peruvian children. However, access to dental services is still scarce, while the number of inhabitants in rural areas who have health insurance is greater than in urban areas[25].

The wealth quintile variable also showed statistical differences in the time since last dental care visit, noting that people with greater economic capacity delayed their dental care less than those who were poorer. Consistently with this finding, a study by Hernández-Vásquez et al. confirmed that, over a 13-year period, the Peruvian population in general increased its use of dental services, while inequities in seeking care narrowed. It is important to note that this narrowing of gaps was not observed in the older age group, where those who benefited the most were the wealthier population. Similarly, an investigation with secondary information on Peruvian adults aged 60 years and older indicated a low frequency of dental care for this age group, noting that the Wealth Index factor determined differences in its use. Likewise, it is mentioned that at national level, oral rehabilitation treatments are not included in programs such as Universal Health Insurance[26,17]. Scientific evidence suggests that oral health is deficient in older adults, who have high prevalence of caries, periodontal disease and high number of missing teeth, leading to complications in the functions of the oral cavity, as well as contributing directly to worsening comorbidities[27].

The limitations of this research include its cross-sectional design, which makes it impossible to link the findings to a causal relationship. Moreover, the present study resorted to the analysis of secondary information, which may have contained inaccuracies at the time of collection, or recall biases by the participants. Nevertheless, the ENDES survey is a useful tool, whose results allow a first approximation to the oral health situation of older Peruvians, in order to propose future public policies that seek to improve the quality of life of this vulnerable population.

The results obtained confirm that older adults delay seeking oral health services, leading to limited access to care. The situation does not seem to be linked to the impact of COVID-19, but rather, is the result of the sustained increase of unattended needs, which were not identified by those responsible. Consequently, the inequity gap for a vulnerable age group increased. All this suggests that older Peruvians have always faced catastrophic scenarios when seeking to meet their health needs, and not only as a result of the emergence of a pandemic. Based on the above discussion, it is concluded that the year 2020 of the COVID-19 pandemic did not have an impact on the time since last dental care visit among elderly Peruvians, but that factors such as area of residence and wealth index were associated with the time since last dental care visit.

DECLARATION OF CONFLICTING INTERESTS
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Use of the Demirjian method to estimate dental age in panoramic radiographs of patients treated at the Buenos Aires University School of Dentistry

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ABSTRACT
The aim of this study was to determine the difference between real age (RA) and dental age (DA) in boys and girls from the Autonomous City of Buenos Aires (C.A.B.A.) by analyzing digital panoramic radiographs from the database of the Imaging Department at the Buenos Aires University School of Dentistry, using the Demirjian Method (DM). The sample consisted of 508 panoramic radiographs of 6- to 14-year old Argentines (268 female and 240 male). The Demirjian method was used to estimate dental age from each panoramic radiograph, and the Wilcoxon test was applied to perform a comparative analysis with the real age recorded in the image database. Average RA was 9.36 years (SD 2.11), and average DA according to the Demirjian method was 10.45 years (SD 2.31). For females, RA was 9.25 (SD 2.12), and DA according to the DM was 10.40 years (SD 2.41). For males, RA was 9.46 (SD 2.10), and DA according to the DM was 10.50 years (SD 2.22). An inter-class correlation coefficient (ICC) calculated as a correlation measure between dental age and real age was ICC = 1.09%. The ICC was 1.04% for the males and 1.15% for females. Significant differences were found between DA and RA (p <0.01) in general and according to sex. Real age was found to be lower than dental age in the study population from Buenos Aires City.

Keywords: *age determination by teeth - panoramic radiography.*

Utilización del Método de Demirjian para estimación de la edad dental en radiografías panorámicas de pacientes atendidos en la Facultad de Odontología de la Universidad de Buenos Aires

RESUMEN
El objetivo del presente trabajo fue determinar la diferencia entre la edad real (ER) y la edad dental (ED) en niños y niñas de la Ciudad de Buenos Aires analizando radiografías panorámicas digitales de la base de datos de la Cátedra de Diagnóstico por imágenes FOUBA, utilizando el Método Demirjian (MD). La muestra consistió en 508 radiografías panorámicas (268 del sexo femenino y 240 del sexo masculino) en una población argentina de 6 a 14 años de edad. Se realizó el cálculo de la edad dental en cada una de ellas, conforme el método de Demirjian. Se efectuó un análisis comparativo con la ER proveniente de la base de datos, utilizando la prueba de Wilcoxon. En las 508 panorámicas se estimó la ED. Se pudo establecer que la ER en promedio es 9.36 años con un desviación estándar (DS) de 2,11 y que la edad según el método de Demirjian es 10,45 años con una DS de 2,31. Para el sexo femenino se obtuvo una ER de 9,25 y una DS de 2,12 y según MD es de 10,40 años con una DS de 2,41. Para el sexo masculino se obtuvo una ER de 9,46 y una DS de 2,10 y según MD es de 10,50 años con una DS de 2,22. Se obtuvo un coeficiente de correlación inter-clase (CCI), como medida de correlación entre edad dental y real, de (CCI 1,09%). El CCI para el sexo masculino fue de (CCI 1,04 %) y en el caso del sexo femenino fue de (CCI 1,15%). Se hallaron diferencias significativas entre ED y ER (p<0,01) en general y también para ambos sexos. Se pudo evidenciar que la edad real es menor que la edad dental en la población de la Ciudad de Buenos Aires.

Palabras clave: *determinación de la edad por los dientes - radiografía panorámica.*

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INTRODUCTION

The ability to estimate age is important in medical studies such as pediatric endocrinology, archeology, anthropology and odontology, and has social, legal and religious connotations. In forensic science, age estimation is used in the identification of human remains. Age estimation is also relevant to living subjects, especially considering the current international context of constantly increasing migratory flows, which leads to an increase in the activity of legal medicine institutes. A person's real age (RA) is linked to their date of birth which, if adequately registered, is accredited legally on the identification document (ID). However, in some countries, birth registries have been reported to have serious difficulties and discrepancies.

As a person grows, their skeletal, dental, anthropological and psychological features enable their age to be estimated. Different study groups recommend using three systems for age analysis of individual development to increase diagnostic precision during forensic procedures and to optimize identification of age development disorders. These systems consider bone age, dental age and development of secondary sexual characteristics[1-3].

In line with the above, in Argentina it has been reported that the Supreme Court's Forensic Medical Center estimates age through a protocol that includes physical examination, periapical scans of the third molars and a scan of the left hand carpus. It is important to consider that dental development is one of the most usual and precise methodologies used to estimate age in teenagers and young adults, while when all teeth are completely formed, associated regressive changes are used[4].

Human dentition follows a reliable, predictable sequence of development, starting at approximately the fourth week after conception and continuing until the beginning of the third decade of life, when the development of all permanent teeth is complete. Dental eruption and mineralization are the main odontological indicators that are studied for the estimation of forensic age in children and young adults. Dental development is a pragmatic measure of maturity and can provide substantial data for any individual. Historically, different authors have proposed methodological strategies to measure the course of the formation and growth of tooth germs to estimate age, considering the degree of mineralization, with an acceptable degree of error in the calculation. In this context, the method advocated by Demirjian, described for first time in 1973 and based on a study conducted on French-Canadian children, uses panoramic scans to analyze development of tooth germs on the seven mandibular left teeth, without considering the third molars. They classified 8 distinctive stages, called AH, defined by morphological changes, without speculative estimations of longitude. The study was based on a scale of maturation outlined by Tanner et al. to estimate real age, which provides a score for each stage of tooth development, for girls and boys. The sum of the scores of the seven teeth provides a value of dental maturity on a scale of 0 to 100, which can be directly converted into dental age by using the percentage tables and curves designed by the authors. Different studies have found that the results obtained for different populations using Demirjian's standards are uneven and contradictory, suggesting the need to create a database for each population in order to gain a clearer picture of human dental maturation. There is a considerable degree of association between dental age and real age, compared to any other biological marker of maturity, because dental age is believed to be less affected by malnutrition. Moreover, evaluation of real age is important to all dental disciplines, for designing treatments for all types of malocclusion, and for pediatric dentists to know how age, sex, weight and height are associated with tooth eruption as events of somatic growth[5-8].

Due to all of the above, members of the Buenos Aires University School of Dentistry from the departments of Image Diagnosis and Legal Odontology decided to analyze digital panoramic radiographs from the database of the Imaging Department using the Demirjian's Method (DM) to determine the difference between real age (RA) and dental age (DA) in boys and girls from Buenos Aires City.

MATERIALS AND METHODS

This was a study with a descriptive, retrospective, analytic design. The sample consisted of 508 panoramic X-ray scans obtained at the Image Diagnosis Department of the Buenos Aires University School of Dentistry between September 2016 and June 2018. Images from patients aged 6 to 14 years (268 females and 240 males) were selected and saved in JPEG format (2440 x 1292 pixels).

Parents or legal guardians signed informed consent before the use of the images for research purposes under anonymity. Fig. 1 shows an example of the cases under study.

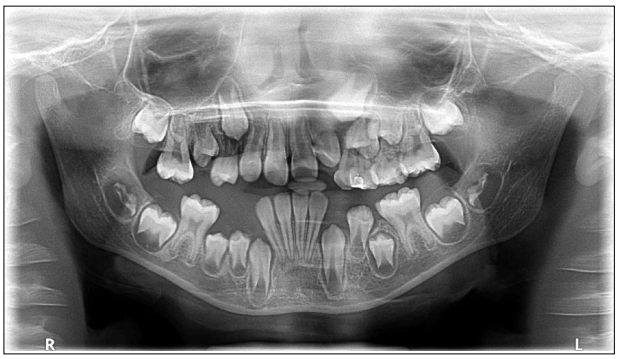

Fig. 1: Example of the images under study. Panoramic x-ray showing the different stages of development of the central incisor, lateral incisor, canine, first and second premolar, first and second molar.

Before the evaluation of the final sample, 43 panoramic scans were selected randomly to calculate intra-evaluator variability. They were analyzed by one observer (IC) twice with a 3-week interval between the first and second evaluation, obtaining a kappa index of 0.8. Exclusion criteria were images with significant distortion, which hinder visualization of the teeth in the area of examination, images with pathologies such as tumors or cysts, scans of patients in orthodontic treatment, and images showing absence of tooth due agenesis or exodontia. Dental age was calculated in each image by the Demirjian method, and compared to real age recorded in the image database, using the Wilcoxon test. The stages of development of the seven mandibular teeth were analyzed in the following order: central incisor, lateral incisor, canine, first and second premolar, first and second molar, called IC, IL, C, 1PM, 2PM, 1M and 2M. A spreadsheet was prepared in Microsoft Office Excel 2007 to record patients, assigning to each case a number (1, 2, 3, 4) and to calculate the scores and dental age. At the time of evaluation, the observer did not know the birth date of each subject. The cases were subsequently separated into two different spreadsheets, one for boys and one for girls. The stages were transformed from letters into numbers (from A, B, C, D, E, F, G, H to 1, 2, 3, 4, 5, 6, 7, 8, respectively) to facilitate the allocation of the maturation score according to the described criteria for each dental stage, and comparing the tooth with diagrams and scans created by Demirjian et al. In case of doubt between two stages, the tooth was assigned the stage of lesser development. Then, the percentage of maturation and dental age were calculated to one decimal place. Real age was calculated to one decimal place by subtracting the birth date from the date of the panoramic scan. The margin of acceptable error generally ranged from 4% to 8% with a confidence interval of 95%. The collected data were remodeled with the program SPSS Version 21 for statistical analysis. To observe the normal distribution of data, the Kolmogorov-Smirnov test was applied, which provided a significant result ($p < 0.05$). The importance of the difference between RA and DA was evaluated.

RESULTS

Dental age was estimated from 508 panoramic scans. Average real age was 9.36 years (SD 2.11) and age according to the Demirjian method was 10.45 years (SD 2.31).

For the females, average RA was 9.25 (SD 2.12) and average age according to DM was 10.40 years (SD 2,41). For males, average RA was 9.46 (SD 2.10) and average age according to DM was 10.50 years (SD 2.22). The inter-class correlation coefficient (ICC), as a measure of correlation between dental and chronological ages was ICC 1.09%. The ICC was 1.04% for males and 1.15% for females. Significant differences were found between DA and RA ($p<0.01$) in general and for both sexes, but mainly in females.

DISCUSSION

From the prenatal stage of dental development to adulthood, it is feasible to estimate age using a radiographic method for ages 2.5 to 18 years. Demirjian's principles promoted understanding of the divergence of dental maturity for individuals, however, it is not precise and not even among all populations. The modification of Demirjian's technique included the analysis of third molars, widening the spectrum of applicability to a group of older French children. Both methods, however, have provided variable results in other populations, with patterns of advance and/or regression in dental maturity with respect to the values found for the original sample. This led to unanimous consensus on the need to redesign the standards for each population sample, given the variability, even within a single geographic area[9-21].

For instance, the Demirjian Method overestimated age in most studies conducted in China, England, Spain, Iran, Tukey, Malaysia, France, Chile and Tunisia[22-26]. Conversely, it was found to underestimate age in Venezuela, Kuwait, Turkey and North China, while it was more precise in Norwegian children[27-31].

One study found coincidence of scores between the original investigations and a study on schoolchildren in some Indian states. The Demirjian method was considered to underestimate the real age for girls and boys in Indian populations[32, 33].

In Argentina, researchers from the University of Cuyo in Mendoza Province calculated dental ages in a population in the Cuyo region using Nolla and Demirjian methods, analyzing 374 panoramic scans of children and teenagers aged 5 to 17 years. They concluded that the Demirjian method overestimated age, in accordance with the present study conducted in Buenos Aires[34].

The effectiveness of the prediction of age is represented by the medium absolute error, which is calculated by subtracting the real age from the age based on dentition. An error of less than one year is considered acceptable, while an error greater than two years would be inaccurate. The methods proposed by Demirjian et al.[5] provided an absolute mean <2 years, therefore, it can be concluded that it estimates dental age precisely.

Many researchers have verified delayed growth in French children compared to other populations. Possible reasons explaining the great variability in the estimation of dental age may be attributed to factors such as ethnicity, dietary habits, environmental factors, and socio-economical standards, which all present notable differences among different populations. The consequence of malnutrition on dental development is still controversial, with inconsistent conclusions that suggest, on the one hand, a high impact factor and on the other hand, little or no impact. Supporting previous research, the present study has verified that dental development matures earlier in the female gender[35-39].

CONCLUSION

Digitized panoramic scans offer the advantage of estimating the different stages of dental development with great precision, and are sensitive to variables such as age distribution, sample size and statistical approach. The current study found that real age was lower than dental age determined by the Demirjian's method in children 6 to 14 years old from Buenos Aires City.

Due to the broad individual variation in dental maturity, the estimation of the real age in children must be complemented with other indicators of biological maturity. For this reason, we propose to promote further research on larger study samples, including younger children than in this research, incorporating children from other geographical areas in Argentina and using the Demirjian Method to compare and evaluate possible differences within the same country.

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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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Efficacy of two low-level laser therapy protocols following lower third molar surgery – a randomized, double-blind, controlled clinical trial

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ABSTRACT

The aim of this study was to test two low-level laser therapy protocols by evaluating pain control, swelling and trismus in the postoperative period of lower third molar surgeries. This was a randomized, double-blind, placebo-controlled, crossover trial. Patients presenting two symmetrically impacted mandibular third molars were included. One side was randomly assigned for LLLT applied immediately after surgery (T1) and then after 24 (T2) and 48 hours (T3) (Protocol A). The other side received LLLT applied immediately after surgery and placebo after 24 and 48 hours (Protocol B). LLLT was given by intraoral application (660nm, 5 J/cm^2, 10 s, 20 mW, 4 points) followed by extraoral application (789 nm, 30 J/cm^2, 20 s, 60 mW, 8 points). The placebo application was similar to that of the experimental side but with laser simulation. The primary outcomes were pain control, swelling and trismus intensity at T1, T2, T3 and 7 days after surgery (T4). Data were analyzed by ANOVA repeated measures and Wilcoxon test ($p< .05$). The final sample consisted of 21 patients (42 teeth). There were no statistical differences for pain level between protocols A and B over time ($p= .909$), although the amount of analgesic medication was lower with protocol A at T2 ($p=.022$). There were no differences in swelling ($p=.958$) or trismus ($p=.837$) between the protocols used over time. Both protocols performed similarly for pain control, swelling and trismus. Therefore, for practical reasons, a single laser application in the immediate postoperative period could be indicated for the management of postoperative discomfort in lower third molar surgery.

Keywords: *third molar - trismus - swelling - pain - low-level laser therapy.*

Eficácia de dois protocolos de terapia a laser de baixa intensidade após cirurgia de terceiros molares inferiores - ensaio clínico duplo-cego, randomizado

RESUMO

O objetivo deste estudo foi testar dois protocolos de terapia com laser de baixa intensidade (LBI) para controle da dor, edema e trismo no período pós-operatório de cirurgias de terceiro molar inferior. Neste estudo randomizado, duplo-cego, controlado, de boca dividida foram incluídos pacientes que apresentavam os terceiros molares inferiores simetricamente. Um lado foi aleatoriamente designado para receber LBI aplicada imediatamente após a cirurgia (T1) e após 24 (T2) e 48 (T3) horas (Protocolo A). O lado oposto recebeu LBI imediatamente após a cirurgia e placebo após 24 e 48 horas (Protocolo B). A aplicação de LBI foi realizada intraoralmente (660nm, 5 J/cm^2, 10 s, 20 mW, 4 pontos), seguida pela aplicação extraoral (789 nm, 30 J/cm^2, 20 s, 60 mW, 8 pontos). O efeito do placebo foi similar ao experimental. Os desfechos primários eram dor, edema e intensidade do trismo nos tempos T1, T2, T3 e 7 após a cirurgia (T4). Os dados foram analisados por ANOVA e teste de Wilcoxon ($p<.05$). A amostra final consistiu de 21 pacientes (43 dentes). Não houve diferença estatística para o nível de dor entre os protocolos A e B ao longo do tempo ($p=.909$), embora a quantidade de medicação analgésica tenha sido menor com o protocolo A em T2 ($p= .022$). Não houve diferença para edema ($p=.958$) ou trismo ($p=.837$) entre os protocolos ao longo do tempo. Em conclusão, a aplicação de LBI imediatamente após a cirurgia e após 24 e 48 horas (Protocolo A) apresenta melhor resultado para controle da dor. Ambos os protocolos foram similares para dor, edema e trismo. Portanto, por razões de praticidade, uma aplicação única de laser imediatamente após a cirurgia pode estar indicada para o manejo do desconforto pós-operatório em cirurgias de terceiros molares inferiores.

Palavras-chave: *terceiro molar - trismo - edema - dor - terapia a laser de baixa intensidade.*

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INTRODUCTION

Surgical procedure to remove impacted or embedded third molars can limit masticatory function and cause, albeit temporarily, severe pain and discomfort[1,2]. Specific therapeutic alternatives have been studied as adjuvants of drugs in controlling pain, swelling and other undesirable postoperative complications. Low-level laser therapy (LLLT) has proven to be effective in reducing postoperative discomfort because of its analgesic, anti-inflammatory and tissue biomodulation effects[2-4].

Three biological effects produced by LLLT have been described: primary effects, classified as biochemical, bioelectric and bioenergetic, which stimulate the production of cellular ATP, causing mitotic acceleration; secondary or indirect effects, with increased blood flow and lymphatic drainage, which interfere with the body's inflammatory response; and general therapeutic effects, such as activation of the immune system[1-5].

There is extensive literature on the benefits of LLLT, including several controlled, randomized studies that evaluate the efficacy of a low-level laser compared to a placebo for the reduction of pain, swelling, and trismus after the removal of lower third molars[1-4]. On the other hand, certain studies that evaluated pain, swelling and trismus after removing lower third molars found no statistically significant difference between the use of LLLT and conventional drug therapy[6,7].

There are different protocols for using LLLT and various study designs for evaluation, and there is still no consensus on the best application method[1-4]. The aim of this study was to test the efficacy of two low-level laser protocols by evaluating pain control, swelling and trismus in the postoperative period of lower third molar surgeries.

MATERIALS AND METHODS

The study project was approved by the Ethical Review Board of Federal University of Goias (#68615217.0.0000.5083). The Declaration of Helsinki on medical protocol and ethics was followed. Patients were only included in the research after signing the institution-approved Free and Informed Consent Form as a means of guaranteeing confidentiality and presenting the risks and benefits of their participation in the research, and having been informed about the entire procedure they would follow.

A randomized, crossover, double-blind clinical trial was performed among patients who received care at the oral surgery service of the School of Dentistry at the Federal University of Goiás (FO-UFG). The inclusion criteria were good general and oral health, and bilaterally impacted lower third molars indicated for extraction with the same classification according to the Pell and Gregory (1933) classification in terms of impaction, and to Winter's (1926) classification in terms of angulation. Exclusion criteria were systemic diseases; using drugs that could interfere with the inflammatory process, pain and healing; infection or pathologies involving the regions of teeth 38 and 48; being allergic to standardized medication; smokers and alcoholics; pregnant or lactating women, and patients who did not follow postoperative recommendations or needed therapeutic supplementation in the postoperative period beyond that recommended for the study. Out of 28 patients selected, seven were excluded, 2 for not consenting to participate and 5 for not following the established guidelines. The final study sample consisted of 21 patients and 42 study sites.

A different protocol was applied on each side of the mouth of each patient:

PROTOCOL A – The LLLT was applied at three different times: immediately after the surgical procedure, and 24 and 48 hours after the surgery. There were four intraoral application points: one on the alveolus, and one each on the buccal, distal and lingual surfaces of the alveolus, 1 cm away from the surgical wound (Fig. 1A). There were 8 points on the face: one pre-auricular point, four points on the Masseter muscle, two submandibular points on lymph nodes and one retromandibular point (Fig. 1B).

PROTOCOL B – When performed on the opposite side, the LLLT was applied in the same way but in one session only immediately after the surgical procedure. Device modulation was identical for both intraoral and extraoral applications. To maintain the reliability of the study, the 24- and 48-hour sessions were performed in the same way, including sound emission, but without laser activation (placebo).

Patients were allocated by simple randomization according to order of arrival in a chart randomly numbered from 0 to 9, with each number appearing in the same quantity. Patients numbered from 0 to 4 were first allocated to Protocol A, while patients

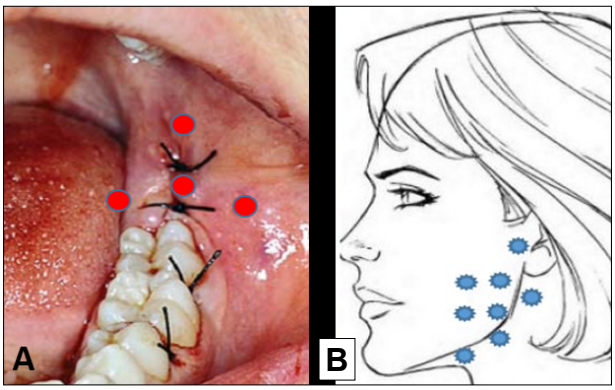

Fig. 1: A- points of application of intraoral laser therapy; B- points of application of extraoral LLLT.

numbered from 5 to 9 first received Protocol B. The patient's right side was always treated first, and the left side received the other protocol. Initial information, such as patient identification, classification of the radiographic positioning of the tooth, facial measurements and mouth opening range were recorded on a specific chart (T0).

To determine swelling and trismus, initial face measurements and mouth opening were collected before (T0) and after surgery. All measurements were taken using a pachymeter (Mitutoyo®, Mitutoyo, Suzano, Brazil). Swelling was measured by means of a simple average of three facial measurements: from the medial canthal ligament to the angle of the mandible, from the tragus to the labial commissure and from the tragus to the menton point (Fig. 2). The range of mouth opening was determined by measuring the mesio-incisal angle of the upper and lower incisors as reference points, measured in millimeters.

The extractions were performed by the same surgeon, thereby standardizing the surgical technique, as well as the anesthetic solution and technique. All patients received a postoperative prescription of Nimesulide 100 mg every 12 hours and Paracetamol 750 mg every 6 hours for two days. They were instructed that the analgesic medication could be taken at a longer time interval if they experienced no pain or mild discomfort. Conversely, if the pain was very intense, this time interval could be safely reduced, and the analgesic could be taken every 4 hours. The quantity and periodicity of analgesic pills taken each day were noted when each patient returned. The surgical procedures on the right and left sides were performed at different times, with a 15-day interval, so that there was no bias in the evaluations,

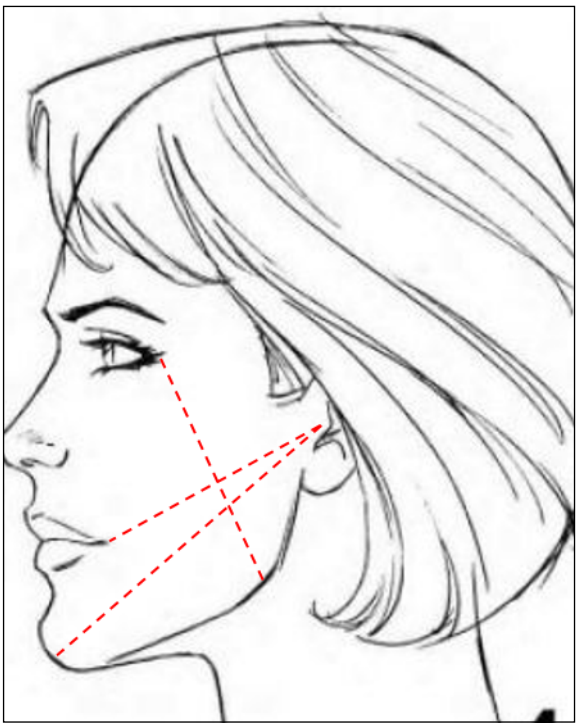

Fig. 2: Facial measurements to determine swelling

particularly in terms of the range of mouth opening. For the laser therapy protocol, a gallium-aluminum-arsenide laser device (Thera Laser, DMC Brasil®, continuous wave, 0.4 mm sized point) was used, with the tip of the device positioned on a surface of intact skin, cleaned by using dry air before application. The applications were performed by an oral and maxillofacial surgeon qualified to perform the procedure, who applied Protocol A on one side of the patient's face and Protocol B on the other. The locations of laser exposure and device modulation were the same for both protocols. For intraoral application, the following technical parameters were used: 660 nm (red), 5 J/cm^2 dose, t 10 s, P 20 mW, E 1.2 J per point. For the extraoral application, device modulation was 780 nm (infra-red), 30 J/cm^2 dose, t 20 s/point, P 60 mW, E 1.2 J per point.

Postoperative evaluations were performed by an evaluator blinded to the type of protocol used, and undertaken immediately after the surgical procedure (T1), 24 hours after (T2), 48 hours afterward (T3) and on the seventh day (T4) after the surgical procedure, for both sides. The chart used for each assessment contained the same information as the initial chart (T0), plus information on the need for analgesic medication and how frequently it was taken. The Visual Analogue Scale (VAS),

validated by Katz and Melzack[8], was used to assess postoperative pain.

The data were treated by SPSS Statistics Version 24 (SPSS Inc., Chicago, USA). The following tests were used: the Shapiro Wilk test to assess the normality of the data, the Levene test for homogeneity of variance, and the ANOVA repeated measures to assess trismus and swelling. The data for postoperative pain and quantity of analgesics used were non-parametric with non-normal distribution, so the Wilcoxon test was used. A significance level of 5% was adopted for all tests.

RESULTS

Table 1 shows the distribution of the patients by sex and the characteristics of the positioning of the 3rd molars.

Regarding pain and use of analgesics, patients reported no pain episodes at T1 and T4. Therefore, only the data obtained at T2 and T3 were included in the statistical tests. There was no difference between protocols A and B for pain evaluation (p = .909). In the evaluation of the amount of analgesic medication used, there was a lower consumption of analgesics at T2 when Protocol A (p= .022) was used (Table 2). Protocols A and B were compared, and no statistically significant difference was found between groups (p=.837). There was a difference between the times for both protocols tested in an inter-group evaluation, with T0 and T4 presenting some difference (*p*=.01). A more significant difference was found when T2 and T3 were compared to T0, T1 and T4 (p<.001) (Fig. 3). Table 3 shows descriptive statistics and p values for protocol A and B comparisons over time. When protocols A and B were compared, there was no statistical difference (*p*=.958) for post-surgical swelling. In an intra-group evaluation, the difference in the means of facial measurements between the times was significant, with the greatest difference found when T1 was compared to T2 and T3 (p<.001). The values at T0 and T1 were very close, as there had not been enough time for a significant swelling to form. In the evaluation of

Table 1 – Descriptive data of the sample in terms of sex and positioning of teeth 38 and 48 (n= 42 third molars)

		Frequency	Percentage Valid (%)
Sex	Male	11	52.4
	Female	10	47.6
Impaction level	Erupted	7	33.3
	Partially impacted	10	47.6
	Fully impacted	4	19.0
WINTER classification (of teeth 38 and 48)	Vertical	16	76.2
	Horizontal	1	4.8
	Mesio-angled	3	14.3
	Disto-angled	1	4.8
PELL and GREGORY classification (of teeth 38 and 48)	I-A	9	42.9
	II-A	2	9.5
	III-A	0	0.0
	I-B	4	19.0
	II-B	4	19.0
	III-B	1	4.8
	I-C	1	4.8
	II-C	0	0.0
	III-C	0	0.0

Table 2- Evaluation of the amount of analgesic medication used in the postoperative period of impacted lower third molars

Randomization	Time	Median	Minimum	Maximum
Protocol A	T2	4	2	4
	T3	4	3	6
Protocol B	T2	4	3	6
	T3	4	3	6

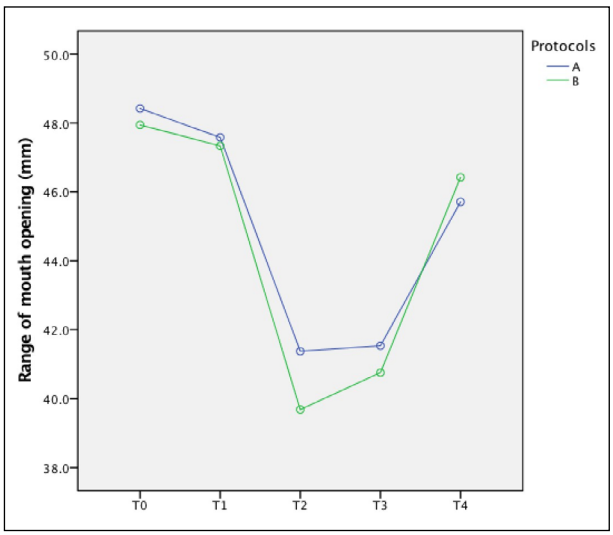

Fig. 3: Graphic representation of the variation in range of mouth opening (mm) during the postoperative period of impacted lower third molar surgeries (at times T0, T1, T2, T3 and T4), using two LLLT protocols.

Table 3 – Evaluation of trismus measured in the range of mouth opening in mm during the postoperative period of impacted lower third molar surgeries, using two LLLT protocols

Time	Protocol	Mean	95% CI		SD	P values
			UL	LL		
T0	A	48.41	44.57	52.26	8.43	.855
	B	47.94	44.12	51.76	8.38	.855
T1	A	47.58	43.81	51.34	8.27	.923
	B	47.33	43.62	51.04	8.14	.923
T2	A	41.37	37.67	45.07	8.13	.511
	B	39.68	35.86	43.51	8.40	.511
T3	A	41.53	37.80	45.6	8.19	.768
	B	40.75	36.75	44.76	8.80	.768
T4	A	45.71	42.26	49.15	7.57	.777
	B	46.42	42.49	50.35	8.64	.777

CI = confidence interval; UL = upper limit; LL = lower limit.

Table 4 – Evaluation of facial swelling in the postoperative period of impacted lower third molar surgeries, using two LLLT protocols

Time	Protocol	Medium	95% CI		SD	*P* values
			UL	LL		
T0	A	121.94	119.04	124.85	6.37	.915
	B	121.75	119.44	124.07	5.07	.915
T1	A	121.69	118.95	124.43	6.02	.868
	B	121.98	119.70	124.25	4.99	.868
T2	A	125.31	122.69	127.92	5.74	.870
	B	125.58	123.37	127.79	4.84	.870
T3	A	125.36	122.54	128.17	6.18	.793
	B	125.83	123.29	128.37	1.25	.794
T4	A	122.85	120.15	125.54	5.91	.813
	B	122.45	120.26	124.64	4.80	.813

CI = confidence interval; UL = upper limit; LL = lower limit.

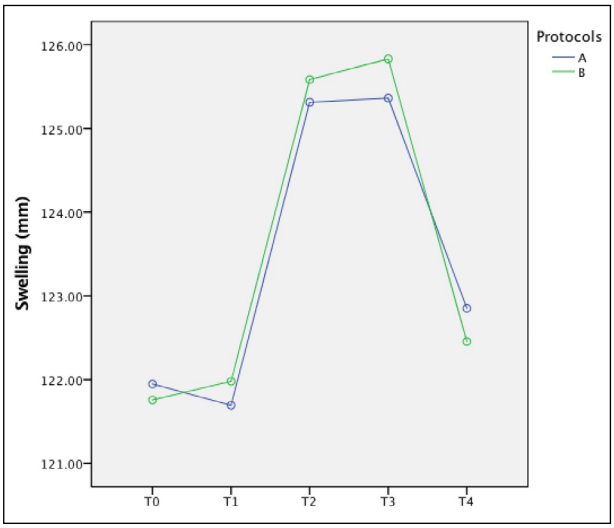

Fig. 4: Graphic representation of the variation in facial swelling (mm) in postopertive surgery of impacted lower third molars, at times T0, T1, T2, T3 and T4, using two LLLT protocols.

the 7-day postoperative evolution, the swelling had reduced to almost initial measurements (Fig. 4). Table 4 shows descriptive statistics and *P* values for protocols A and B comparisons over time.

DISCUSSION

This study shows no critical difference between LLLT applied in one or three times. Although protocol A leads to less use of analgesics, it was not relevant for swelling and trismus.

Concerning methodology, panoramic radiography of the jaws was used to evaluate and classify the third molar positions and surgical planning. According to Guerrero *et al.*[9], other imaging tests are not needed for this type of surgery. Careful radiological planning followed by an adequate surgical approach is essential for a good resolution of cases, irrespective of whether 2D or 3D preoperative images are used[10]. Postoperative swelling is mainly influenced by the surgical protocol used, and the length of time and degree of difficulty involved in the surgery[11]. In addition, the surgical technique used to remove impacted third molars affects the quality of the postoperative period, with some studies showing the importance of the care that must be taken with each step of the procedure[12,13]. To reduce bias in our study, a Split-Mouth design trial with a standardized surgical protocol was performed by a dental surgery specialist. The surgeries were conducted in the most atraumatic manner possible and resulted in lower levels of swelling and pain associated with laser therapy treatment. Similar measures of care have also been adopted in various comparable studies[14,15]. There is consensus on the prescription of preemptive or therapeutic antibiotics in cases where the patient has a systemic or local condition leading to an infection that might develop or worsen with third molar surgery[15,16]. However, studies show that if a good surgical technique is used and the aseptic chain is maintained, there is no need to use antibiotics in third molar surgeries[12]. Based on that evidence, antibiotics were not used in this study, as the patients presented neither local nor systemic conditions which would have justified such use.

Postoperative care is crucial in reducing the

discomfort caused by the surgical procedure. Pain, trismus and swelling are complications resulting from the inflammatory processes which occur after third molar surgery. Drugs are recommended to minimize the inflammatory response[1,2,13,17]. However, although medication is satisfactory in relieving pain, it also has adverse effects[16]. Adjuvant therapies, such as laser therapy, have been used to enhance postoperative comfort while at the same time reducing drug consumption[1-4,7,14].

The analgesic medication of choice used by patients who underwent surgery was Paracetamol 750mg due to its low rate of adverse reactions and its efficacy in controlling postoperative pain, even though it does not have a high degree of analgesic potential[13]. Other therapies to control inflammatory reactions were not adopted in this study so that more precise answers regarding the effects of LLLT could be obtained. The placebo was used specifically to evaluate the role of single or three LLLT applications.

Regarding pain, it was found that the consumption of analgesics was lower at T2 for protocol A compared to the quantity of analgesics used at T2 when protocol B was applied. This agrees with the results reported by Kazancioglu *et al.*[17], who also found a lower consumption of analgesics by patients who received LLLT in the postoperative period. Our results indicate that, in general, there was little difference between the protocols used.

Despite the decrease in analgesic consumption at T2 for protocol A, the difference was not statistically significant by VAS assessment. Other authors[7,18] also found no statistically significant difference in the pain presented by patients when using LLLT. Other studies found a difference in the pain profile of patients who used LLLT in the postoperative period of impacted third molar surgeries compared to the control group[1,2,19-22].

Variations in the medication protocols could explain these differences. In the studies by Fernando *et al.*[7], Landucci *et al.*[23], Eshghpour *et al.*[14], and Sierra *et al.*[24], the protocol used for postoperative medication included Amoxicillin 500mg for seven days, Ibuprofen 600mg for three days and Chlorhexidine 0.12% mouthwash. Markovic & Todrivic[1,2] and Amarillas-Escobar *et al.*[20] administered Dexamethasone in different doses for each group. There was also a difference in the laser therapy protocol. In our study, Paracetamol was prescribed at standard intervals (every six hours for two days). However, the patients were instructed that the medication could be taken at longer intervals if they felt less discomfort or no postoperative pain, and that the interval could be safely reduced in case of more severe pain. Well-established results of pain reduction found in studies on mucositis and other mucosal ulcerations could be related to the superficiality of the lesion[25]. The use of laser in bone alterations, such as postoperative surgery for third molars, demands further research to evaluate the in-depth effect of LLLT[26].

The trismus evaluation showed no statistical difference in the postoperative period between protocols A and B. Similar results were found in the literature[17,18,20,21], which also showed no difference in the trismus evaluation between a single dose of LLLT in the immediate postoperative period compared to applying it in 3 (three) sessions, as occurred in our study. However, different medication protocols and LLLT doses were used. Studies evaluating trismus when LLLT was used in the postoperative period of impacted third molar surgeries, which included groups that did not receive the laser treatment[5,27-29], showed positive results with lower trismus in the group where LLLT was applied. This shows that, even though our study found no statistical difference between protocols A and B, the use of LLLT in the postoperative period of impacted third molar surgeries can be beneficial, providing greater post-surgical comfort. The evaluation showed that there was no clinical difference between the protocols when swelling was compared. Of the factors analyzed, swelling seems to respond least to LLLT, a finding also reported in other studies[10,17,20].

The difficulty in comparing results published in studies is mainly due to the lack of standardization in the use of LLLT[17,18]. There are great variations between the technical parameters, time and place of application, and type of laser used. Some apparent benefit has been found, especially in relation to a reduction in trismus and pain, as noted in several studies[4,27-29]. Further research could contribute to clarify a better cost-benefit ratio of using only one LLLT session in the immediate postoperative period or adding other LLLT sessions to reduce pain.

In conclusion, this study found no difference between applying LLLT in 3 sessions (immediately, 24 and 48 hours after surgery) and applying it only in the immediate postoperative period. We consider that protocol A could be more challenging due to the need to apply it 24 and 48 hours after

surgery. Therefore, for practical reasons, a single laser application in the immediate postoperative period could be effective to manage postoperative discomfort in lower third molar surgery.

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Ability of two reciprocating Nickel-Titanium instruments for gutta-percha/sealer removal in simulated curved root canals

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ABSTRACT

The aim of this study was to compare the capacity of two reciprocating NiTi instruments in removing gutta-percha/sealer material from simulated curved root canals (SCRC). The time required for filling material removal was also recorded. Twenty SCRCs were divided into two groups of 10 (n=10) samples each. In Group 1, the SCRC were prepared to a R25 Reciproc Blue instrument (RCPB; VDW, Munich, Germany). In Group 2 the SCRC were prepared to a Primary WaveOne Gold instrument (PWOG; Dentsply, Ballaigues, Switzerland). In both groups, the canals were filled with matched-taper single gutta-percha cones and AH Plus sealer. Filling materials were removed with R25 RCPB (Group 1) and PWOG (Group 2). The amount of remaining gutta-percha/sealer was calculated at three predetermined levels of evaluation located at 2, 6 and 10 mm from the WL and expressed in percentages. Canals re-treated with RCPB contained significantly less remaining gutta-percha/sealer compared to canals prepared with PWOG (P=0.02). The RCPB instruments required significantly less time to complete the retreatment procedures (P<0.01). No unwinding or instrument separation was noted. RCPB instruments removed significantly more gutta-percha/sealer from simulated curved root canals than PWOG. However, neither of the tested instruments completely removed all filling materials.

Keywords: *Endodontics - gutta-percha - root canal therapy - retreatment.*

Análisis de la capacidad de dos instrumentos reciprocantes de Niquel-Titanio para la remoción de gutapercha/sellador en conductos curvos simulados

RESUMEN

El propósito del presente estudio fue comparar la capacidad de dos instrumentos de NiTi de movimiento reciproco para remover la obturación de gutapercha/sellador durante el retratamiento de conductos curvos simulados (SCRC). El tiempo requerido para la remoción del material fue también registrado. Se utilizaron veinte (n=20) SCRC divididos en dos grupos de diez especimenes (n=10) cada uno. En el Grupo 1 los SCRC se prepararon hasta un instrumento Reciproc Blue R25 (RCPB; VDW, Munich, Germany). En el Grupo 2, los SCRC se prepararon hasta un instrumento WaveOne Gold Primary (PWOG; Dentsply, Ballaigues, Switzerland). En ambos grupos los conductos se obturaron con cono único de gutapercha de conicidad creciente y el sellador AH Plus. La remoción de los materiales de obturación se realizó mediante los instrumentos RCPB R25 (Grupo 1) o PWOG (Grupo 2). La cantidad de gutapercha/sellador remanente se calculó en tres niveles de evaluación predeterminados ubicados a 2, 6 y 10 mm de la LT, y finalmente fue expresada en porcentajes. La cantidad de gutapercha/sellador remanente en los SCRC retratados con RCPB fue significativamente menor en comparación con los que fueron retratados con PWOG (P=0.02). Los instrumentos RCPB requirieron un tiempo significativamente menor para completar el retratamiento (P<0.01). No se observaron deformaciones o separación de los instrumentos.

Los instrumentos RCPB removieron una cantidad significativamente mayor de gutapercha/sellador que los instrumentos PWOG en conductos curvos simulados. Sin embargo, ninguno de los instrumentos ensayados removió completamente los materiales de obturación.

Palabras clave: *Endodoncia - gutapercha - tratamiento del conducto radicular - retratamiento.*

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INTRODUCTION

One reason for endodontic failures may be the persistence of bacteria in the root canal system[1]. This may be due to insufficient cleaning, the inability of the practitioner to identify and treat extra canals, or inadequate obturation, leading to the development of periapical pathosis. To re-establish the normal status of the periapical tissues, a non-surgical retreatment approach is the procedure of choice[2-4]. Complete removal of the pre-existing root canal filling materials followed by reinstrumentation, disinfection and obturation of the root canal system is essential for a successful outcome[5]. Historically, different methods have been used for removing filling materials, including hand-operated and engine-driven rotary instruments, some of which have been specifically designed for gutta-percha removal[6-10]. In this respect, two recently introduced reciprocating root canal preparation systems called Reciproc Blue (RCPB; VDW, Munich, Germany) and Wave One Gold (WOG; Dentsply Sirona, Ballaigues, Switzerland) were suggested for non surgical endodontic retreatment[11-13]. The RCPB system consists of three single-use instruments: R25 (25/.08), R40 (40/.06) and R50 (50/.05), which are manufactured using a special heat treatment technology. They have an S-shaped cross section, variable taper and a non-cutting tip[14]. The WOG system consists of four single-use instruments with a parallelogram-shaped cross section: Small (20/.07), Primary (25/.07), Medium (35/.06) and Large (45/.05). The WOG instruments were originally manufactured with heat treated M-Wire alloy, but have recently been changed to GOLD alloy technology. The metallurgical improvements in the RCPB and WOG instruments for increasing their flexibility and resistance to cyclic fatigue have been reported by Keskin et al.[15] and Sarefoglu et al.[16]. To the best of our knowledge, little information is available with respect to the efficacy of RCPB and WOG for removing gutta-percha/sealer.

The purpose of this study was to compare the capacity of RCPB and WOG NiTi instruments in removing gutta-percha/sealer material from simulated curved root canals (SCRC). The null hypothesis was that there would be no significant difference between the instruments in their ability to remove gutta-percha/sealer, and that there would be no difference in the total time required for retreatment.

MATERIALS AND METHODS

Twenty (n=20) Endo Training Resin Blocks (ETRB; Dentsply Maillefer, Ballaigues, Switzerland) with standardized SCRC 16 mm in length with a round cross-section, a 0.02 continuous taper and a curvature of 40° ± 0.5 were used in this study. After the canals were explored with #10 K-Files (Dentsply Maillefer), the working length (WL) was established from the top of the access opening to the stop end of the simulated canals (16 mm). The ETRB were then randomly divided into two groups of 10 (n=10) samples each.

Canal preparation and obturation

To avoid variation, all samples were prepared by a single trained operator using an electric X-Smart IQ motor (Dentsply Sirona) following the predetermined programs for each RCPB or PWOG instrument and according to the manufacturer's instructions. For both groups, the ETRB with the SCRC were mounted on a fixed custom attachment simulating a standardized clinical position. In Group 1, the SCRC were prepared to a R25 RCPB in a reciprocating crown-down motion. The instruments were introduced into the canals until resistance was felt and then used with 3 in-and-out-pecking movements and light apical pressure. The instruments were then removed and cleaned. After irrigation with 3 mL distilled water, the instruments were used again with in-and-out-pecking movements until the WL was reached. The canals were then irrigated with 3 mL distilled water and dried with paper points. The instruments were discarded after each canal preparation. In Group 2, the SCRC were prepared to a Primary WOG instrument (PWOG) using the same operative procedures as in Group 1.

In both groups, the SCRC were filled with the single gutta-percha cone technique[17] and AH Plus sealer (Dentsply, Ballaigues) prepared according to the manufacturers' instructions. The canal walls were coated with a thin layer of the sealer. A single matched-taper gutta-percha cone corresponding to each of the last instruments used for canal preparation was then coated with the sealer and slowly inserted into the canals until the WL was reached. Excess gutta-percha/sealer at the canal orifice was removed with a heated instrument followed by compacting with a plugger (Dentsply Maillefer). After obturation, all filled SCRC were kept at 37°C and 100% relative humidity for 14 days to allow the sealer to set completely.

Canal Retreatment

In Group 1, retreatment consisted of gutta-percha/sealer removal with R25 RCPB using a slow in-and-out-pecking motion until the WL was reached. The instruments were used along the entire length of the canal in an in-and-out-pecking motion with a brushing circumferential movement while pressing against the canal walls. This procedure continued until no filling material was observed on the flutes of the instruments as observed with an operating microscope (Newton MEC XXI, BA Argentina). If any gutta-percha/sealer remnants were visible, the process was repeated until no remnants of material were observed. A new instrument was used for each canal and then discarded. In Group 2, retreatment was performed with PWOG. The operative procedures were similar to those described for Group 1. The RCPB and PWOG were used at a fixed speed of 500 rpm with the manufacturer's recommended torque. In both groups, irrigation was performed throughout removal of the filling material using a total 5 mL distilled water per canal. The total time required for retreatment (including irrigation) was measured from the time when the canal was entered until no gutta-percha/sealer was visible on the instrument surfaces. The effective operating time was recorded in minutes using a digital chronometer. In addition, if unwinding or instrument separation occurred, it was also recorded.

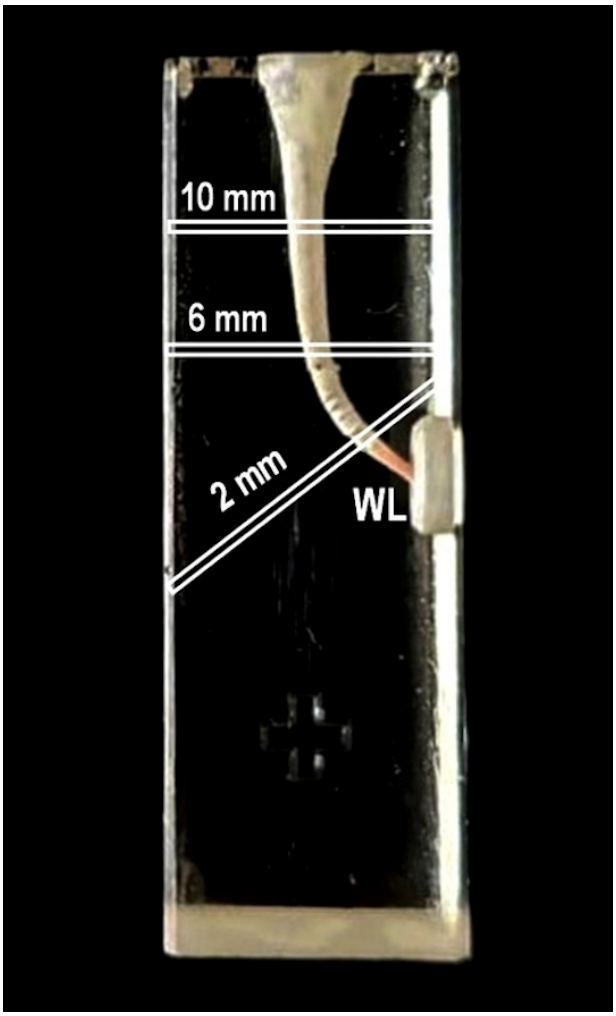

Fig. 1: Image of a resin block with simulated curved root canal after preparation showing a schematic drawing of the cross sections located at 2, 6 and 10 mm from the WL.

Filling removal evaluation

After retreatment, the SCRC were cross sectioned at 2, 6 and 10 mm from the WL, which were considered as evaluation levels (EL). Sections 1 mm thick were cut at low speed under constant irrigation with distilled water using a diamond wafering blade 0.3 mm thick mounted on a Precision Micro Disc NH-6P cutting machine (DHUC Ing, BA, Argentina). The cuts were made perpendicularly to the long axis of the SCRC (Fig. 1). All sections were photographed under reflected light at x10 magnification using a Sony Cyber-shot DSC-W180 digital camera (Sony Corporation, Tokio, Japan) coupled to an Axio Imager A1m stereomicroscopic loupe (Carl Zeiss, Oberkochen, Germany). The photographs were taken at a fixed focal distance of 5 cm and transferred to a computer. For each SCRC, the area covered by remaining filling material and the total canal area at each of the predetermined levels of the canal were outlined (Fig. 2) and measured

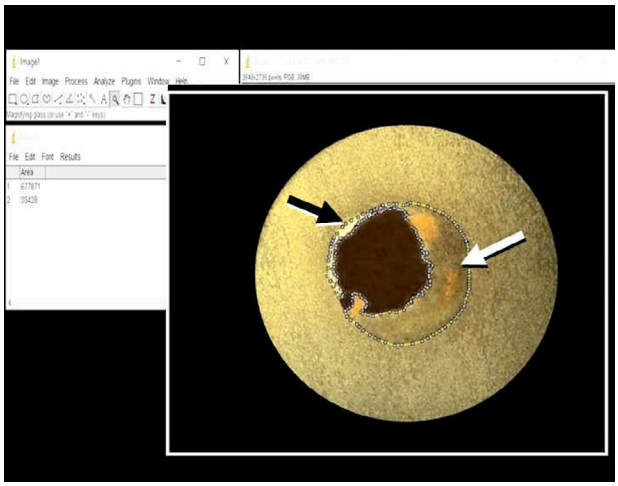

Fig. 2: Example of gutta-percha and sealer remaining on the root canal walls after retreatment. Note the outlined areas occupied by the materials, calculated using the Image J Program. Black arrow: sealer remnants; White arrow: Gutta-percha remnants. Original magnification X10.

using the Image J 1.38x Image Analysis Software (National Institutes of Health, Bethesda, MD). The images were analyzed by two trained examiners who were blind to group assignment. Any cases for which there was disagreement were discussed until agreement was reached. Using the digital images, the area of remaining filling material was calculated as percentage. The measurements were repeated 3 times and the mean percentage values for each group were analyzed comparatively.

Statistical analysis

The data were analyzed using the SPSS Version 21 (IBM Corp, Chicago IL) at a 5% significance level. To identify any significant differences between groups a Two-way Analysis of Variance for repeated measures was used. The time required by RCPB and PWOG instruments to complete the retreatment procedures was analyzed by Student's *t*-test.

RESULTS

The mean percentage of remaining filling material and the results for the total time required for retreatment are shown in Table 1. There were significant differences ($p<0.01$) between the two groups with respect to the time required for retreatment (4.6 ± 0.6 and 6.6 ± 1.4 min for RCPB and PWOG, respectively). In both groups, remaining filling material was observed on the canal walls of all specimens. When considering the type of instrument for Retreatment (groups), the RCBP left significantly less filling material than PWOG ($p=0.02$). With respect to the action of the instruments at each EL, there were significant differences among all of them ($p<0.05$). The most significant amount of remaining material in both groups was found at 2 mm from the WL ($p<0.01$). No event of instrument unwinding or separation was noted throughout the experiment.

DISCUSSION

The success of endodontic retreatment depends on the thorough removal of the old filling material and the elimination of remaining infected tissues that contribute to endodontic failure. The present study compared the capacity of R25 RCPB and PWOG reciprocating instruments for gutta-percha/sealer removal as well as the required operating time for root canal retreatment. For the experiment, resin blocks with standardized SCRC were used in order to avoid any anatomic variations that are normally present in root canals of natural teeth. The degree of homogeneity (baseline) of SCRC with respect to canal volume, surface area and canal length before canal preparation enabled standardization of the groups, thus enhancing the validity of the study[18-22]. However, the results of the study cannot be fullyextrapolated to natural teeth because clinical cases normally involve multiple variables.

In the present study, the matched-taper single gutta-percha cone technique was used for root canal obturation[17]. According to Gordon et al.[23] and Schäfer et al., [24] the tapered cones match the prepared root canal geometry. The technique effectively fills most narrow, curved canals, and has become widely accepted as an equivalent alternative to other obturation techniques[17,24]. As per protocol, the amount of remaining filling material was assessed by cross-sectioning the canals at three different levels. In comparison to other evaluation methods,[7, 11, 25] cross sections allow visualization of the entire circumference of a canal[23], thereby enabling comprehensive assessment of the areas with remaining filling material. Interestingly, no unwinding or instrument separation was observed for RCPB or PWOG during the experiment. Our results agree with those of other authors [15, 16, 26], and suggest that this could be because of the specific heat treatment of the NiTi alloy, which improves instrument resistance to fatigue and flexibility. Moreover, as reported by Ruddle et al.[27], the shape

Table 1. Means ± SD of remaining filling material (expressed as percentage) at 2, 6 and 10 mm from the WL, and total time required for retreatment.

GROUP	n	2 mm	6 mm	10 mm	Time (min)
1. RCPB	10	28.5 ± 8.3^{aA}	18.4 ± 7.5^{aB}	6.5 ± 3.2^{aC}	4.6 ± 0.6^{a}
2. PWOG	10	51.3 ± 28.8^{bA}	22.1 ± 8.9^{bB}	12.9 ± 13.3^{bC}	6.6 ± 1.4^{b}

Different lowercase letters represent significant differences between groups. Different uppercase letters represent significant differences within the same group. SD: Standard deviation

of the cross-section of the RCPB and PWOG appears to provide sufficient space between the flutes and the canal walls to avoid engagement during transportation of debris in coronal direction.

Our findings showed significantly more remaining gutta-percha and sealer at the level of 2 mm from the WL. In this respect, the results are in line with those of Ersev et al.[9] and Khedmat et al.[10], who suggest that similar results could be expected when removing the filling materials with NiTi instruments up to the WL. According to Hülsman and Bluhm[7], this may be because during retreatment, it is difficult, if not impossible, to direct NiTi instruments toward certain aspects of the root canal walls, especially in the apical third. Moreover, the last instruments used for gutta-percha/sealer removal could be insufficient to remove all traces of the filling materials. Because the aim of the present study was limited to experimental comparison of the capacity of RCPB and PWOG for gutta-percha/sealer removal, the effects of further refining the canal walls with larger sized instruments were not tested in our protocol. However, it should be noted that from a clinical point of view, endodontic retreatment procedures require not only removal of filling materials, but also removal of infected dentine. Thus, further re-instrumentation is recommended to ensure better cleaning of the root canal space[28]. The results of this study demonstrated significant differences between RCPB and PWOG with respect to their ability to remove filling material, as well as the total time required for retreatment. Therefore, the null hypothesis was rejected. Our findings agree with other authors [7-10, 26, 29, 30] who report that to date, none of the currently available techniques or instruments are capable of completely removing filling material from root canals. Further research is needed on instruments and retreatment techniques with the aim of identifying which ones are more effective for the removal of pre-existing filling material.

CONCLUSION

Within the limitations of the present study, RCPB proved to be more capable and faster than PWOG for gutta-percha/sealer removal. Although both instruments were useful and safe, they did not completely remove filling material from laboratory models with simulated curved root canals.

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DECLARATION OF CONFLICTING INTERESTS

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Cyberchondria and Associated Factors Among Brazilian and Portuguese Dentists

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ABSTRACT

Cyberchondria is a psychopathological behavior that affects people who compulsively consult the internet, by searching the symptoms of different pathologies from which they believe they are suffering, and when influenced by what they read, are sure they have some of these diseases. The aim of this study was to assess the cyberchondria level and associated factors among Brazilian and Portuguese dentists. A total 597 Brazilian and Portuguese dentists participated in this cross-sectional study. They were contacted via WhatsApp and asked to complete an online questionnaire on the Google Forms platform, from January 17 to 31, 2021, during the COVID-19 pandemic. Sociodemographic information was collected and cyberchondria was measured using the Portuguese language version of the Cyberchondria Severity Scale. Binary logistic regression models were used to estimate the unadjusted and adjusted Odds Ratio (OR) and corresponding 95% confidence interval (CI) for the association of cyberchondria and covariates. Each covariate was individually included in the regression model, and the unadjusted OR (95% CI) was estimated. Most participants were Brazilian (62.8%), women (75.5%), married (60.5%) and with children (55.6%). Average age was 42.1 years (+ 12.5). In the final model, it was found that with each increase of one year in age, the chance of a high level of cyberchondria decreased (OR=0.97; 95% CI 0.95-0.98). Brazilian dentists were 1.85 times more likely (95% CI 1.25-2.75) to have a high level of cyberchondria than Portuguese dentists. Women were 1.62 times more likely (95% CI 1.07-2.44) to have a high level of cyberchondria than men. It was concluded that young age, Brazilian nationality, and female gender favored the high level of cyberchondria among the participants in this sample during COVID-19 pandemic.

Keywords: *behavior - somatoform disorder - dentistry - epidemiology - COVID-19 pandemic.*

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Cibercondria e fatores associados entre dentistas brasileiros e portugueses

RESUMO

A cibercondria é um comportamento psicopatológico que atinge as pessoas que consultam compulsivamente a internet, pesquisando os sintomas das diferentes patologias de que acreditam estar sofrendo e, quando influenciadas pelo que leem, têm a certeza de possuir alguma dessas doenças. O objetivo deste estudo foi avaliar o nível de cibercondria e fatores associados entre dentistas brasileiros e portugueses. Participaram deste estudo transversal 597 dentistas brasileiros e portugueses. Eles foram contatados via WhatsApp e solicitados a preencher um questionário online na plataforma do Google Forms, no período de 17 a 31 de janeiro de 2021, durante a pandemia de COVID-19. Informações sociodemográficas foram coletadas e a cibercondria foi mensurada através da versão em língua portuguesa da Cyberchondria Severity Scale. Modelos de regressão logística binária foram usados para estimar a Odds Ratio (OR) não ajustada e ajustada e o intervalo de confiança (IC) de 95% correspondente para a associação de cibercondria e covariáveis. Cada covariável foi incluída individualmente no modelo de regressão, e o OR não ajustado (IC 95%) foi estimado. A maioria dos participantes eram brasileiros (62,8%), mulheres (75,5%), casados (60,5%) e com filhos (55,6%). A média de idade foi de 42,1 anos (+ 12,5). No modelo final, verificou-se que a cada aumento de um ano de vida, a chance de um nível elevado de cibercondria diminuía (OR = 0,97; IC95% 0,95-0,98). Os dentistas brasileiros tiveram 1,85 vezes mais probabilidade (IC95% 1,25-2,75) de apresentar alto índice de cibercondria quando comparados aos portugueses. As mulheres tinham 1,62 vezes mais probabilidade (IC 95% 1,07-2,44) de ter um alto nível de cibercondria em comparação com os homens. Concluiu-se que a idade mais jovem, a nacionalidade brasileira e o sexo feminino favoreceram o alto índice de cibercondria entre os participantes desta amostra durante a pandemia COVID-19.

Palavras-chave: *comportamento - transtorno somatoforme - odontologia – epidemiologi -- pandemia COVID-19.*

INTRODUCTION

The term "Cyberchondria" was coined by joining the words cyber and hypochondria to characterize a psychopathological behavior, a somatoform disorder, now also called digital hypochondria. A cyberchondriac is a person who performs repeated internet searches for health information, which are associated with increasing levels of health anxiety[1]. The impact of internet on access to health information has increased over time[2], and although finding information on the characteristics, prevention and self-care of illnesses has enabled people to improve their health outcomes[3], it should be noted that internet can provide access to both trustworthy and untrustworthy content[2].

Cyberchondria entails excessive online searches to seek reassurance about the nonthreatening nature of the self-perceived signs and symptoms. This condition has been associated with obsessive-compulsive disorders, hypochondriasis, and health anxiety, and can be a risk factor for the development of more mental illness[4]. People with cyberchondria have heightened anxiety, often because of new pathologies that they have discovered online, and this generates new concerns that are amplified in the mind of the cyberchondriac. Anxiety disorders can lead to discomfort, accelerated heartbeat, cold sweat and poor sleep quality[5].

Cyberchondria has been studied since 2000[1]. Since then, professionals have sought to analyze this behavior that can be adopted by anyone, including students of health sciences. Students of medicine, nursing, dentistry, and other health sciences may incorrectly believe that they have contracted certain diseases they have studied[6]. This can also be observed in the form of anxiety. Health anxiety, cyberchondria and sociodemographic factors may show specific behavior patterns among students of health sciences. Medical students in Turkey had high scores of health anxiety and men were more suspicious of doctors, seeking information on the internet prior to going for medical consultations[6]. This mistrustful behavior can compromise the promotion of physical and emotional health[6]. A study on Indian computer technicians found a prevalence of 55.6% of high level of cyberchondria[7], and the authors emphasized that this high prevalence deserved preventive care. Cyberchondria compromises mental health, so finding ways to prevent it can help promote public health[7].

In view of the relevance and scarcity of data about the topic in the field of dentistry, the aim of this study was to assess the level of cyberchondria and factors associated with it among Brazilian and Portuguese dentists.

MATERIALS AND METHODS

Ethical Aspects

This study was conducted in compliance with the principles of the Declaration of Helsinki (revised in World Medical Association 2013), and approved by the Human Research Ethics Committee of the Federal University of Minas Gerais (Brazil) (Protocol #91561018.5.0000.5149).

Design, Population, and Study Sample

This study included Brazilian and Portuguese dentists who consented to answer an online questionnaire posted on the Google Forms platform from January 17 to 31, 2021. Subjects were invited to participate by means of a link disseminated via WhatsApp, Facebook Messenger, and online forums for Dentists. Dentists were contacted through both Brazilian and Portuguese dental class societies.

The questionnaire was structured into two sections: one for collecting sociodemographic information, and another to measure cyberchondria using the Portuguese language version of the Cyberchondria Severity Scale (CSS)[8]. The original CSS was developed by Irish psychologists with undergraduate students[9].

To participate in the study, each participant was asked to complete and sign a free and informed consent form. Only dentists were included. The exclusion criterion was anyone who was not a dentist from Portugal or Brazil. Each participant received his/her personal score for the CSS.

Data Collection and Variables

Participants answered a questionnaire with 40 closed questions. The questionnaire included seven questions to collect participants' demographic information.

The CSS includes 33 statements that allow each participant to define his/her typical behavior, using a five-point scale: Never; Rarely; Sometimes; Frequently; Always[8]. The total score ranged from zero to 132 points. The higher the score, the higher the level of cyberchondria[8].

Pilot Study

A pilot study to evaluate the proposed methodology was conducted on 21 dentists. The results of the pilot

study demonstrated that there was no need to modify the proposed methods of the main study. Pilot study participants were not included in the main study.

Statistical Analysis

Descriptive data analysis was performed with the calculation of proportions, central tendency and variability measures. The dependent variable was the Cyberchondria score, which ranged from 0 to 132. The score was categorized by the median, into low level of Cyberchondria (0-35), and high level of Cyberchondria (36-132).

Binary logistic regression models were used to estimate the unadjusted and adjusted Odds Ratio (OR) and corresponding 95% Confidence Interval (CI) for the association of Cyberchondria and covariates. Each covariate was individually included in the regression model, and the unadjusted OR (95% CI) was estimated. All covariates were simultaneously included in the final binary logistic model, in which only variables with P values lower than 0.05 remained significant. Linearity of logit of quantitative variables was also checked. Collinearity diagnosis was made by the Variation Inflation Factor. The Hosmer & Lemeshow test was used to check the goodness of fit of the final model. All statistical analysis was performed using SPSS software for Windows version 26.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

A total 597 dentists participated in this study, of whom 62.8% were Brazilian, and 37.2%, Portuguese. The majority had graduate degrees (57.2%), were women (75.5%), married (60.5%) and had children (55.6%). Age ranged from 22 to 77 years, with mean age 42.1 (+12.5) (Table 1). The power of the sample

Table 1- Descriptive analysis of the study variables

Quantitative variables	**Mean (Median)**	**SD**	**Min-Max**
Cyberchondria score (N=597)	36.8 (35.0)	17.4	0-110
Age (N=596)	42.1 (41.0)	12.5	22-77
Years since graduation (N=597)	18.4 (17.0)	12.6	0-51
Hours of sleep per night (N=597)	6.9 (7.0)	0.9	3-10
Categorical variables	**Frequency**		**%**
Country (N=597)			
Brazil	375		62.8
Portugal	222		37.2
Sex (N=597)			
Female	451		75.5
Male	146		24.5
Marital status (N=597)			
single / divorced / widowed	236		39.5
married / cohabitation	361		60.5
BMI (N=597)			
Low weight	15		2.5
Overweight or Obesity	219		36.7
Suitable Weight	363		60.8
Graduate degree (N=596)			
Master/PhD/post-doctoral stage	341		57.2
Specialization	172		28.9
Undergraduate	83		13.9
Children (N=597)			
No	265		44.4
Yes	332		55.6

Note: Not all participants answered all questions. SD= standard deviation

test was calculated, and when analyzing the variable referring to the countries Brazil and Portugal, a power of 78.5% was found.

The final model showed that for each one-year increase in age, the chance of high level of cyberchondria decreased (OR = 0.97; 95% CI 0.95-0.98). Brazilian dentists were 1.85 (95% CI 1.25-2.75) times more likely to have a high level of cyberchondria than Portuguese dentists. Women had 1.62 (95% CI 1.07-2.44) times greater chance of high level of cyberchondria than men. When evaluating multicollinearity, the variable ‘’graduated a given number of years ago” was removed from the final model VIF> 10 (Table 2). After this removal, there were no further collinearity problems in the final model. The assumption of linearity of the logarithm for the age variable was not violated. The assessment of influential cases by Cook’s distance did not reveal values higher than one, indicating the absence of influential cases. The final model was

Table 2- Analysis of the association between cyberchondria scores and study variables between Brazilian and Portuguese dentists

Variables	Frequency (%) Cyberchondria score higher than the median (36-110)	Unadjusted Odds Ratio (CI 95%)	P	Adjusted Odds Ratio (CI 95%)	P
Age		0.97 (0.96-0.99)	<0.001	0.97 (0.95-0.98)	<0.001
Years since graduation		0.98 (0.96-0.99)	0.001		
Hours of sleep per night		0.96 (0.81-1.14)	0.649	0.93 (0.77-1.12)	0.467
Country (N=597)					
Brazil	52.3	1.46 (1.05-2.04)	0.025	1.85 (1.25-2.75)	0.002*
Portugal	42.8	1		1	
Sex (N=597)					
Female	51.9	1.68 (1.15-2.46)	0.007	1.62 (1.07-2.44)	0.023*
Male	39.0	1		1	
Marital status (N=597)					
single / divorced / widowed	57.6	1.81 (1.30-2.52)	<0.001	1.43 (0.95-2.15)	0.084
married / cohabitation	42.9	1		1	
BMI (N=597)					
Low weight	53.3	1.21 (0.43-3.42)	0.713	1.00 (0.34-2.92)	0.993
Overweight or Obesity	48.9	1.02 (0.73-1.42)	0.930	1.25 (0.85-1.83)	0.254
Suitable Weight	48.5	1		1	
Graduate Degree (N=596)					
Master/PhD/post-doctoral stage	50.1	1.08 (0.67-1.75)	0.750	1.19 (0.71-1.99)	0.515
Specialization	45.9	0.91 (0.54-1.54)	0.734	1.00 (0.56-1.78)	0.994
Undergraduate	48.2	1		1	
Children (N=597)					
No (N=265)	55.8	1.67 (1.21-2.32)	0.002	0.97 (0.61-1.52)	0.881
Yes (N=332)	43.1	1		1	

Note: Not all participants answered all questions. CI = confidence interval; p=probability value; BMI= body mass index. *: statistically significant values.

adequate according to the assessment made by the Hosmer & Lemeshow test (p = 0.498).

DISCUSSION

The aim of this study was to assess the level of cyberchondria, and factors associated with it, among Brazilian and Portuguese dentists. Our results demonstrated that a high level of cyberchondria was more prevalent among women, among younger ages and among Brazilian dentists. These results will be discussed below.

The study of cyberchondria is still very recent. There are controversies about gender and age differences[10]. Our findings showed a higher level of cyberchondria among women, which was in agreement with the findings of Atikinson et al. (2009)[11]. However, other studies have found no differences between the genders[11-14]. These differences in results may be justified by the cultural differences in the studies mentioned.

A systematic review found that women tended to use the internet more frequently than men[15]. On the internet, the behavior between genders differs, as women tend to seek more information on health and social networking sites while men tended to consult video. Furthermore, women tend to be more anxious than men[16]. Studies have suggested that anxious people spend more time on the Internet[11-14]. With the already present fear of contracting numerous diseases, anxious people become even more concerned about their health as a result of online medical searches, and this is characteristic of cyberchondriacs[12]. People with a high level of anxiety are more likely to exaggerate the perception of signs and symptoms of their own or those of loved ones[11]. The more emotionally overwhelmed someone feels, the more the person tends to spend time looking for the specific symptom, and the more they search for disease symptoms, the more they convinced are that they are ill[13].

Internet use was found to decrease as age increased[17]. Young people are more familiar than the elderly with websites, software and social networks[1,17], and consequently, there are more younger than older cyberchondriacs, as seen in our findings. This result was in line with those reported by McMullan et al.[1]. The number of internet users in the world has increased in recent decades. In 2021, to date, there are 145.83 million internet users in Brazil and 7.7 million in Portugal. Brazil is the larger country, with a surface area of 8.516.000 sq km and population 211 million, while Portugal has a surface area of 92.212 sq km and population 10 million[18]. The proportion of internet users in both countries is similar, at 70%. The territorial and housing differences therefore do not explain the greater number of cyberchondriacs in Brazil than in Portugal. Our findings therefore suggest a cultural difference.

It is also important to emphasize that data were collected during the COVID-19 pandemic. Social distancing was in place in both countries at the time of data collection. However, Brazil was experiencing an uncontrolled situation of disease, with an average 3000 deaths every 24 hours. Portugal had already started the vaccination campaign against COVID-19, while Brazil had not. This increases anxiety and can influence the behavior of cyberchondriacs[2,10].

Limitations were noted in this study. The cross-sectional design allows analysis of association and does not allow analysis of cause and effect. Future studies with other longitudinal and qualitative designs should be encouraged. The sample, made up of dentists, limits the extrapolation of results to other communities and other professionals. The study of cyberchondria is still very recent and there are few studies with which to compare results, which would lead to a more in-depth discussion[19]. Our findings highlight the importance of educational campaigns on cyberchondria. Dentists are important health promoters[20] and should be encouraged to take care of their own physical and mental health.

CONCLUSION

Based on the results, it may be concluded that women, younger dentists, and those of Brazilian nationality seem to be more prone to high levels of cyberchondria than men, older persons and dentists of Portuguese nationality.

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

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Saliva sampling methods. Cariogenic streptococci count using two different methods of saliva collection in children

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ABSTRACT

The aim of this study is to compare the efficacy of two methods for collecting saliva samples from infants under 2 years of age for cariogenic streptococci (CS) count. Two collection methods were applied in 11 infants. In Method (A), saliva samples were collected by swabbing the inner cheek mucosa and floor of the mouth in figure of eight motions with a sterile cotton swab until it was soaked. In method (B), saliva samples were collected by aspiration of 1 ml of saliva with a sterile plastic syringe on the floor of the mouth, after stimulation with glove. The samples were cultured in modified Gold's broth (MSMG), and on trypticase, yeast extract, sucrose, cystine and bacitracin culture medium (TYSCB). In method (A), the swab with the sample was unloaded in situ on TYSCB and placed in PBS medium for transport. Then, 100 µl of the eluate was seeded in MSMG. In method (B) 100 µl were seeded in TYSCB and 100 µl in MSMG. Both culture media were incubated under capnophilic conditions for 48 hours at 37 °C. Colony forming units (CFU/ml) were counted by calibrated operators (kappa = 0.75). The presence of cariogenic streptococci (CS) (Streptococcus mutans-Streptococcus sobrinus) was determined by qPCR in the samples collected by both methods. The CFU/ml counts in MSMG differed significantly between methods (p = 0.021). In TYSCB, the recovery of CFU/ml was higher in method (A), without significant difference (p = 0.705). The molecular technique detected presence of CS, with no difference between collection methods.

Collecting saliva samples by swabbing proved more effective in terms of recovery of microorganisms, and did not affect the detection of presence of CS by molecular techniques..

Keywords: *Saliva - Streptococci mutans - Streptococci sobrinus - Infants.*

Recuento de estreptococos cariogénicos a partir de dos métodos de obtención de saliva en niños

RESUMEN

El objetivo de este estudio es comparar la eficacia de dos métodos de obtención de muestras salivales, en infantes menores de 2 años para el recuento de estreptococos cariogénicos (EC). Se aplicaron dos métodos de recolección en 11 infantes, el método (A), consistió en la recolección de muestras de saliva con hisopos de algodón estériles, realizando movimientos en ocho sobre la mucosa del carrillo y piso de boca, hasta embeber el hisopo. En el método (B) la recolección de las muestras se realizó por aspiración con jeringa plástica estéril en piso de boca hasta obtener 1 ml, luego de estimulación con guante. Las muestras fueron cultivadas en caldo de Gold modificado (MSMG) y medio de cultivo TYSCB (tripticasa, extracto de levadura, sacarosa, cistina y bacitracina). En (A), el hisopo con la muestra fue descargado in situ en TYSCB y colocado en medio de transporte PBS. 100 µl del eluato se sembró en MSMG. En (B) 100 µl fueron sembrados en TYSCB y 100 µl en MSMG. Ambos medios de cultivo fueron incubados en condiciones de capnofilia por 48 hs. a 37°C. El recuento de unidades formadoras de colonias (UFC/ml) se realizó por operadores calibrados (kappa= 0.75). La presencia de EC (Streptococcus mutans - Streptococcus sobrinus) fue determinada por qPCR en las muestras obtenidas por ambos métodos. Los resultados mostraron que los recuentos de UFC/ml en MSMG presentaron diferencias significativas entre ambos métodos (p=0.021) En TYSCB la recuperación de UFC/ml fue mayor en el método (A), sin observarse diferencias significativas (p=0.705). Se detectó la presencia de EC por técnica molecular, sin mostrar diferencias entre los métodos empleados.

La recolección de muestra de saliva con hisopo presentó mayor eficacia en términos de recuperación de microorganismos, sin alterar la detección de presencia de EC por técnicas moleculares.

Palabras clave: *Saliva - Streptococcus mutans - Streptococcus sobrinus - Infantes.*

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INTRODUCTION

The oral cavity is an easily accessible site from which to collect biological material for studying and diagnosing systemic and oral diseases, and analyzing of microbial communities[1]. Microbial counts in specimens of saliva and dental plaque have provided information for estimating caries risk in adults and children, showing a positive correlation between caries experience and cariogenic streptococci (CS) counts[2]. The methods proposed for counting bacteria include culture in selective media, biochemical tests, immunological and genetic tests with DNA probes, enzyme-linked immunosorbent assay, and polymerase chain reaction (PCR)[3]. Culture in selective media is a useful tool for morphotyping, counting colony-forming units (CFU/ml) and obtaining bacterial strains for molecular processes to enable genomic studies. Media such as Mitis Salivarius Agar (MSB) and Tryptic Soy Agar (TSY20B) are often used in studies to demonstrate the correlation between cariogenic streptococci counts and caries lesions[4]. Gliosca et al.[5] proposed another technique for counting bacteria by means of an adhesion test (AA-MSMG) using the selective culture medium modified Gold's broth (MSMG-20% sucrose) to evaluate cariogenic risk. This method has satisfactory predictive value and is a valid instrument to categorize patient risk. In addition, molecular techniques such as real-time polymerase chain reaction (qPCR), due to their high sensitivity, specificity and speed, are also effective for detecting and quantifying bacterial species[6].

Regardless of the microbiological analysis methods, saliva sampling needs to be performed using simple, affordable, noninvasive techniques, which are standardized, valid and reproducible.

Some salivary components vary according to the saliva collection method. Therefore, before beginning with a study, a technique must be selected to optimize sample collection according to the study objective and/or the biomarker to be analyzed[7], taking into account the type of saliva to be collected (stimulated or unstimulated) and the context in which the study is to be performed. Results will thus be comparable to those of other studies.

Saliva samples can be obtained using absorbent or non-absorbent methods. Absorbent methods include collecting samples using different materials such as paper strips, cotton rolls and polystyrene and polyethylene swabs. Non-absorbent methods include collecting biological material by spitting or passive drooling into sterile collection containers, and sampling by aspiration with devices such as plastic syringes, among others[8,9].

There are some studies evaluating the efficiency of saliva collection methods for studying different biomarkers[10,11]; however, they have not been validated for detecting and identifying microorganisms in children.

The aim of this study is to compare the efficacy of two methods for collecting saliva samples from infants under two years old for cariogenic streptococci (CS) count. Microorganisms were counted using two differential selective culture mediums and by molecular detection.

MATERIALS AND METHODS

This study was conducted on 11 infants aged 6 to 28 months who attended an early childhood center in Buenos Aires City. This was part of the project "Horizontal transmission and early colonization of mutans group Streptococci in infants who attend mother-and child educational centers", approved by the Ethics Committee at the Buenos Aires University School of Dentistry (CUDAP: EXP-UBA: 0072332/201 7 Nº O12/2018 C.ÉTICA FOUBA).

Saliva specimens were collected from each participant using two different methods: an absorbent method (A), and a non-absorbent method (B). The methods were applied in alternative order on different infants to avoid the possibility of the second method collecting a smaller specimen due to the child being more tired or less cooperative.

In Method (A), saliva samples were collected by swabbing the inner cheek mucosa and floor of the mouth in figure of eight motions with a sterile cotton swab until it was soaked. Then the swab was unloaded in situ by plating on Petri dishes containing TYSCB (Tryptone Yeast Extract Cystine Sucrose and Bacitracin) culture medium, and then in Eppendorf-type tubes containing phosphate-buffered solution (PBS), for transfer. In method (B), saliva samples were collected by aspiration of 1 ml of saliva with a sterile plastic syringe on the floor of the mouth, after stimulation with glove. The content of the plastic syringe was unloaded in Eppendorf-type tubes. The samples were taken to the Microbiological Diagnosis Laboratory at Buenos Aires University's School of Dentistry within 2 hours of sampling.

The saliva samples were vortexed and seeded in two differential selective culture mediums for colony-forming unit count (CFU/ml).

The samples taken using method A were plated in TYSCB (in situ), and 100ul of the elute in PBS was seeded in modified Gold's broth (MSMG)[5]. Equivalent aliquots of the samples taken using method B were seeded in TYSCB and MSMG.

Cultures were incubated under anaerobiosis (GasPack - Mitsubishi®) for 48 hours at 36 ºC ± 1 ºC. After incubation, calibrated personnel (Kappa >0.75) observed the cultures under stereoscopic microscope ("Arcano" ST30-L binocular stereo microscope) at 50X magnification. Counts were performed considering the morphological characteristics of the colonies described for cariogenic streptococci under the study conditions. On TYSCB, counts were performed up to a maximum 300 characteristic colonies per plate, and values higher than this were not recorded. Adhered colonies applying the AA-MSMG were counted in 3 areas of $1cm^2$, using a grid covering the entire contact the area of the culture bottle.

Molecular processing to determine cariogenic streptococci (Streptococcus mutans – Streptococcus sobrinus) was performed using species-specific primers for real-time polymerase chain reaction (qPCR) method. The genomic material from the samples from methods A and B was obtained by following the instructions provided by the manufacturer of the commercial kit (Presto™ Mini gDNA Bacteria Kit, Geneaid). DNA integrity was quantified and evaluated by spectrometry (CYTATION 3 Cell Imaging reader, Biotek). The DNA samples employed had values between 1.7 and 2.0 (ratio 260/280 nm) and their concentrations were normalized at 20 ng/microliter.

Detection and quantification of Streptococcus mutans (S. mutans) and Streptococcus sobrinus (S. sobrinus) were performed by qPCR in a CFX96TM Real Time System thermocycler (Bio-Rad Laboratories, Inc.). Species-specific primers were employed, using as a target the gene encoding the following glycotransferase enzymes: gtfB, for S. mutans and gtfT S. sobrinus[12]. The reactions were performed in triplicate, using Sso Advanced Universal SYBR Green Supermix (Bio-Rad Laboratories, Inc.) in a final volume of 10 microliters.

Statistical processing consisted of calculating the mean rank for each collection method and each culture medium, and comparing the results by two-way analysis of variance by ranks (Friedman's test). For the molecular technique, the percentages of positive results for S. mutans and S. sobrinus for each collection method were calculated, and differences were analyzed using McNemar's test.

RESULTS

Seven females and four males took part in this test. Average age was 14.9 months (SD±4.67).

Mean rank for the CFU/ml count for AA-MSMG was 1.83 for method A, and 1.17 for method B (Fig. 1), with statistically significant differences (p=0.021).

Mean rank for the count on cultures on selective TYSCB medium was 1.54 for method A, and 1.46 for method B, without statistically significant difference (p=0.705) (Table 1), though recovery was greater in method A (Fig. 2).

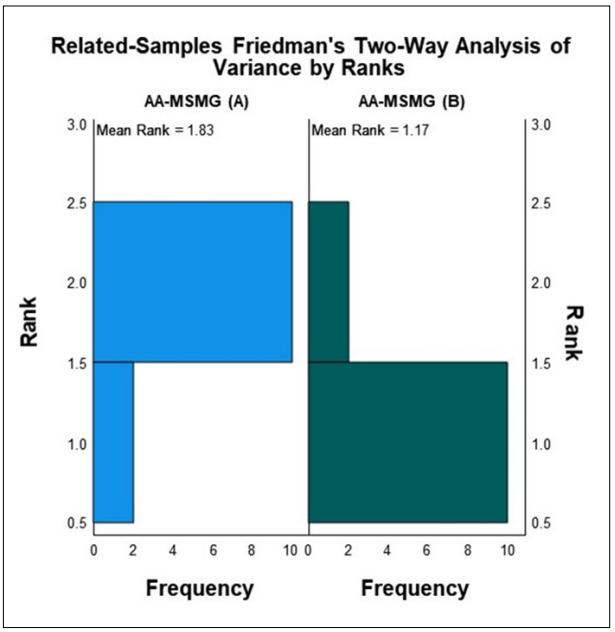

Fig.1: Mean rank of CFU/ml according to a sampling method. AA-MSMG: Culture on Gold Broth modified with 20% of sucrose (A): absorbent method, (B): non-absorbent method.

Table 1. Cariogenic streptococcus count in TYSCB for collection methods A and B

	Median	Percentile 25	Percentile 75	p
TYSCB (A)	158	0	300	0.705
TYSCB (B)	6	2	154	

TYSCB: Culture in Tryptone Yeast Extract Cystine Sucrose and Bacitracin medium. (A): Absorbent method, (B): Non-absorbent method.

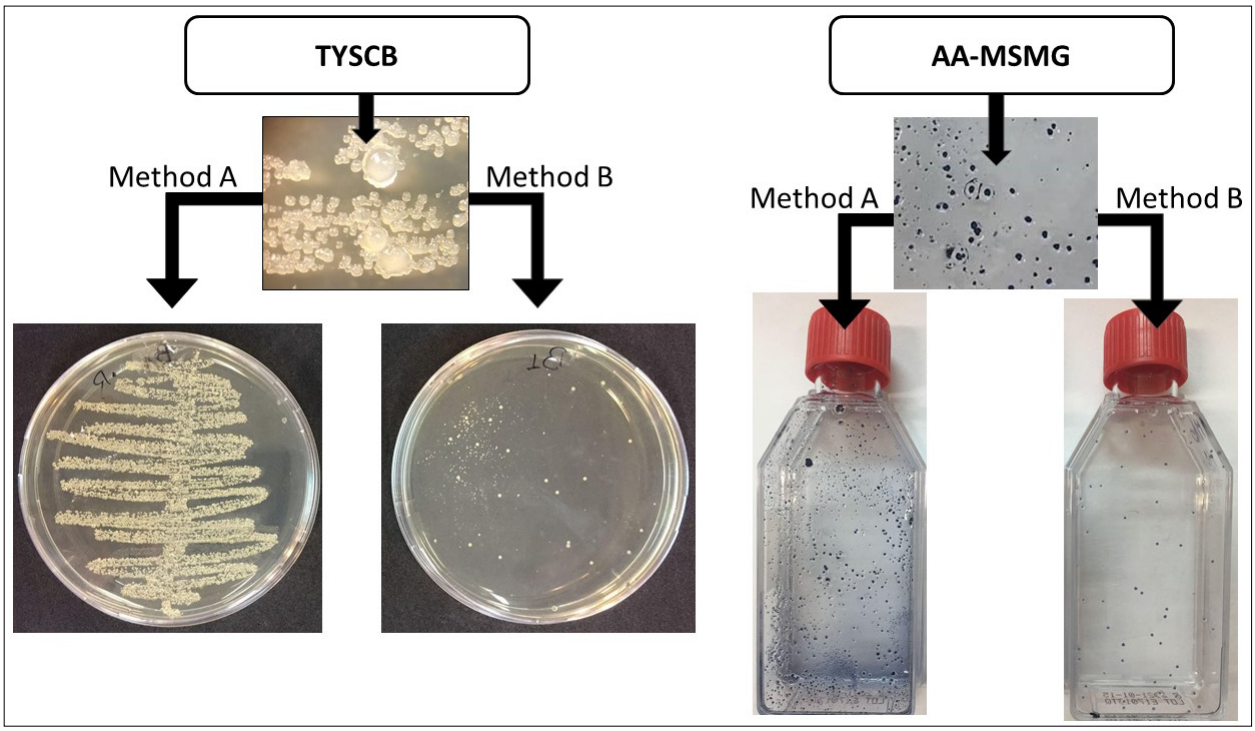

Fig.2: Culture on Tryptone Yeast Extract Cystine Sucrose and Bacitracin (TYSCB) and Gold Broth modified with 20% sucrose (AA-MSMG). Method A = absorbent. Method B = non-absorbent.

With qPCR, positive results for S. sobrinus and S. mutans were 36.4% and 45.5%, respectively, for method A; and 75% and 41.7%, respectively, for method B. For qPCR, no significant difference was found between sampling methods (S. sobrinus: p=0.21; S. mutans: p=0.941) (Table 2).

Table 2 - Detection of cariogenic streptococcus by qPCR using collection methods A and B

Method	Positive	% Positive	p
S. *sob* qPCR A	4	36.4%	0.219
S. *sob* qPCR B	9	75.0%	
S. *m* qPCR A	5	45.5%	0.941
S. *m* qPCR B	5	41.7%	
qPCR A	8	72.7%	1.000
qPCR B	10	83.3%	

McNemar Test
S. sob = Streptococcus sobrinus, S. m = Streptococcus mutans

DISCUSSION

Microbiological studies on saliva specimens are needed to establish cariogenic risk level and identify microorganisms that may be associated with the dental caries process. However, taking samples from infants may involve difficulties related to children's cooperation or unfavorable perception of sampling methods by aspiration with syringes.

Our study compared a suction sampling method and an absorbent sampling method, finding that they provided similar results for colony counts and genetic material. The absorbent method enabled greater detection of CFU/ml in the AA-MSMG culture technique.

The absence of significant differences between methods when the count was performed on TYSCB may be explained by the fact that the count parameters established for this method did not assign an exact numerical value when counts were higher than 300 colonies, which was taken as maximum value.

We assume that the differences observed could only be attributable to the intrinsic characteristics of each method, because the samples were taken simultaneously under similar conditions.

This result was in agreement with other reports[14,15] which used swabbing to collect specimens from infants aged 0 to 30 months and 0 to 6 months.

However, Motisuki et al.[13], for stimulated saliva simples in a population of children aged 5 to 13 years, found a significantly lower CS CFU/ml count when samples were collected by swabbing than when collected by methods using dental biofilm or drooling

into sterile collector tubes. This difference may be explained by the fact that our swabbing method applied motion in the infant's oral cavity, which may have caused detachment of microorganisms in the oral cavity during the procedure.

Some studies have compared the ability to detect specific bacteria in unstimulated and stimulated saliva samples collected using different techniques and cultured in different mediums under the hypothesis that the bacterial component in saliva varies according to type of sample collected.

One study on 3-year-olds[16] determined the presence of S. mutans in unstimulated saliva samples collected by soaking a cotton swab under the tongue compared to samples taken by moving a swab around in the oral cavity. It found that presence of S. mutans was higher in oral swab samples than in unstimulated saliva samples. This agrees with the results of our study, which found that the oral swab technique produced higher CS recovery.

Saliva is increasingly being used as a biological material in which to study the oral microbiome. It is therefore necessary to create a protocol for collection methods, considering potential interference factors. A recent study by Omori[17] reports finding similar percentages of relative abundance of streptococci for the drool method and the swab method, recommending the cotton swab method to study the microbiome in subjects who cannot produce saliva or have difficulty in spitting.

There is evidence that stimulated saliva samples could be used as a substitute for unstimulated saliva for oral microbiota studies and that bacterial profile would not vary significantly according to type of saliva specimen used[18]. Another study[19] comparing the composition of oral microbiota between stimulated saliva (with paraffin block) and unstimulated saliva (paper points) found significant differences, with stimulated saliva containing an estimated number of species three times higher than unstimulated saliva.

Different authors have suggested that some absorbent collection devices can introduce bias and errors in the subsequent data analysis, mainly in relation to immunoglobulin assays and studies focusing on certain steroid hormones[20-22]. Our study found that swabbing did not alter the detection of cariogenic streptococci identified by molecular analysis (qPCR), in agreement with other authors[23,24] who established that microbial profiles in saliva are minimally affected by the collection method.

Taking saliva samples in young children is challenging as a result of situations such as the time involved in obtaining specimen volumes large enough for subsequent processing and analysis, compliance with protocols prior to sampling (fluid, food and medication intake, and mouthwash before sampling), sleep cycles, and the child's predisposition to the practice. For example, Granger et al.[25] reported difficulties in collecting saliva from children aged 6 to 15 months related to ethnicity and the socioeconomic level of the families.

Although the aim of the current study was not to establish association between sampling and aspects related to family income level, it is worth noting that the early childhood center where it was conducted is attended by children from low-income families. The center provides meals (breakfast, lunch and afternoon snack), sleep time (30 to 60 minutes to rest) and play activities. These situations justified the performance of this study to identify the most appropriate method for collecting samples in the context.

In developed countries today, there are available commercial devices that are easy to use, cause minimum discomfort to participants, and obtain adequate quantities of specimens for subsequent processing. However, in developing countries, where financial and bureaucratic limitations hamper the purchase of complex devices, the standardization of protocols with low-cost supplies offers alternatives for conducting microbiological studies.

This study shows that the oral swab method for collecting saliva samples is more effective in terms of recovering microorganisms, and does not alter CS detection by molecular methods. It thus provides preliminary evidence contributing to the development of protocols and methods for obtaining saliva specimens from infants.

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DECLARATION OF CONFLICTING INTERESTS
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Do non-clinical subjective factors influence the treatment decision-making of Brazilian dentists?

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ABSTRACT

The literature contains little information on several non-clinical factors such as the association between graduate residency programs and the application of minimally invasive dentistry, or on dentists' clinical decision-making processes for replacing restorations for esthetic reasons. This study evaluated whether non-clinical subjective factors influence the treatment decisions made by Brazilian dentists regarding technical and esthetic matters. Dentists were invited to participate in a cross-sectional survey by answering an electronic questionnaire containing clinical cases, regarding what treatment they would select for: T1 - a molar tooth with significant crown destruction and spontaneous pain, and T2 - premolar teeth with extensive amalgam restorations and no carious lesion or associated complaint. The survey also included questions about subjective variants (sociodemographic and professional). Chi Square test and Fischer's Exact test were used to analyze the answers to T1, and one-factor analysis of variance and post-hoc Tamhane were applied to T2. The significance level was set at 5% for all analyses. A total 302 professionals participated in the study. For T1, it was found that clinical decision-making was influenced by the Brazilian region of clinical practice (p=0.005). For T2, a significant association was found between increased loss of patient tooth tissues and whether the professional had completed a residency program in Operative Dentistry (p=0.035), worked in a private practice (p=0.033), or if most of his/her patients belonged to a high estimated socioeconomic level (household income above $4350) (p=0.002). In conclusion, the clinical decision-making of Brazilian dentists varies according to professional profile, mainly with relation to the replacement of restorations due to esthetic concerns.

Keywords: *cross-sectional studies - esthetics, dental - dentistry, operative - private practice - surveys and questionnaires.*

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Fatores subjetivos não clínicos influenciam na tomada de decisão do tratamento de cirurgiões-dentistas brasileiros?

RESUMO

Vários fatores não clínicos, como a associação entre programas de especialização e a aplicação da odontologia minimamente invasiva, ainda são escassos na literatura. Outro aspecto relevante é a tomada de decisão clínica do dentista quanto à substituição de restaurações em função da aparência estética. Este estudo avaliou se fatores subjetivos não clínicos influenciam na tomada de decisão clínica de dentistas brasileiros com base em questões técnicas e estéticas. Foi realizado um estudo transversal com um questionário eletrônico contendo casos clínicos que foram apresentados a uma lista de profissionais. No questionário, interrogou-se o tratamento proposto para um dente molar com destruição coronária significativa e dor espontânea (T1). Também foi questionado o tratamento proposto para dentes pré-molares com extensas restaurações de amálgama e sem lesão cariosa ou queixas associadas (T2). Em seguida, foram questionadas as variantes subjetivas (sociodemográfica e profissional). Na análise de T1, foram utilizados os testes Qui Quadrado e Exato de Fischer. Em T2, foi aplicada a análise de variância de um fator e post-hoc Tamhane. Para todas as análises, o nível de significância foi estabelecido em 5%. Um total de 302 profissionais participaram deste estudo. A tomada de decisão clínica para T1 foi influenciada pela região brasileira de prática clínica (p = 0,005). Em T2, realizar especialização em Dentística Operatória (p = 0,035), trabalhar em consultório particular (p = 0,033) e a maioria dos pacientes apresentar nível socioeconômico estimado elevado (renda familiar acima de R$10.000,00) (p = 0,002) aumentou significativamente a perda de tecidos dentários. Em conclusão, a tomada de decisão clínica dos dentistas brasileiros varia de acordo com o perfil dos profissionais, principalmente no que se refere à substituição de restaurações por questões estéticas.

Palavras-chave: *estudos transversais - estética dentária - dentística operatória - prática privada - inquéritos e questionários.*

INTRODUCTION

Clinical decision-making, which is a relevant area of healthcare, is based on both clinical and non-clinical factors[1]. Non-clinical factors are subjective determinants such as time since graduation or patient socioeconomic status[1]. Thus, the clinical decisions regarding techniques and materials may vary according to the profile of both patients and professionals. Knowledge of the factors involved in this process is relevant to defining behavior and implementing more effective strategies for promoting healthcare services[2].

Dental professionals are strongly encouraged to apply evidence-based dentistry (EBD) when making decisions[3]. Nonetheless, most dental treatments cannot be truly described as based on experimental evidence[3]. Diagnostic errors are common and recognized as a source of preventable adverse events, but they are rarely evaluated because of the complex decision-making process[4]. In this regard, the factors associated with dental clinical decision-making still need to be explored.

The concept of EBD advocates minimally invasive dentistry, and adoption of a philosophy of prevention and avoidance of invasive treatments, with minimum removal of healthy tissues[5]. Some studies have associated several subjective determinants to dentists' decisions on whether to treat patients with either more aggressive or more conservative approaches[1,2]. However, literature is still scarce on other factors such as the association between graduate programs and the application of minimally invasive dentistry.

Another point that requires further study is dental professionals' clinical decision-making regarding esthetics. Some studies have detected the controversial replacement of satisfactory amalgam restorations by composite resins because of amalgam's inferior esthetics[2,6]. The current study evaluates whether non-clinical subjective factors influence the treatment decision-making of Brazilian dentists based on technical and esthetic matters.

MATERIALS AND METHODS

This study was approved by the University Ethics Committee (32149220.7.0000.5291). The present research is a cross-sectional analysis based on a self-administered electronic questionnaire.

Study design and target population

The target population consisted of professionally active dentists throughout the Brazilian territory. Participants were invited to take part in this study via e-mail contacts provided by the Regional Dentistry Council and via available dentistry-related social media.

Development and structure of the data collection instrument

The data collection instrument was developed following a strict order to ensure reliability of results: (1) Establishment of conceptual structure, objectives of the instrument and target population, (2) Preparation of the questionnaire, (3) Application of the questionnaire to scientific consultants, (4) Pre-testing of the questionnaire with the target population, (6) Sample calculation, and (7) Data collection.

The data collection instrument was prepared according to the definitions and objectives established. The instrument was divided into 2 sections: the consent form and the questionnaire. After receiving an invitation to participate in the survey, participants accessed the informed consent form, which presented the objectives, methods, risks, and benefits of participating in the study. The professionals were only redirected to the questionnaire if they chose to participate voluntarily in the research. For the second section, extraoral and intraoral photographs obtained with informed consent from 2 patients were used to produce a clinical case with technical and esthetic questions. Treatment 1 (T1) of the clinical case involved the right mandibular first molar, which showed significant destruction of more than 2/3 of the crown and associated spontaneous pain (Fig. 1). Treatment 2 (T2) involved the adjacent right mandibular premolars, which had extensive Class II cavities with amalgam restorations and no carious lesions or any other associated complaint (Fig. 2). After preparing the clinical case, questions were developed based on a previously validated questionnaire[2] and literature review. The most appropriate treatment decisions for T1 and T2 were defined as root canal treatment followed by indirect restoration[7] and no treatment[2,8], respectively.

Application of the questionnaire to scientific consultants

Once the initial questionnaire had been designed, it was applied to scientific consultants to test the hypothesis that the prepared items represented and adequately contemplated the domains of the desired construct[9]. A committee of 20 scientific consultants (reply rate = 85%), made up of dentistry university professors, was invited to make comments, suggestions and modifications on the questionnaire. Next, a quantitative evaluation was performed, in which the scientific committee's agreement rate (%) was calculated for each question as the ratio between the number of consultants that made a suggestion for improvement and the total number of consultants. An agreement rate lower than

Fig. 1: Clinical case 1 presented in the questionnaire (T1), showing the intraoral occlusal view of the right mandibular first molar with extensive crown destruction.

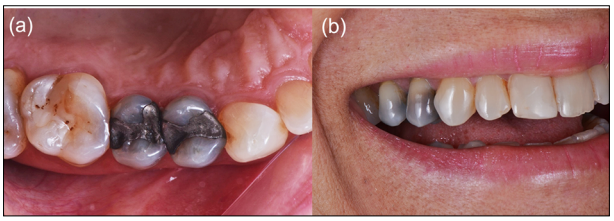

Fig. 2: Clinical case 2 presented in the questionnaire (T2). (a) Intraoral occlusal view of the right adjacent mandibular premolars showing extensive Class II cavities with amalgam restorations. (b) Extraoral right buccal view focusing on amalgam pigmentation of premolars.

90% determined that the question needed to be discussed and modified, while an agreement rate of 90% or higher indicated that the question was adequate. In addition, a qualitative assessment was performed, in which the suggestions of scientific consultants were discussed to ensure the necessary changes.

Pre-testing of the questionnaire with the target population

A pilot study was carried out in December 2020 with 29 professionally active dentists in different regions of Brazil to assess the understanding, adequacy and applicability of the questionnaire. After each question, participants were asked about their understanding of the items, and to make any suggestions in the space provided. Based on the performance of pre-testing, the questionnaire was considered adequate and reliable. Participants' answers and suggestions of were analyzed and considered for improvement of the questionnaire.

Questionnaire and data collection

The electronic questionnaire was structured using the QuestionPro survey software (Question Pro, Seattle, WA, USA) and applied from January 2021 to March 2021. The final version of the questionnaire consisted of 2 technical questions, in which the previously prepared clinical case was presented and the participants were asked about clinical decision-making. The patient's socioeconomic status was not disclosed to avoid possible bias in the treatment selected. The following clinical case was presented: "A 40-year-old female patient visited the dental care service complaining of spontaneous pain in the right mandibular first molar. What would be your first treatment option for this tooth?". The treatment options were (a) Extraction, (b) Extraction followed by three-unit fixed prostheses, (c) Extraction followed by dental implant, (d) Root canal treatment followed by indirect dental restoration, and (e) Root canal treatment followed by direct dental restoration. Then, the second question was presented: "The presence of amalgam restorations was also identified in the right maxillary premolars. The patient did not report any symptoms or complaints associated with these teeth. The radiographic examination did not show the presence of a carious lesion. What would be your first treatment option for these teeth?". The treatment options were (a) Replacement with new amalgam restoration, (b) Replacement with composite resin restoration, (c) Replacement with ceramic onlay, (d) Replacement with ceramic crown, (e) Finishing and polishing, and (f) No treatment required. Professionals could select any treatment option for the cases presented, without restrictions.

The questionnaire also included 7 questions on dentists' sociodemographic and professional characteristics, to characterize their profile. The following sociodemographic and professional determinants were evaluated: (1) Time since graduation, (2) Area(s) of Post-Graduate Program/ dental residency Program, (3) Area(s) of Master's and Doctorate Graduate Program, (4) Brazilian region of clinical practice, (5) Ethnicity of the dentist, (6) Sector of clinical practice, and (7) Estimated socioeconomic level of patients.

Sample size calculation

Based on a previous study with similar method[2], in which an 83% agreement was obtained for a similar clinical approach, and using the Cochran equation, the standard significance level of 5% was applied and the minimum total sample size was determined as 217 individuals.

Statistical analysis

Data were processed using Excel software (Microsoft, Redmond, WA, USA) and analyzed with IBM SPSS 22 statistical software (IBM Corporation, Armonk, NY, USA). Chi-square test and Fisher's exact test were used to analyze T1. For analysis of T2, a qualitative index presenting scores of 1 to 5 was developed to categorize the proposed treatment according to the loss

of tooth tissues. Score 1 represented the lowest level of tooth tissue destruction (No treatment necessary), whereas score 5 characterized the most aggressive approach (Replacement with ceramic crown). Then, one-way analysis of variance was applied and, for the pairwise comparison of groups, the post-hoc Tamhane test was used. The significance level established for all analyses was α = 5%.

Table 1. Sociodemographic and professional characteristics of the study population (n = 302 dentists):

Subjective Determinants		**% (*n*)**
Time since graduation	0-5 years	9.3% (28)
	6-10 years	8.9% (27)
	11-19 years	16.2% (49)
	More than 20 years	61.6% (186)
	No answer	4.0% (12)
Area(s) of Dental Residency Graduate Program	Has not completed a program	7.6% (23)
	Operative Dentistry	23.2% (70)
	Prosthodontics	14.9% (45)
	Endodontics	11.9% (36)
	Dental Implant Surgery	20.9% (63)
	Pediatrics	7.6% (23)
	Oral and Maxillofacial Surgery	7.0% (21)
	Periodontics	11.9% (36)
	Others	32.5% (98)
Area(s) of Master's and Doctorate Graduate Program	Has not completed a program	37.4% (113)
	Master's degree (Professional Program)	15.6% (47)
	Master's degree (Academic Program)	17.5% (53)
	Doctorate degree	6.3% (19)
	Master's (Professional Program) and Doctorate degree	5.6% (17)
	Master's (Academic Program) and Doctorate degree	14.2% (43)
	No answer	3.3% (10)
Brazilian region of clinical practice	Midwest	2.6% (8)
	Northeast	11.3% (34)
	North	3.0% (9)
	Southeast	67.5% (204)
	South	12.6% (38)
	No answer	3.0% (9)
Ethnicity of the dentist	Asian	1.0% (3)
	White	85.4% (258)
	Native American	0% (0)
	Brown	12.6% (38)
	Black	1.0% (3)
Sector of clinical practice	Private practice	64.6% (195)
	Public health service	8.6% (24)
	University	19.9% (60)
	Other	3.6% (11)
	Has not practiced in the last 12 months	2.6% (8)
Estimated socioeconomic level of patients	Class A/B1 (household income above $4350)	32.5% (98)
	Class B2 (household income close to $2610)	33.1% (100)
	Class C (household income close to $1090)	16.9% (51)
	Class D/E (household income lower than $565)	17.5% (53)

RESULTS

The electronic questionnaire was viewed by a total 1157 participants, of whom 385 started and 302 completed it (Completion rate = 78.4%). Average time spent answering the questionnaire was 4 minutes. The sociodemographic and professional characteristics of the participants are shown in Table 1. Most participants graduated 20+ years prior to this survey (61.6%) and worked in a private practice (64.6%). Most participants had completed a Post-Graduate Program/dental residency (92.4%).

The main choices for T1 and T2 were, respectively, "Root canal treatment followed by indirect restoration" (73.5%) and "No treatment needed" (55.3%) (Table 2).

Table 2. Distribution of treatments proposed by dentists for the clinical cases presented in the questionnaire:

T1 (Molar)	% (*n*)	T2 (Premolars)	% (*n*)
Extraction	0% (0)	Replacement with ceramic dental crown	4.0% (12)
Extraction followed by three-unit fixed prostheses	0% (0)	Replacement with ceramic onlay	3.0% (9)
Extraction followed by dental implant	1.0% (3)	Replacement with new amalgam restoration	0% (0)
Root canal treatment followed by indirect dental restoration	73.5% (222)	Replacement with composite resin restoration	12.9% (39)
Root canal treatment followed by direct dental restoration	25.5% (77)	Finishing and polishing	24.2% (73)
-	-	No treatment necessary	55.3% (167)

Table 3. Treatments proposed for T1 (molar) according to the Brazilian region of clinical practice:

Treatments proposed - T1 (molar)	Brazilian region of clinical practice				
	Midwest	Northeast	North	Southeast	South
Extraction	0% (0)	0% (0)	0% (0)	0% (0)	0% (0)
Extraction followed by three-unit fixed prostheses	0% (0)	0% (0)	0% (0)	0% (0)	0% (0)
Extraction followed by dental implant	0% (0)	3.0% (1)	11.1% (1)	0.5% (1)	0% (0)
Root canal treatment followed by indirect dental restoration	87.5% (7)	58.8% (20)	66.7% (6)	79.4% (162)	52.6% (20)
Root canal treatment followed by direct dental restoration	12.5% (1)	38.2% (13)	22.2% (2)	20.1% (41)	47.4% (18)
Total % (*n*)	**100% (8)**	**100% (34)**	**100 (9%)**	**100% (204)**	**100% (38)**

Table 4. Treatments proposed for T2 (Premolars) according to the area of Dental Residency Graduate Program of the study participants:

Treatment proposed – T2 (Premolars)	Area(s) of Dental Residency Graduate Program(s)								
	Has not completed a program	Operative Dentistry	Prosthodontics	Endodontics	Dental Implants Surgery	Pediatric Dentistry	Oral and Maxillofacial Surgery	Periodontics	Others
Replacement with ceramic dental crown	8.7% (2)	4.3% (3)	4.4% (2)	0% (0)	4.8% (3)	9.1% (2)	5.0% (1)	5.6% (2)	3.1% (3)
Replacement with ceramic onlay	4.3% (1)	1.4% (1)	4.4% (2)	2.8% (1)	3.2% (2)	13.6% (3)	0% (0)	5.6% (2)	3.1% (3)
Replacement with new amalgam restoration	0% (0)	0% (0)	0% (0)	0% (0)	0% (0)	0% (0)	0% (0)	0% (0)	0% (0)
Replacement with composite resin restoration	8.7% (2)	21.4% (15)	15.6% (7)	11.1% (4)	7.9% (5)	13.6% (3)	10.0% (2)	8.3% (3)	11.2% (11)
Finishing and polishing	13.0% (3)	34.3% (24)	33.3% (15)	16.7% (6)	27.0% (17)	4.5% (1)	20.0% (4)	22.2% (8)	22.4% (22)
No treatment necessary	65.3% (15)	38.6% (27)	42.3% (19)	69.4% (25)	57.1% (36)	59.2% (13)	65.0% (13)	58.3% (21)	60.2% (59)
Total % (*n*)	**100% (23)**	**100% (70)**	**100% (45)**	**100% (36)**	**100% (63)**	**100% (22)**	**100% (20)**	**100% (36)**	**100% (98)**

The clinical decision-making for T1 was significantly influenced by the Brazilian region of clinical practice (p=0.005) (Table 3). In T2, having completed a residency program in Operative Dentistry (p=0.035) (Table 4), private practice (p=0.033) and most patients having higher estimated socioeconomic level represented by class A/B1 (household income above $4350) (p=0.002), significantly increased the loss of tooth tissues with the treatment proposed (Table 5). Most specialists in Operative Dentistry (58.6%) had graduated 20+ years ago, followed by 11 to 19 years for 16.8% of the respondents.

Table 5. Analysis of subjective determinants that showed differences between the treatments proposed in T2 (Premolars):

Subjective Determinants		Mean (standard deviation)	N	Statistical difference
Area(s) of Dental Residency Graduate Program(s)	Has not completed a program	1.8 (1.3)	23	B
	Operative Dentistry	2.0 (1.0)	70	A
	Prosthodontics	2.0 (1.1)	45	B
	Endodontics	1.5 (0.8)	36	B
	Dental Implants Surgery	1.7 (1.1)	63	B
	Pediatric Dentistry	2.1 (1.5)	22	B
	Oral and Maxillofacial Surgery	1.6 (1.0)	20	B
	Periodontics	1.8 (1.2)	36	B
	Others	1.7 (1.0)	98	B
Sector of clinical practice	Private practice	1.8 (1.1)	193	A
	Public health service	1.4 (0.6)	26	B
	University	1.6 (0.8)	60	B
	Other	2.2 (1.3)	19	B
Estimated socioeconomic level of patients	Class A/B1 (household income above $4350)	2.1 (1.2)	98	A
	Class B2 (household income close to $2610)	1.7 (0.9)	98	B
	Class C (household income close to $1090)	1.5 (0.9)	51	B
	Class D/E (household income lower than $565)	1.5 (0.9)	53	B

Different letters in the statistical difference column indicate significant differences between groups in the post-hoc Tamhane test (p<0.05).

DISCUSSION

The results of this study indicated an association between some of the non-clinical factors tested and the treatments proposed in both T1 and T2. This is supported by the literature, in which determinants such as patients' skin color were also associated to dentists' clinical decision-making[1,2]. The treatment proposed for the mandibular molar with extensive crown destruction and spontaneous pain indicative of irreversible pulpitis, presented in T1, had greater agreement among the study participants than T2. Only 3 professionals (1.0%) indicated T1 as tooth extraction followed by dental implant, whereas 299 dentists (99.0%) opted for more conservative treatments (root canal treatment followed by some type of crown restoration). This finding is favorable because the option of tooth extraction is considered an overtreatment for the case presented[7]. The only sociodemographic determinant that influenced the results of T1 was the Brazilian region of clinical practice. The growing regional differences in the index of decayed, missing and filled teeth (DMFT) of the Brazilian population may reflect the clinical routine and beliefs of professionals regarding treatments. The difference in the DMFT index among the Brazilian north and southeast regions was close to 20% in 1986, but this percentage increased considerably to 26% in 2003 and 43% in 2010[10]. The worst DMFT indexes are in the north and northeast regions due to spatial autocorrelation with low levels of access to dental care and higher levels of poverty, illiteracy, and lower levels of education[10,11]. As a result, the northeast and north regions present a higher level of caries disease and a lower proportion of restored teeth compared to the southeast[10,11].

In T2, the most prevalent treatment selected for the maxillary premolars with amalgam restorations was "no treatment necessary" (55.3%), followed by "finishing and polishing" (24.2%). These options are also

consistent with current concepts of minimally invasive dentistry supporting the removal of the least possible amount of healthy dental tissues. However, replacement of the satisfactory amalgam restoration by composite resin, ceramic dental crown, and ceramic onlay were selected, respectively, by 39 (12.9%), 12 (4.0%), and 9 dentists (3.0%); adding up to the considerable number of 60 dentists (19.9% of the total sample) selecting a procedure that is not justified. The esthetic component is subjective and depends on individual perception, and no complaint was associated to the restorations.

The motivation and effects of the specific barriers in the implementation of evidence-based dentistry should be investigated in future studies because dentists may be overestimating treatment options when compared to laypeople's perception of smile. The clinical decision to replace restorations, particularly those that do not have an associated infection, should always be based on high professional criteria and consideration of patient feedback to avoid misconceptions. Restoration substitutions should be avoided because they usually result in increasing cavity size, and thus in loss of healthy tooth structure, easily leading to a costly, repetitive restorative cycle[8,12]. Esthetics play an important role in people's lives and are directly associated with quality of life[13]. Some authors have suggested that the cosmetic industry and the dental profession have leveraged this importance to increase demand and, consequently, profits[2,6].

The clinical decisions made for T2 showed greater influence of subjective determinants. Having completed a residency program in Operative Dentistry significantly raised the mean aggressiveness of the treatment proposed. Most Operative Dentistry specialists would recommend "no treatment necessary" (38.6%), but this percentage was the lowest among all areas of Dental Residency Program. At the same time, Operative Dentistry professionals were the most likely to select the treatment options "finishing and polishing" (34.3%) and "replacement with composite resin" (21.4%), involving higher scores for tooth tissue loss, compared to other professionals. Generally, Operative Dentistry specialists routinely check restorations and esthetic dental procedures, resulting in a high level of smile esthetic demand[14]. The finishing and polishing procedure is considered favorable because it reverses the decision to replace old amalgam restorations and may extend the durability of restorations[15–17], but it can cause loss of tooth tissue, contradicting the principles of minimally invasive, evidence-based dentistry. The choice of Operative Dentistry professionals to replace the presented amalgam restoration with composite resin may be explained by the differences among dentists in the esthetic perception of smiles, which is significantly impacted by the highest degree they have earned and area of clinical practice[18,19].

Other determinants that increased the rate of tooth tissue loss in T2 were private practice and having more patients of high socioeconomic level (as estimated by respondents). The high cost of esthetic dental treatments and the difference in DMFT index according to socioeconomic status may explain these findings[11].

The choice of treatment was more conservative in the present study than in the study by Chisini et al.[2]. This may be related to the information provided, considering that our study stated that the patient did not report any symptoms or complaints associated with these teeth, including esthetic factors. Another explanation may be related to the population evaluated, considering that the present study surveyed the entire national territory, not just the northeast and south regions.

This study used an electronic questionnaire because it provides fast, accurate data; is affordable, and follows the technological and dynamic trends of scientific method[20,21]. Based on these characteristics, electronic questionnaires are preferred by most participants in epidemiological studies[22]. Traditional approaches (e.g., face-to-face interviews, telephone interviews and printed questionnaires) have shown a gradual reduction in participation rates, mainly in the last decade[23,24]. Among the reasons suggested for the drop in response rates are the greater demand for participation in surveys, the use of smartphones, and a general decrease in volunteering[24]. It is also important to consider the challenges of conducting traditional interviews during the coronavirus (COVID-19) pandemic, which has had unprecedented effects on society. COVID-19 led to a massive rise in survey-based analysis, and the resources to conduct ethical, reliable, accurate research on digital platforms are increasingly being emphasized and explored[21].

The strict sequence used to develop the data collection instrument for this study aimed to ensure the reliability of the method[25]. Questionnaire items were constructed and organized based on a pre-existing questionnaire and literature review[2]. Literature review is the most frequently used method for developing survey-based analysis instruments, though it is also appropriate to use an existing questionnaire resource because it ensures that the questions have been previously tested for psychometric qualities[25]. The application of the questionnaire to scientific consultants and the pre-testing stage with the target population, prior to data collection, are essential steps in survey-based methods.

In conclusion, dentists' clinical decision-making varies according to professional profile, mainly regarding

the replacement of restorations because of esthetic concerns. Having completed a residency program in Operative Dentistry, working in the private sector, and most patients having a high estimated socioeconomic level are factors that reduce the use of minimally invasive dentistry in esthetic treatments.

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DECLARATION OF CONFLICTING INTERESTS
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Accelerated artificial aging and color stability in resin-based cements

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ABSTRACT

The aim of this study was to determine color change after accelerated artificial ageing (AAA) of different composite cements that are used with veneers. Five cylindrical test specimens, 15 mm in diameter and 2 mm thick, were made from a single layer of each of the following: RelyX Veneer 3M ESPE (RX), Paracore White Coltene (PC), Solocem White Opaque Coltene (SO), Resin Duo Cement Densell (DC), Panavia V5 Paste Kuraray Noritake (PA) and Panavia F2.0 Kuraray Noritake (PF) (30 specimens altogether). The specimens were light cured following manufacturers' instructions using a Coltolux LED (Coltene) unit. Initial color was determined using an Easyshade - Vita Zahnfabrik Spectrophotometer. Then, the specimens were subjected to AAA for two weeks (336 hours) with cycles of 4 hours of UV light at 60 °C and 4 hours of vapor condensation at 50 °C, successively, after which color was recorded again. Color change was determined for each specimen according to the difference in shade on the Vita scale before and after AAA. Results were analyzed using Kruskal Wallis test. Mean and standard deviation for each group were: RX 8.40 (1.52); PC 8.60 (3.13); SO 6.40 (3.51); DC 10.00 (0.00); PA 7.60 (3.29); PF 2.00 (0.00). The Kruskal Wallis test showed significant difference for material ($p<0.05$), and comparison of means showed difference between Panavia F2.0 and the other materials. A table providing equivalence between the Vita Classic and CIELAB scales was used to transfer the recorded colors to the CIELAB scale, and the color difference ΔE was calculated for each group, where ΔL, Δa and Δb are the differences in the L, a and b values before and after the AAA. The mean and standard deviation were analyzed statistically by the ANOVA test and Tukey's test. Mean and standard deviation for each group were: RX 14.94 (2.02); PC 14.51 (4.02); SO 12.08 (4.53); DC 16.31 (0.00); PA 10.9 (3.38); PF 7.24 (0.00). The ANOVA test showed significant difference for material ($p<0.05$). Tukey's test showed two groups (PF-DC, RX, PA). Under the experimental conditions of this study, it can be concluded that accelerated ageing significantly affects the color stability of the resin based cements tested.

Keywords: *resin cements - color - dental veneers.*

Envejecimiento artificial acelerado y estabilidad de color de medios de fijación a base de resinas

RESUMEN

El objetivo de este trabajo fue determinar el cambio de coloración de distintos medios de fijación a base de resinas con indicación para carillas estéticas luego de someterse al envejecimiento artificial acelerado (AAA). Se confeccionaron 30 probetas cilíndricas de 15 mm de diámetro y 2 mm de espesor con RelyX Veneer 3M ESPE (RX), Paracore White Coltene (PC), Solocem White Opaque Coltene (SO), Resin Duo Cement Densell (DC), Panavia V5 Paste Kuraray Noritake (PA) y Panavia F2.0 Kuraray Noritake (PF) de una sola capa de material. Se polimerizaron según las indicaciones del fabricante con unidad de curado Coltolux LED (Coltene). Cada grupo quedó conformado con 5 probetas de cada material. Se tomó el color con el espectrofotómetro Easyshade de Vita Zahnfabrik. A continuación, se sometieron a AAA por dos semanas (336 horas) con ciclos de 4 horas de radiaciones ultravioletas a 60°C Y 4 horas de condensación de vapor de agua a 50°C sucesivamente. Una vez terminado este proceso se volvió a registrar el color. El cambio de color se evaluó dentro de la escala de color, ordenada en función del valor. El dato registrado fue la diferencia en la posición inicial y final en esta escala. Los resultados obtenidos para cada grupo fueron analizados por medio de la prueba de Kruskal Wallis. Los valores de media y desvío estándar de cada grupo fueron: RX 8,40 (1,52); PC 8,60 (3,13); SO 6,40 (3,51); DC 10,00 (0,00); PA 7,60 (3,29); PF 2,00 (0,00). El análisis con la prueba de Kruskal Wallis mostró diferencia significativa para el factor material ($p<0.05$) y la comparación de medias mostró diferencia entre PF y el resto de los materiales. Por otra parte, con una tabla de equivalencia entre los colores en escala de Vita Classic y CIE Lab, se hicieron el pasaje de los valores registrados a la escala de CIE Lab y se calculó la diferencia de color ΔE de cada grupo antes y después del AAA. La media y el desvío estándar fueron analizados estadísticamente por el test de ANOVA y prueba de Tukey. Los valores de media y desvío estándar de cada grupo fueron: RX 14,94 (2,02); PC 14,51 (4,02); SO 12,08 (4,53); DC 16,31 (0,00); PA 10,9 (3,38); PF 7,24 (0,00). En la evaluación de los resultados con ANOVA se encontró diferencia significativa entre los materiales evaluados ($p<0,05$). La comparación de medias con prueba de Tukey mostró la presencia de dos grupos (PF-DC, RX, PA). En las condiciones experimentales de este trabajo puede concluirse que el envejecimiento acelerado afecta significativamente la estabilidad de color de los cementos utilizados en este trabajo.

Palabras clave: *cementos de resinas - color- coronas con frente estético.*

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INTRODUCTION

Veneers are indirect restorations made with conservative techniques with the aim of harmonizing smiles by restoring the color, shape and adequate function of esthetically compromised teeth. Dental ceramic has excellent properties, including color stability, translucence and high capacity to imitate tooth structure. It is the restorative material with the most similar optical properties to those of teeth[1]. There is currently a wide range of available dental ceramics for indirect restorations, which can be used to make veneers, including sintered or machined feldspathic ceramics, injected or machined glass ceramics, resin matrix ceramics such as resin nano ceramics (Lava Ultimate) and glass ceramic in a resin interpenetrating matrix (Vita Enamic)[2,3]. Highly translucent ceramic restorations allow passage and dispersion of light, especially when they are thin. Successful clinical treatment therefore depends on the perfect combination of colors between the restoration and the tooth. Many variables are involved, including color of the tooth structure, thickness, color and type of ceramic and resin cement, as well as the translucence of the restorative material as a result of refracted and transmitted light.[4,5]

Light-activated resin-based cement is used for bonding ceramic veneers. Controlling its activation by light makes it possible to optimize the technique and lengthen work time. It also provides color stability because there is no redox reaction of the tertiary aromatic amines that are present in chemically cured materials[4]. It must be considered that light-cured materials may be affected by irradiance of the curing unit. In clinical practice, they may be affected by the type and thickness of the ceramic, as well as by other factors that determine the translucence of the ceramic, such as color, thickness, surface smoothness, polish, and volume ratio between amorphous and crystalline phases[6]. Almeida et al. concluded that light-cured resins (including resin cement, fluid composite and preheated restoration composite) had greater color stability than dual-cured resin[7]. Many other authors (e.g., Marchionatti, Ghavam, Magalhães) have reported that, due to their polymerization efficacy and high degree of conversion, dual-cured resins do not undergo significant color change, similarly to light-cured resins [8-11].

The color stability of composite resins depends on intrinsic factors owed to the material's physicochemical reactions, and extrinsic factors related to diet[12,13]. Color can change in resin-based materials as a result of aging, and has been studied in vitro using methods such as exposure to UV radiation/condensation, thermocycling and storage in water[14-16]. Accelerated Artificial Aging (AAA) has been shown to be efficient for evaluating the longevity of different materials used in dentistry. AAA simulates the clinical parameters, as far as possible, due to the action of different conditions to which the material is subjected, such as UV light, and continuous alterations in temperature and humidity, and it has been used to evaluate the color stability of dental restorations. Color undergoes change due to degradation of the polymer matrix and unreacted components in the system, as well as the type of initiators, inhibiting agents, polymerization systems, filling types and free double bonds[17,18]. Moharamzadeh et al.[19] say that residual monomers are the main components released into the environment. Therefore, color changes in the resin cement used for bonding may become visible, affecting the final esthetic appearance of the restoration and leading to failed treatment[14].

The choice of color for each patient has always been a great challenge in dentistry. Dentists have traditionally determined tooth color by comparing it to a sampler of artificial tooth colors. However, it is difficult to unify the perception of a given color by different observers. Variables such as lighting, experience, fatigue of the human eye, and color-blindness interfere with the objective definition of color[20,21]. Today, instruments that analyze color objectively and quantitively –such as colorimeters, spectrophotometers, spectroradiometers, and digital cameras– can be used to measure tooth color. A spectrophotometer is a standardized scientific colorimetric device for measuring colors which provides information on the reflectance curve as a function of wavelengths throughout the visible range, specifying the perceived color numerically or on a scale[22]. The CIELAB system is a 3-dimensional system that identifies any color in the entire visible spectrum by means of three numbers. The parameter L* represents lightness, with L* equal to 0 being black and L* equal to 100 being white. The parameter a* indicates red to green, with lower values indicating green and higher values indicating

red. The parameter b* indicates the position between yellow and blue, with lower values corresponding blue and higher values to yellow[23, 24].

Ceramic crown color stability has been thoroughly studied. However, for restorations with thin, translucent veneers, resin color stability too, is of the utmost importance and may affect long-term clinical success. The aim of this study was to determine color change after accelerated artificial aging of different composite resin cements indicated for esthetic veneers.

MATERIALS AND METHODS

Five cylindrical test specimens 15 mm in diameter and 2 mm thick were made from each of the following six materials (total 30 specimens): RelyX Veneer by 3M ESPE (RX), Paracore White by Coltene (PC), Solocem White Opaque by Coltene (SO), Resin Duo Cement by Densell (DC), and Panavia V5 Paste (PA) and Panavia F 2.0 (PF) by Kuraray Noritake (Table 1). They were all prepared following the manufacturers' instructions. To make the test specimens, material was placed in a silicone-coated mold, and pressed with a glass slide to minimize inclusion of bubbles that would affect specimen shade. Excess material was removed. The specimens were light-cured using a COLTOLUX Coltene LED light-curing unit (1200 mW/cm^2). Then they were fixed to a strip of double-sided adhesive tape attached to a sheet of white paper, and numbered from 1 to 5 for each material. Color was measured with a Vita Easyshade spectrophotometer which had been calibrated before use following the manufacturer's instructions. The spectrophotometer has a color temperature of 6500 K (D65). Color was measured in the center of each test specimen (Fig. 1) against a white background, according to the Vita Classic and Vita 3D Master color scale and considering the Vita Classic sampler

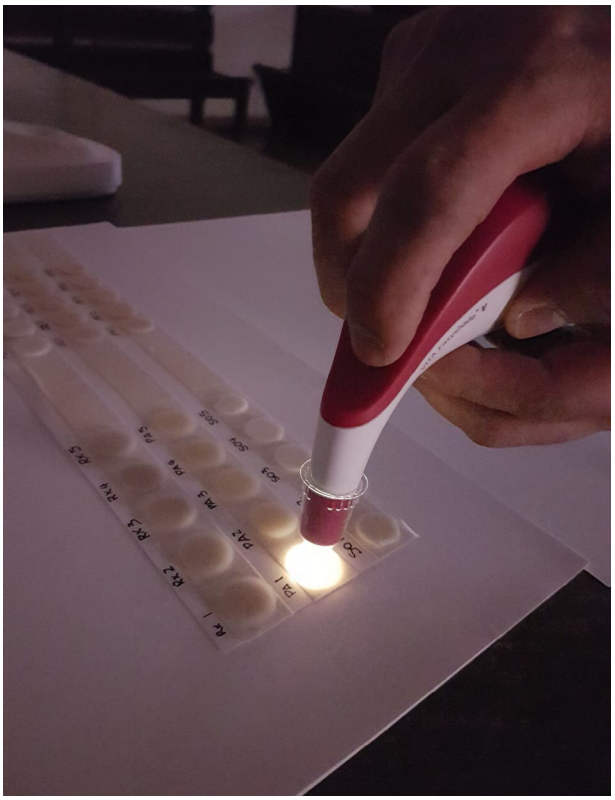

Fig. 1: Measuring sample color with a spectrophotometer.

(Fig. 2). Specimens were stored in a closed box to prevent contact with light. They were subsequently subjected to an accelerated artificial aging procedure at the National Institute of Industrial Technology (*Instituto Nacional de Tecnología Industrial*, INTI), in a chamber where they were exposed for 14 days to cycles of 4 hours of UV radiation with a fluorescent lamp at 60 °C and 4 hours of water vapor condensation at 50 °C. After the aging procedure, the color of each specimen was recorded again in the same way. Color change was evaluated on the color scale, ordered according to shade. The differences between the initial and final colors for each group

Table 1. Luting products that were studied

RESIN CEMENT	TYPE	MANUFACTURER	BATCH NUMBER
RelyX Veneer	Light-cured	3M ESPE	N875397
Paracore White	Dual-cured	Coltene	G06870
Solocem White Opaque	Dual-cured	Coltene	64549
Resin Dúo Cement	Dual-cured	Densell	QH0373-B (Base) QH0373-C (Cat)
Panavia V5 Paste	Dual-cured	Kuraray Noritake	IU000I
Panavia F2.0	Dual-cured	Kuraray Noritake	5A0159 (Base paste) 5A0038 (Catalyst)

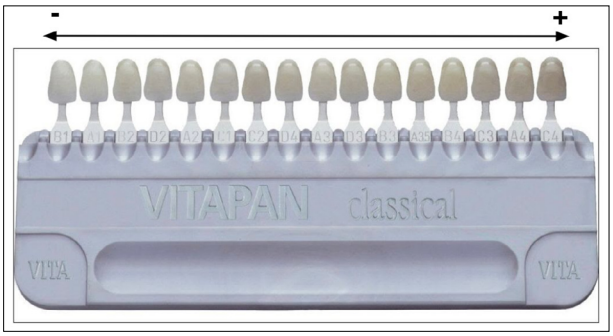

Fig. 2: Vita Classic scale ordered according to shade.

were analyzed using the Kruskal Wallis test and multiple comparisons, with significance level $p<0.05$. A table providing equivalencies between colors on the Vita Classic and CIELAB scales[25] (Table 2) was used to transform the recorded colors to equivalent values on the CIELAB scale. The difference in color (ΔE) was calculated for each group according to the following formula:

$$\Delta E\ (Lab) = \sqrt{[(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2]}$$

where ΔL, Δa and Δb are the differences in the values of L, a and b before and after AAA. Mean and standard deviation were analyzed statistically using ANOVA and Tukey's test. Significance level was set at $p<0.05$.

Table 2: Equivalence between Vita Classic and CieLAB scales

Shades	L*	a*	b*
B1	59.85	4.24	7.34
A1	63.46	5.05	9.11
B2	61.90	6.09	12.55
D2	59.42	5.59	8.59
A2	60.55	6.99	12.46
C1	55.87	5.15	8.81
C2	54.83	6.87	13.40
D4	55.57	6.18	14.40
D3	55.65	7.19	11.69
A3	56.16	7.96	14.58
B3	49.28	7.97	16.83
A3.5	48.94	8.49	15.70
B4	50.02	8.17	18.33
C3	46.29	6.78	12.88
A4	43.05	8.34	14.95
C4	34.92	7.23	12.87

Source: Thanathornwong B, Suebnukarn S, Ouivirach K. Decision support system for predicting color change after tooth whitening. Comput Methods Programs Biomed. 2016; 125:88-93. https://doi.org/10.1016/j.cmpb.2015.11.004

RESULTS

Mean and standard deviation values for the difference in Vita Classical Shades for each group were: RX 8.40 (1.52); PC 8.60 (3.13); SO 6.40 (3.51); DC 10.00 (0.00); PA 7.60 (3.29); PF 2.00 (0.00) (Fig. 3). The Kruskal Wallis test showed significant difference for the factor 'material' ($p<0.05$) and comparison of means showed a difference between PF and the rest of the materials.

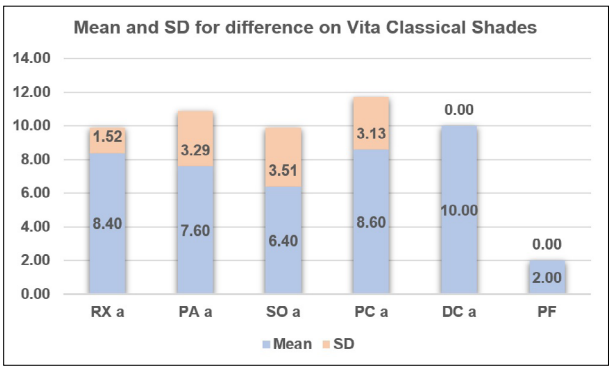

Fig. 3: Arithmetic mean and standard deviation of the difference on the Vita Classic scale. Differences between groups identified with the same lowercase letters are not statistically significant.

Mean and standard deviation values for the difference in in color ΔE for each group were: RX 14.94 (2.01); PC 14.51 (4.01); SO 12.07 (4.53); DC 16.31 (0.00); PA 10.90 (3.38); PF 7.23 (0.00) (Fig. 4). Analysis with ANOVA showed significant differences among the materials evaluated ($p<0.05$). Comparison of means with Tukey's test showed the presence of two groups (PF-DC, RX, PA).

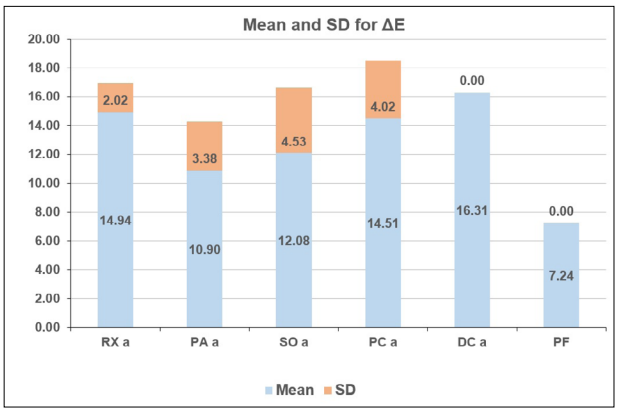

Fig. 4: Arithmetic mean and standard deviation of the difference on the CIELAB ΔE scale. Differences between groups identified with the same lowercase letters are not statistically significant.

DISCUSSION

Analysis based on CIELAB showed that all test specimens underwent darkening, with reduction of L* values. This may be associated to the presence of oxides or unreacted components in the polymerization system, which can act as intrinsic pigments[17,18]. There was an increase in a* values, indicating a trend towards reddish discoloration. For the b* coordinate, all specimens presented a yellowing effect during aging.

Composite resin color stability involves both intrinsic factors owed to the material's physicochemical reactions and extrinsic factors[12]. Regarding composition, most bonding agents used in this study contain TEGDMA and Bis-GMA monomers with structural chemical groups prone to hydrolysis and/or hydrogen bond with water: ether in TEGDMA, hydroxyl in Bis-GMA, and ester in both. Hydrolytic degradation and hygroscopic effects are determinant factors in color variation in resin-based materials. Color changes may be related to the nature of the organic matrix[13]. TEGDMA-based resins release larger quantities of monomers in aqueous environments than do Bis-GMA- and UDMA-based materials. Regarding water absorption, Bis-GMA-based resins are more absorbent than TEGDMA. UDMA has lower absorbance and greater color stability[19]. Resins with larger filler particles are more susceptible to color change, possibly because color perception is directly related to dispersion. Since the resin-filler interface is one of the weakest points of the material, with high sensitivity to water absorption, it may be assumed that hydrolytic degradation of the interface could modify the way in which light is dispersed by the particles. Differently sized particles may determine differences in the degree of dispersion due to aging in water[13]. In the current study, both light-cured and dual resin-based cements underwent significant color change. Many studies have reported that the mode of polymerization activation (dual polymerization and photopolymerization) had no influence on color change[8-10]. However, other authors, such as Almeida, noted that dual polymerization resin-based cement underwent significant color change. Color change in composite resins has been associated to hydrolytic degradation of organic components in resin-based materials, especially with chemical degradation of polymerization promoters[7].

Dual polymerization resin-based bonding agents are believed to be more prone to color change due to oxidation of tertiary aromatic amines present in their composition and of inhibitors, while light-cured resin-based bonding agents have greater color stability due to their aliphatic amines being less susceptible to oxidation[14]. The chemical reaction between amine and benzoyl peroxide is not very efficient because it depends on the physical bonding between molecules during the polymerization reaction[17]. Initiator mobility becomes restricted as the material polymerizes and its viscosity increases, leaving unreacted initiators in the polymer network[9]. Kilinc et al. say that inhibitor breakdown leads to yellowing[15]. Some authors report that the proportion between light-cured and chemical-cured components in the system is determinant. When there is a high concentration of light-sensitive components, greater color stability may be expected, while greater presence of chemical activation components leads to greater color change[4,11,14]. However, manufacturer information regarding the quantity of each component is unclear. Based on the results of the current study showing significant color change in dual resin-based bonding agents, it may be inferred that they contain a high proportion of self-curing components.

The initiator in the light-cured resin-based material used in the current study, RelyX Venner, is camphorquinone, which is yellowish, and produces slight color changes during polymerization. The effectiveness of photopolymerization depends on the quantity of amine molecules available to bond with the camphorquinone, and is directly related to the material's mechanical and optical properties. Photopolymerization materials increase the yellowish appearance during the first 24 hours following polymerization because the free camphorquinone molecules return to their original yellow color[7].

The AAA procedure consists of aggressive conditions of humidity, ultraviolet radiation and high temperature. It is not yet possible to reliably simulate the clinical situation, since factors such as absorption of pigments from food, bacterial plaque accumulation and mechanical loading are excluded[8]. Pissaia et al. used the method involving immersion in distilled water, and concluded that color changed less in light-cured resin-based cements than in dual-cured ciments[1]. The aging procedure can be used to compare different materials to each other, but

cannot be correlated to a clinical time frame[15]. Some authors estimate that the color changes within the first 300 hours of AAA at 37 °C are equivalent to one clinical year[10, 26].

Spectrophotometers are considered to be objective instruments for measuring color, and due to their precision, sensitivity, reproducibility and ease for communication, have been used in many studies to quantify tooth color. Despite these advantages, the translucence of the tooth structure and curved surfaces of teeth can lead to systematic errors in measurement[20]. The current study measured test specimens with flat surfaces, avoiding this type of measurement error.

Based on the individual capacity of the human eye to appreciate differences in colors, three different intervals are used to distinguish changes in color values: $\Delta E<1$ is imperceptible to the human eye; $1<\Delta E<3.3$ is clinically acceptable, and $\Delta E>3.3$ is not clinically acceptable. Restorations may thus be considered clinically acceptable when ΔE is less than 3.3. Turgut et al. studied color change using 0.5 mm ceramic blocks and cement with a thickness of 0.1 mm, finding that although there was a change in color, the change in the block was clinically acceptable according to ΔE values. They suggested that the resin-based cement was the main cause of color change in restoration treatments with ceramic veneers because ceramic materials have high color stability[4]. Ceramic veneers are thinner than ceramic crowns, and therefore present greater color change[5]. In the current study, ΔE was greater than 3.3 for all materials, which may be attributed to the greater thickness of solid cement specimens. The literature shows that discoloration is less in resin-based cements that are polymerized beneath ceramic structures than in solid cement specimens, because color change of the entire block is minimized, and possibly also due to the cement being protected by the surface layer of porcelain, the low volume of cement, and its low degree of direct exposure to the environment[6], all of which are favorable to reducing color change. Tests on solid resins show higher pigmentation due to the intrinsic properties of the material. The current study used cement specimens 2 mm thick, which is much thicker than the amount used clinically, and it was not covered by a ceramic layer, which may affect color change.

Although an in vitro study cannot be directly transferred to the clinical situation, it provides an interesting level of evidence. It would be ideal to perform this analysis using a different design, to evaluate the effect of the same variables on color change. Resin-based cements are known to undergo color change, and further studies could analyze whether color change with the ceramic is significant.

CONCLUSION

Under the experimental conditions used in this study, it can be concluded that accelerated artificial aging significantly affects color stability in the resin-based cements tested.

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DECLARATION OF CONFLICTING INTERESTS

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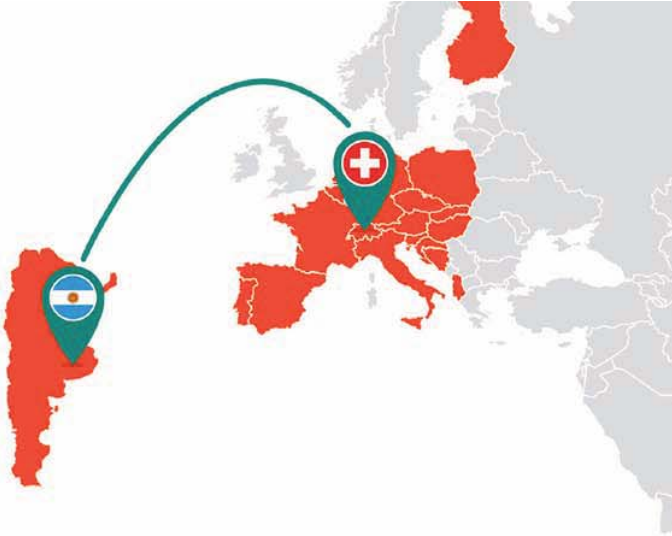

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