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CONTENTS / *ÍNDICE*

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In vitro evaluation of apical microleakage in retrofillings with different resection angles

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ABSTRACT

Paraendodontic surgery is a procedure that aims to solve problems that could not be solved by, or when it is not possible to perform conventional endodontic treatment. The aim of this experimental study was to compare the apical microleakage of teeth sectioned at 45° or 90° to the long axis of the tooth and root-end filled with mineral trioxide aggregate (MTA) using stereomicroscopy. In this study, 26 maxillary central incisors were used. Cleaning and shaping were performed with use of the Oregon technique and the samples were randomly divided into two groups. In Group A (n=10) apical section was performed at an angle of 90°, making a retrocavity with an ultrasonic tip and retrofilling with MTA. In group B (n=10) the same procedures were performed, but the apical section was at a 45° angle. Then the samples were immersed in a dye (India ink), placed in an oven at 37° for 48 h before applying the clearing technique. Afterwards the teeth were assessed by stereomicroscope at 20x magnification to analyze dye leakage. Data were submitted to the Student's-t test with significance level p< 0.05. There was statistically significant difference between groups. Group B showed higher apical microleakage values compared with group A (P= 0.004), but both groups showed dye leakage. The results showed that the 90° apical section promoted lower dye microleakage values at the dentin-retrofilling material interface than the 45°-section and could be considered the most effective technique for apical preparation in paraendodontic surgery.

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Keywords: *Periapical diseases, retrograde obturation, mineral trioxide aggregate.*

Avaliação in vitro da microinfiltração apical de retrobturações com diferentes ângulos de ressecção

RESUMO

A cirurgia paraendodôntica é um procedimento que visa resolver problemas que não poderiam ser resolvidos, ou quando não é possível realizar o tratamento endodôntico convencional. O objetivo deste estudo experimental foi comparar a microinfiltração apical de dentes seccionados a 45° ou 90° em relação ao longo eixo do dente e extremidade radicular preenchida com agregado de trióxido mineral (MTA) utilizando estereomicroscopia. Neste estudo, 26 incisivos centrais superiores foram utilizados. Limpeza e modelagem foram realizadas com o uso da técnica de Oregon e as amostras foram divididas aleatoriamente em dois grupos. No Grupo A (n = 10) foi realizada seção apical em ângulo de 90°, realizando retrocavidade com ponta ultrassônica e retrobturação com MTA. No grupo B (n = 10), os mesmos procedimentos foram realizados, mas a seção apical estava em um ângulo de 45°. Em seguida, as amostras foram imersas em um corante (nanquim), colocadas em estufa a 37°C por 48 h, antes da aplicação da técnica de clareamento. Posteriormente, os dentes foram avaliados por estereomicroscópio, com aumento de 20x, para análise do vazamento de corante. Os dados foram submetidos ao teste t de Student, com nível de significância p <0,05. Houve diferença estatisticamente significante entre os grupos. O grupo B apresentou maiores valores de microinfiltração apical em comparação ao grupo A (P = 0,004), mas ambos os grupos apresentaram vazamento de corante. Os resultados mostraram que a seção apical de 90° promoveu menores valores de microinfiltração de corante na interface do material retrobturador dentinário do que a seção 45° e pode ser considerada a técnica mais efetiva para preparo apical em cirurgia paraendodôntica.

Palavras-chave: *Doenças da polpa dental, obturação retrógrada, MTA.*

INTRODUCTION

In spite of the controversy regarding success rates of conventional endodontic therapy, it is a fact that with the continuous improvement of instruments and techniques, high indexes are obtained, approximately 88%[1]. In case of failure, clinicians may and must decide for endodontic retreatment, that has 80% success rates, an evidence of its effectiveness[2].

When there is failure in retreatment or when this practice is contraindicated, surgical therapy may be a solution for teeth maintenance. Such procedure consists in exposing the apex of the root, in performing apical resection, apical retropreparation and then sealing with retrofilling material[3,4].

Apical sealing is the most important surgical success factor resulting from the use of retrofilling material, which can prevent bacterial activity and bacterial by-products inside the root canal and in periapical tissues[5,6]. Periapical infections may be resistant to antimicrobial therapy and only paraendodontic surgery can produce disinfection and enhance tissue repair[7]. Success indexes of paraendodontic surgeries performed according to modern concepts account for 88,4%[8] .

Materials have been developed through time and their properties have been improved. This has changed the materials of choice for retrofilling. Mineral Trioxide Aggregate (MTA) – developed at the University of Loma Linda in the early 1990's [9] -presents advantages vis a vis previously used materials, due to its sealing ability, marginal adaptation and biocompatibility[10-12]. MTA has been the most widely used retrofilling material, yet, it has some limitations, such as long setting time, difficulty in handling and maintaining the mixture consistency[13,14].

Retrocavity preparation may be performed after resection at an angle of 45° or 90° with respect to the long axis of the tooth. For many years, the 45° -angled section was used as it enabled the visual and manual access to the apical region[15]. This angling enhances the exposure of dentinal tubules, enabling higher microleakage levels and resulting in keeping the lingual/palatal root portion without the adequade treatment[16,17]. In the case of the 90° resection, it enables a better crown/root proportion as it preserves more dental structure and promotes less leakage[18,19].

Retrocavities need to have at least a 3-mm depth for a more effective and a safer sealing action[16]. The use of spherical burs for making these retrocavities means that a root resection at 45° should be made for chamfering the root and having manual access, which tends to promote a higher periapical permeability[20,21]. However, by using angled ultrasound tips for the apical retropreparation, it was possible to get a more even and conservative preparation, when compared with the spherical burs [22] making it unnecessary to chamfer the root portion and permitting that a 90°-angle resection be made[20].

The purpose of this research was to evaluate apical microleakage in diaphonized and MTA-retrofilled teeth, by using 45° and 90° apical section angles, through the dyeing (India Ink) method analysed by stereo microscope.

MATERIALS AND METHODS

The research project was submitted to the Ethics Committee in Research with Humans of the University of Itaúna. The project was approved and protocol N° 421.819 was then assigned.

Twenty-six central superior, uniradicular incisors, with a completely formed apex, were used in the research study. The teeth were cleaned, sterilized in autoclave and previously submerged in saline solution, at the beginning of the procedures. Periapical X-Rays were made for evaluating the absence of calcifications, reabsorptions or of any previous endodontic treatment.

After selecting the teeth, access to their crowns was performed using the high rotation carbide bur 1557 (KG Sorensen, São Paulo, Brazil), and subsequently, the ceiling of the pulp chamber was removed, with the high rotation Endo-Z bur (Dentsply Maillefer, Ballaigues, Switzerland). The teeth were instrumented using the Oregon Technique, with manual K-type files. The patency was established by means of the K #10 file (Dentsply Maillefer, Ballaigues, Switzerland) and the length of the canal was determined through visualization of the K #15 file (Maillefer, Ballaigues, Switzerland) in the apical foramen. The length of the task was determined as being 1 mm beyond the apical foramen. Gates-Glidden #2 and #4 Burs (Maillefer, Ballaigues, Switzerland) were used for preparing the two coronal thirds of the dental roots; the apical third was standardized in all the teeth with a K-type #45 file (Maillefer, Ballaigues, Switzerland). During instrumentation, 2 ml of a NaOCL solution were used for canal irrigation. The final irrigation was done with 3 ml of Ethylenediaminetetraacetic acid (EDTA) 17% (Formula & Action, São Paulo, Brazil) for 60 s, followed by 2 ml of NaOCl 2,5%. Before the filling procedure, the canals were completely dried with absorbent paper cones (Dentisply Maillefer, Ballaigues, Switzerland). The filling procedure was done through the lateral condensation technique by using a digital spacer digital #25 (Odous de Deus, Belo Horizonte, Brazil), Endofill cement filling (Dentisply Maillefer,

Ballaigues, Switzerland) and gutta-percha cones #45 and accessory cones R1 (Dentisply Maillefer, Ballaigues, Switzerland). Three millimeters of the root crown portion were removed and, then, sealing was done by using IRM (Dentsply / Caulk, Milford, USA).

Subsequently, the samples were divided into two groups: A (n=10) and B (n=10). By using a millimetered ruler, in the apical third of the teeth a 3 mm standard marking was made at the points where the samples were resected. In Group A, the root-end was resected at an angle of 90° with the long axis of the tooth, by means of a high rotation multilaminated Zekrya bur (Dentsply-Maillefer, Ballaigues, Switzerland), with constant irrigation. The section was done from the mesial surface up to the end of the root distal surface. In Group B, the same procedures were applied, however, the apical resection was performed at a 45° cutting angle with the axis of the tooth.

After apicoectomy, the cavities were shaped with the Retro-D700 ultrasonic tip adapted to the ENAC ultrasonic device (Osada, Tokyo, Japan), by applying medium-power and under constant irrigation, with standardized 3 mm cavity depth and diameters. The samples were covered with two layers of enamel, except for the apical 3 mm portion. Six teeth were used as a negative control and all the surfaces of the dental structure were covered with two layers of enamel, showing the effectiveness of the enamel as a barrier to dye penetration. Retrofillings were irrigated with EDTA, 24%, during 3 minutes and then carefully rinsed with water for removal of excess of EDTA. The chosen retrofilling material was white MTA (Ângelus, Londrina, Brazil), handled according to the manufacturer's label and inserted into the cavities by using an MTA applicator (Ângelus, Londrina, Brazil). After the handling procedure, and according to the manufacturer's label, an initial 15-minute lapse was considered for letting the MTA set.

After these procedures, the teeth were submerged in Indian ink and placed in a microbiological oven at 37°C, and 100% Relative Humidity, during 48 hours. Subsequently, the samples were withdrawn from the oven and placed on surgical bandage to remove the excess of dye. Then, they were left at room temperature during 24 hours for dye fixation. Subsequently, the teeth were decalcified in hydrochloric acid, 5%, during 3 days, rinsed during 24 hours, and dehydrated in incremental alcohol solutions (70%, 80%, 90% and 100%, respectively) during 4 hours, a diaphonization technique previously described by Vertucci [23]. Then, the teeth were clarified using methyl salicylate, and remained like this until the analyses[24]. An examiner, trained and calibrated for identifying lineal dye penetration, performed the analyses of the samples.

Then, the teeth were photographed and evaluated in a stereosmicroscope (Leica Microsystems, Heerbrugg, Switzerland) with a 20X magnification, for observing the dye penetration process along the root-end surfaces. The lineal distance of the dye penetration was measured using the Image J software. This was transferred to a Microsoft Office Excel sheet and, then, to SigmaPlot program (Systat Software Inc. version 8.0, San Jose, CA, USA). The measurements of the lineal dye microleakage in both groups were analysed using a Student's-t test, with a $p<0.05$ significance level.

RESULTS

The measurements of the lineal dye microleakage of both groups are exhibited in Fig. 1 and illustrated in Fig. 2. The statistical Analysis showed a significant difference between Groups A and B ($p=0.004$). Both sections showed apical microleakage, yet, the group resected at 90° showed less leakage than the group with a 45° section in relation to the long axis of the tooth.

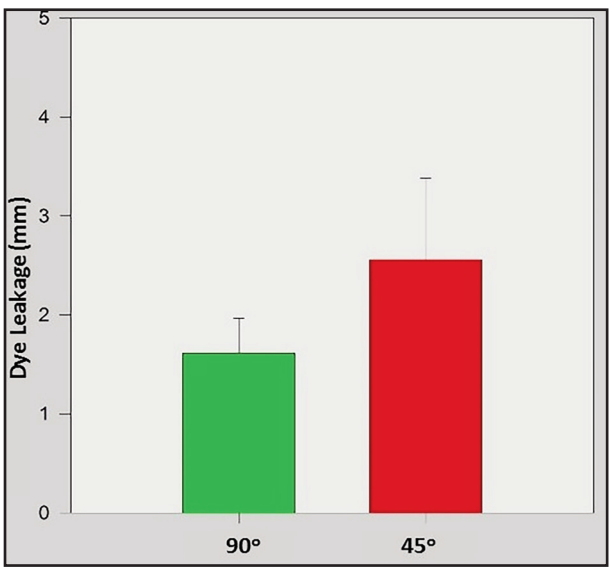

Fig. 1: Measures (arithmetical means) of microleakage in Group A (green column) and Group B (red column) in millimeters (mm). The vertical lines stand for standard deviations.

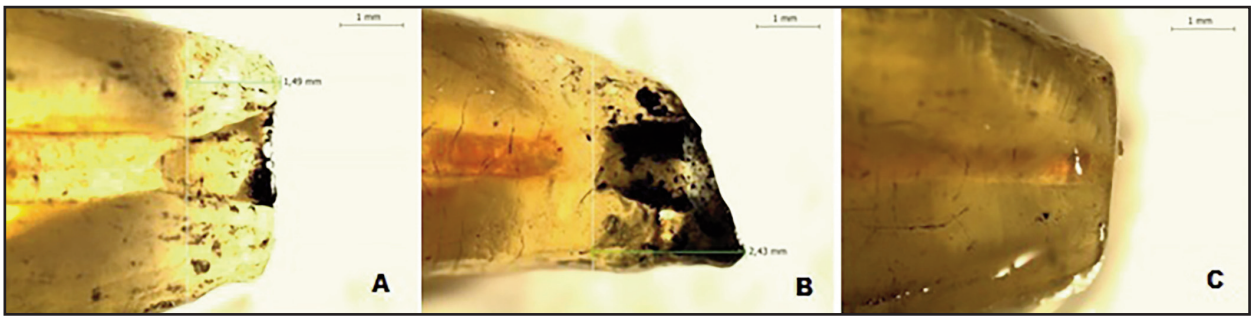

Fig. 2: Stereomicroscope with an original 20X magnification showing Indian ink penetration in the analysis groups and absence of leakage in the control group: A) Group A; B) Group B; C) Control Group.

DISCUSSION

In spite of the limitations of *in vitro* research studies, such studies are very valuable for developing endodontic techniques and materials, leading to relevant information for clinical practice[25].

Analysis using diaphonization permits the direct visualization of the internal anatomy of the root canal system and enables measuring lineal dye leakage in the interface dentin/retrofilling material. Diaphonization, which was used in previous Endodontia research studies, is a user-friendly method, with good sensitivity levels and no need for sophisticated methods and equipment. The dye used has a penetration capability similar to that of endodontic bacteria. This permits associating the outcomes of this *in vitro* study with those of the clinical study[24-26].

The relation between apical section angling and microleakage has been approached in several research studies, through different methodologies[3-7,9]. In this research, diaphonization was the analytical method chosen as it enables the internal visualization of retrofilled teeth and of the apical third dyed by penetration of Indian ink. By means of diaphonization, it was possible to have a 3D view of all the surfaces of the tooth and, consequently, to have a good access for determining the penetration of the dye[27,28]. In literature, there are studies in which the chosen methodology was vertical dental resection for analysis of microleakage, yet, this technique restricts the visual access to retrocavity peripheral areas and deteriorates the adequate evaluation of dye penetration, not simulating a real clinical situation[18,29].

Periapical surgeries are additional therapeutic procedures for teeth maintenance that may and must be applied in cases of failure of treatment and/or of endodontic retreatment, provided they are well prescribed and performed[8, 20].

With the development of dental materials, more desirable properties have been obtained for different clinical scenarios. Among such materials, MTA should be pointed out. MTA is undergoing extensive research and has shown to have physical and chemical features that make it appropriate for retrofilling, specially because it is biocompatible and helpful in tissue repair processes[9-13]. It is considered that this favorable biological response of MTA results in hydroxyapatite formation, useful in post-surgical bone repair[28].

The manufacture of ultrasonic angled tips has turned apical bevelling unnecessary as these tips allow clinicians to perform more uniform retropreparation, with parallel walls that make it easier to insert and adapt the filling material. Besides, it minimizes major leakage episodes associated with the augmented exposure of tubules caused by the 45° resection, as shown in this research study[17,18,30,31].

Lin et al. compared two retropreparation techniques - with ultrasonic tips and with the traditional spherical bur technique - using stereomicroscopy for evaluating the quality of the shape and size of a given retropreparation. The outcomes of such research study showed that the preparations with ultrasonic tip were more conservative and that there was a smaller number of root perforations than in the preparations with the spherical drills[19].

CONCLUSION

Irrespective of the technique used for performing apical resection, there was presence of microleakage. Nevertheless, the 90° section with the long axis of the tooth produced lower microleakage values when compared with the 45° section.

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Effect of flowable composite or glass ionomer liners on shrinkage stress of a composite resin

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ABSTRACT

The aim of this study was to evaluate the effect of flowable composite or glass ionomer liners on the shrinkage stress of a restorative composite resin. Fifteen previously sandblasted metal boxes were attached to a universal mechanical testing machine (INSTRON 1011, Instron Corporation). Five of these boxes were filled with Filtek Z350 XT (FXT) Universal Restorative A2 (3M ESPE) (Group 1 or Control). Two further groups of 5 boxes were prepared by interposing a layer of Vitrebond Light Cure Glass Ionomer 3M ESPE (VGI) (Group 2 or G.I.) or Filtek Z350 XT Flowable Restorative A2 3M ESPE (FFR) (Group 3 or Flowable) between the box and the composite resin, completing with the same volume of composite as in Group 1. Upon activating light-curing, the filled boxes mounted on the testing machine were videoed for 60 seconds (40 s photoactivation and 20 s post-curing), timed with a digital chronometer. Force values were recorded in newtons and converted into stress according to contact surface. Stress values were recorded every 10 s. Results were analyzed using repeated measures ANOVA. Mean and standard deviation in kPa (stress) recorded for each group were: Control group: 126.2 (30.8); G.I.: 48.4 (18); Flowable: 27.9 (19.5). Statistical analysis showed significant differences between the control group and the rest ($p<0.01$), with no significant difference between groups with glass ionomer liners and flowable resin liners (G.I. and Flowable). Under the experimental conditions of this study, it can be concluded that polymerization shrinkage stress can be reduced by the presence of a liner between the preparation and the restorative material.

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Keywords: *Composite resins, dental curing lights, glass ionomer, liner.*

Efecto de recubrimientos con resinas de baja viscosidad o ionómero vitreo sobre la tensión por contracción de una resina compuesta

RESUMEN

El objetivo de este trabajo fue evaluar el efecto de la colocación de una capa de Composite flow o Ionómero vítreo sobre la tensión de contracción de un composite para restauración. Se utilizaron 15 cajas metálicas previamente arenadas y conectadas a la máquina universal para ensayos mecánicos (INSTRON 1011, Instron Corporation). Cinco de estas cajas (G1) se rellenaron con Filtek Z350 XT (FXT) Universal Restorative A2 3M ESPE. Al iniciar la activación de la unidad de curado se comenzaba a registrar con una cámara de video y un cronómetro digital desde el comienzo de la activación de la lámpara hasta 60 s después, registrando los valores post curado durante 20 s. Los valores de fuerza generados por la polimerización fueron registrados en newton de cada 10 s para los 15 ensayos. Los valores fueron convertidos en tensión de contracción según la superficie de contacto. Se realizaron además dos grupos de cajas (5 en cada una) en los cuales se colocaron una capa inicial de Vitrebond Light Cure Glass Ionomer 3M ESPE (VGI) (G2 o IV) y Filtek Z350 XT Flowable Restorative A2 3M ESPE (FFR) (G3 o Flow) y se completó con el mismo volumen de composite de las del GI. Los resultados obtenidos fueron analizados por medio de ANOVA para mediciones repetidas. La media y la desviación estándar en kPa (tensión o estrés de contracción) registrado para cada grupo fueron: Grupo control: 126.2 (30.8); IV: 48.4(18); Flow: 27.9(19.5). El análisis estadístico mostró diferencias estadísticamente significativas entre el grupo control y el resto ($p=0.00$), pero no hubo diferencias significativas entre la presencia de Ionómero vítreo o Composite Flow (IV y Flow). En las condiciones experimentales de este trabajo puede concluirse que la tensión de contracción generada durante la polimerización puede ser disminuida por la presencia de algún material interpuesto entre la preparación y el composite restaurador.

Palabras clave: *Resinas compuestas, dispositivos para fotocurado, ionómero vítreo, recubrimiento.*

INTRODUCTION

Composite materials are being increasingly used in dentistry. Over the years, their composition and properties have changed to meet esthetic and mechanical requirements[1-3]. However, the greatest current challenge is still how to manage shrinkage stress, which depends on multiple factors such as polymerization shrinkage and the material's elastic modulus, which are directly related to the organic matrix and the quantity and type of filler; the speed

of conversion and degree of conversion [4,5] related to polymerization activation mode, and the type and quantity of initiators. These factors are directly affected by the composition of the material. [6,7]

Composites consist of an organic matrix, usually dimethyl acrylate, reinforced with ceramic fillers treated superficially with a vinyl-silane agent to adhere to the organic matrix and agents that promote the polymerization reaction. The base monomer, usually bis-GMA or some other diacrylate, has high molecular weight and, due to its high viscosity is mixed with other dimethyl acrylates of lower molecular weight (TEGDMA, UDMA) as diluents. It can be cured by chemical, physical or dual activation.[8,9] The composite sets as a result of polymerization, when the monomer chains crosslink to produce a final thermoset structure. During this process, the intermolecular distance between monomers is reduced, causing shrinkage. Linear and volumetric shrinkage of restoration composites have ranges of 0.5% to 2.0% and 1.0% to 4.0%, respectively. Shrinkage stress is 0.5 MPa to 8 MPa, depending on variables such as inorganic filler, monomer characteristics, material insertion technique, photoactivation methods and design of the preparation[10]. During polymerization gel point, the composite's elastic modulus increases such that the dissipation or deformation capacity is reduced to compensate the shrinkage. The adhesion to the tooth wall and the shrinkage of a composite restoration generate shrinkage stress, which is transmitted to the adhesive interface or dental substrate, generating clinical problems such as marginal gap, tooth fissures and/or fractures, secondary caries, postoperative sensitivity, marginal pigmentation, etc.[11-13]

The activation mode, as well as the types and concentrations of initiators, regulate the degree of conversion and kinetics of the reaction[14]. The higher the degree of conversion, the greater the shrinkage and the elastic modulus, both of which contribute to producing greater stress. Faster polymerization rates mean that the monomers move faster than the critical conversion, causing rapid setting and at the same time reducing flowability. A higher speed of reaction is associated to faster growth in the module before and after the gel point, and translates into faster development of stress compared to what would be produced by using a slower curing regime. [15,16]

There are several techniques to reduce shrinkage stress, such as using fillers or liners with low elastic modulus, incremental placement of composite resins, low intensity during the initiation of photopolymerization and modification of the composition of the material. Placing a liner material with lower elastic modulus such as a flowable composite or hybrid glass ionomer enables the size of the preparation, as well as shrinkage, to be reduced. Since both these materials are more flexible, shrinkage stress transmitted to the adhesive interface and/or the tooth is reduced. However, the results of studies on flowable composite as a liner are controversial. The elastic modulus of flowable composites varies, and can sometimes be higher than that of the composites themselves. The flowable composites with lower elastic modulus reduce shrinkage stress and better results were achieved even in some resins without ceramic filler.

Several authors have shown that using a filler reduces microleakage and increases adhesion and resistance values. Aggarwal et al.[17] studied marginal adaptation of composite resins with flowable composite and glass ionomer liners on third lower molars with different adhesive systems. Leevailoj et al. [18] evaluated marginal microleakage in class II restorations with high-viscosity composites (packable) with and without flowable composite liners in natural teeth. Montes et al. [19], evaluated bond strength of restorations with flowable composite with adhesives with or without ceramic fillers in bovine teeth. However, other studies report that using fillers has no beneficial effect on the margin of the material and the dentin due to the low content of filler and high polymerization shrinkage, e.g., the papers by Braga, Choi, Kwon and Caldenaro[20-23].

There are many studies on glass ionomer related to reduction of shrinkage stress. Bryant et al. [24] evaluated shrinkage of different types of glass ionomers and composite resins, finding that the shrinkage of glass ionomers is comparable to that of composites: about 2% to 3% in the long term without contact with moisture. Chutinan et al. [25] evaluated glass ionomers under conditions of moisture, reporting that as from 56 days, glass ionomers undergo expansion. Feilzer et al. [26] evaluated the influence of water sorption on shrinkage stress in resin-modified glass ionomer cements. Although the material initially shrinks after the setting reaction, subsequent hygroscopic

expansion of the glass ionomer due to conditions of moisture and according to time positions it better compared to flowable composites, in agreement with the conclusions reported by Kemp-Scholte[27] and Tolidis[28]. Competition between addition polymerization and the acid-base reaction of the resin-modified glass ionomers may affect the dimensional change of the material and its capacity to dissipate shrinkage stress, as suggested in the papers by Berzins, Kakaboura and Young[29-31].

In order to contribute to knowledge on this topic, the aim of this study was to assess the effect of using a layer of flowable composite or hybrid glass ionomer on shrinkage stress of a composite for restoration.

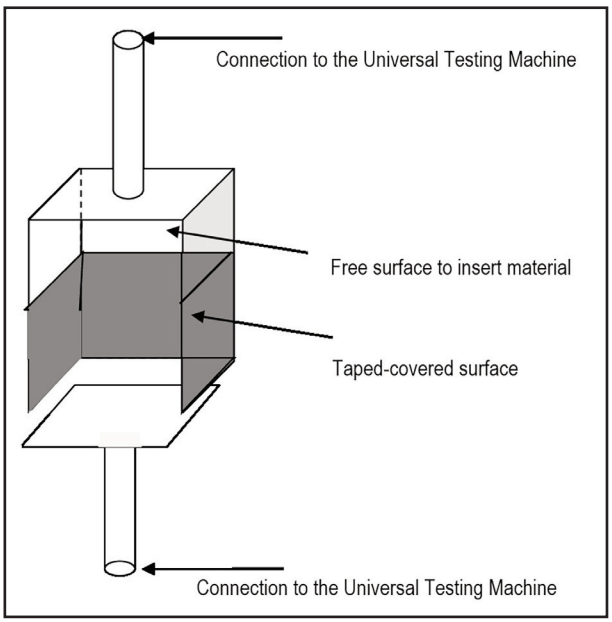

Fig. 1: Open box system used to evaluate shrinkage stress.

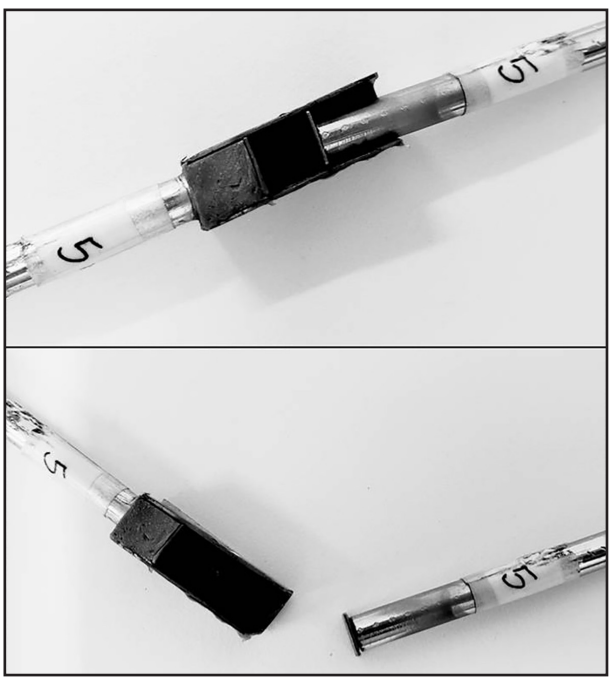

Fig. 2: Devices used to evaluate shrinkage stress; assembled (top) and disassembled (bottom). The two ends were connected to the universal mechanical testing machine.

MATERIALS AND METHODS

We used a nanofilled composite (FXT) (Filtek Z350 XT A2 from 3M), a resin-modified glass ionomer (VGI) (Vitrebond Light Cure Glass Ionomer from 3M) and a Flowable Composite (FFR) (Filtek Z350 XT Flowable Restorative A2 from 3M ESPE). The light-curing unit was a tungsten quartz lamp (XL-3000, 3M/ESPE).

We made metal boxes in which 4 of the sides were attached to each other. The top side was welded to a metal rod 4 mm in diameter. The bottom side was not attached to the rest of the box, but welded to another metal rod 4 mm in diameter (see Figures 1 and 2). Its edges were polished to enable it to slide freely through the box. The back surface was divided into two areas: one of 9 mm^2 on which to place the material, and the rest covered in paper tape. Composite was inserted through the open side. The opposite side was called floor, and its surface area was 20.25 mm^2. The metal rods were used to connect the pieces to a universal mechanical testing machine (INSTRON 1011, Instron Corporation, Japan).

Before using the boxes, we sandblasted them with aluminum oxide 50 μm in diameter using a Bio-Art microblaster for 10 s from a distance of 2 cm with air pressure 7 bar (102.5 psi). Then we washed them with distilled water in a Teslab® tb02 ultrasonic cleaner for 1 min at a power of 80 W and a frequency of 40 kHz, and dried them with air from a triple syringe.

We prepared three groups of 5 metal boxes: Group 1 (control group), filled with FXT; Group 2 (G.I.) lined with a resin-modified glass ionomer VGI and filled with FXT; and Group 3 (or Flowable) lined with FFR and filled with FXT.

For all three groups, each box was filled with 67.8 mg of composite, weighed with an OHAUS® Analytical Standard precision balance. The contact surface area was 56.25 mm^2 in all three groups. In Group 2, the Centrix system was used to line the boxes with a layer 1 mm thick of VGI, which was

cured according to the manufacturer's instructions. Immediately, the composite Filtek Z350 XT A2 (3M) was applied in a horizontal layer 2 mm thick, in contact with all five sides of the box, and cured for 40 s. The same was done for FFR. All assays were performed by a single operator.

The boxes were mounted on the testing machine such that the free-moving side was attached to the 0.5 kN load cell – adjusted in a 50N full scale – (the base of the free area was situated towards the floor of the box), while the rest of the box was attached to the base of the machine. The force values generated by polymerization were recorded every 10 s in newtons and converted to stress values (in kilopascals) according to contact surface area. Each procedure was recorded with a video camera and a digital chronometer from the beginning of composite activation with the lamp for 60 s (40 s photoactivation and 20 s post-curing).

The videos were used to record force values every 10 s for the fifteen tests performed. The results and the values converted to stress were analyzed statistically by ANOVA for repeated measurements and Tukey's test.

RESULTS

Table 1 shows mean and standard deviation (kPa) for the maximum stress values recorded for each group and evaluation time. Figure 3 shows mean stress values recorded for each group according to time. The ANOVA test, in linear and quadratic

Table 1: Mean and standard deviation (kPa) of maximum stress value recorded per group and time.

Time		10 s	20 s	30 s	40 s	50 s	60 s
Control	MEAN	27.4	78.0	94.1	99.5	118.0	126.2
	SD	28.1	18.6	21.6	24.1	28.5	30.8
G.I.	MEAN	20.4	28.7	29.6	30.1	42.7	48.4
	SD	9.2	14.3	16.8	19.1	20.0	18.0
Flowable	MEAN	-8.4	-1.1	2.4	3.4	19.8	27.9
	SD	4.8	7.3	10.3	11.5	17.0	19.5

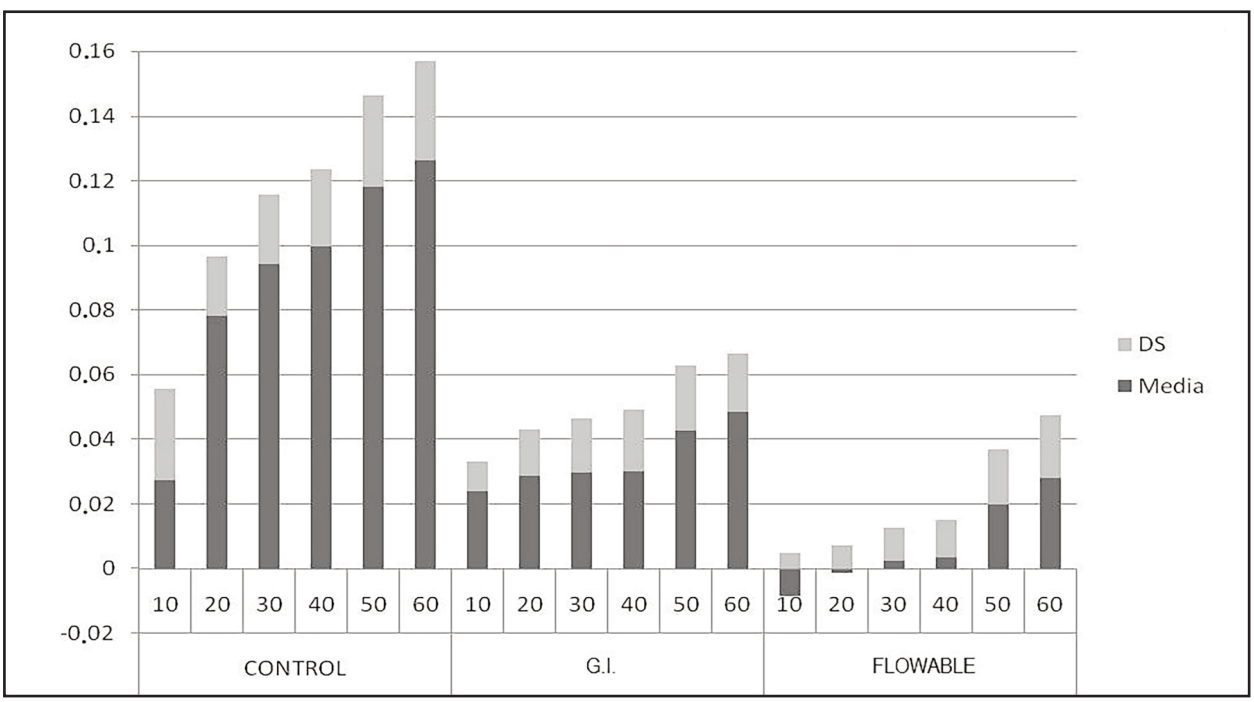

Fig. 3: Mean (kPa) per time and group (Control, GI and Flowable). Time: 1=10s, 2=20s, 3=30s, 4=40s, 5=50s and 6=60s.

function, showed statistically significant differences ($p<0.05$) according to material. Comparison of means using Tukey's test showed statistically significant differences between the control group (restorative material only) and the other two groups ($p<0.01$), but there was no significant difference between glass ionomer and flowable composite (Groups 2 and 3). Analysis of confidence intervals established that there was no statistically significant difference at the first evaluation time (10 seconds), but in the Control Group, shrinkage stress increased significantly as from 20 s compared to the groups with flowable composite or glass ionomer liners, with no difference between the latter two. This behavior is clearly visible in Figure 3, which shows that Groups 2 and 3 behave similarly, while in the Control Group, the stress generated increases progressively.

DISCUSSION

This study evaluated stress from the time of polymerization until 20 s post-curing, for the composite and for composite with a liner. It did not evaluate the individual behavior of each base or liner material, although their elastic modulus and stress are known to increase hours and even days after they have completely set[1].

It has been suggested that placing a liner with lower elastic modulus between the composite restoration and the substrate, used together with other precautions, may reduce the shrinkage stress[18,19]. Flowable composites and resin-modified glass ionomer are used as liners, but their use is still controversial according to results published to date. Because resin-modified glass ionomers (RMGIs) have fewer monomers that polymerize and therefore less shrinkage than composites, their use has long been suggested for reducing stress [14,24-26]. Kemp-Scholte & Davidson report that polymerization shrinkage stress was relieved by 20% to 50% as a result of the various flexible liner materials such as glass ionomer [27]. Tolidis K et al. also report that RMGIs liners reduce shrinkage stress. RMGIs set more gradually and slowly than resins, up to 48 h later, with better stress dissipation[28]. Bryant & Mahler report that at 30 min, the volumetric shrinkage of both conventional and hybrid ionomers is very similar to that of composites [24]. If shrinkage is similar, it may be deduced that stress would be similar, although our study found significant differences with and without liners, but no significant difference in stress relief for glass ionomer compared to flowable composite.

The competition between the acid-base reaction and addition polymerization can modify final structure and stress-dissipating ability. The two types of setting inhibit each other, i.e., if addition polymerization is activated, the rate and extent of the acid-base reaction is inhibited. Similarly, the polymerization reaction is affected by the polar nature of the ionomer medium, and as ionomer opacity increases as a result of acid-base neutralization, physical activation by light is attenuated[29]. It has been shown that the efficiency of curing decreases when it is applied after 20 min,[30] even though this situation is clinically quite unlikely to occur. But if activation is delayed by 3 min and 15 s, there will only be 85% polymerization compared to immediate activation.

RMGIs are susceptible to water uptake and release [27]. The movement of water may occur while the material sets under sealed conditions as a base or liner. Two chemical reactions have been reported. One is the intrinsic use of water during initial setting and the other is extrinsic water sorption by the acid-base reaction [31]. In the model applied herein, where RMGIs are used as bases or liners in metal boxes, there is no extrinsic water effect, and therefore the material shrinks. This could explain why no difference was found between glass ionomer and flowable composite.

According to Braga RR, Ferracane JL & Hilton TJ[20], flowable resins produce similar stress levels to composites. Most flowable composites do not produce significant stress reduction when used under composites [20] and there are even studies that report an increase in stress with flowable composite or RMGI liners[21-23]. Volumetric shrinkage and elastic modulus are inversely related and depend on the material's ceramic filler. Composites with high ceramic load have less organic matrix, and therefore less shrinkage due to the formation of crosslinked polymer chains, but in turn, they prevent elastic deformation for dissipating stress due to the high rigidity of the ceramic filler. A flowable composite follows these theories, but is a more fluid material due to the addition of monomers of smaller molecular size, and would theoretically have greater volumetric shrinkage. In turn, there are flowable composites with high ceramic loads which dissipate less stress due to

their high elastic modulus. Thus, they do not dissipate stress due to the change in mechanical properties of the material. It would be helpful to know to what extent the final stress is caused by the quantity and size of organic molecules and the elastic modulus of the composite when it has set.

The current study used metal boxes in a moisture-free environment. The boxes were sandblasted to increase micromechanical adhesion of the materials by applying a model similar to the one used by Pires-de-Souza et al. [32], with the difference that they used glass rods instead of metal boxes, mounted in the same way to the testing machine to record data. It should be noted that the substrate and adhesion are unlike the clinical situation. However, the design enables the behavior of materials and combinations to be evaluated, beyond the variables involved in the clinical situation. It would be interesting to ascertain the influence of the stress caused by each of them and the effect of the final resulting stress with the composite until the material hardens completely.

CONCLUSION

Under the experimental conditions in this study, it may be concluded that shrinkage stress generated during polymerization may be reduced by a liner placed between the preparation and the restorative composite.

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Relationship between Molar Incisor Hypomineralization (MIH) severity and cavitated carious lesions in schoolchildren

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ABSTRACT

The aim of this study was to identify the relationship between Molar Incisor Hypomineralization (MIH) severity and cavitated carious lesions in schoolchildren. This cross-sectional study included 506 schoolchildren selected from public schools. The prevalence and severity of MIH was evaluated using the European Academy of Pediatric Dentistry criteria (EAPD), while the prevalence and severity of caries was evaluated by applying the ICDAS (International Caries Detection and Assessment System). The prevalence of MIH was 42.4%, with a severity of 21.7% mild, 7.7% moderate, and 13.0% severe. Prevalence was 61.6% for incipient lesions and 34.0% for cavitated lesions. The prevalence of ICDAS II ≥4 lesions was higher in schoolchildren with MIH than in those without MIH (55.8% vs. 44.2%, p< 0.001). In the multinomial model, children with MIH in the moderate/ severe category were more likely (OR= 3.28; CI95% 1.01 – 10.6, p< 0.048) to present cavitated lesions. The presence of MIH at mild levels was not associated with cavitated carious lesions. A high prevalence of MIH was observed. Moderate and severe levels of MIH were associated with cavitated carious lesions. To prevent dental caries, it is important to identify MIH in children, particularly in the moderate and severe categories.

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Keywords: *Dental enamel hypoplasia, severity of illness index caries, oral hygiene.*

Asociación entre la severidad de Hipomineralización Incisivo-Molar (HIM) y lesiones cavitadas de caries en escolares

Resumen

El objetivo de este estudio fue identificar la asociación entre la severidad de la Hipomineralización Incisivo-Molar (HIM) y las lesiones cavitadas de caries en escolares. Estudio transversal que incluyó a 506 escolares seleccionados de escuelas públicas. La prevalencia y la severidad de HIM se evaluó utilizando los criterios de la European Academy of Pediatric Dentistry (EAPD), mientras que la prevalencia y severidad de caries se evaluó mediante los criterios del ICDAS (International Caries Detection and Assessment System). La prevalencia de HIM fue del 42.4%, por severidad: 21.7% leve; 7.7% moderado y 13.0% severo. La prevalencia de lesiones incipientes fue de 61.6% y 34.0% para lesiones cavitadas de caries. La prevalencia de ICDAS II ≥4 lesiones fue mayor en escolares con HIM que en aquellos sin HIM (55.8% vs 44.2%, p< 0.001). En el modelo multinomial, los niños con HIM en la categoría moderada / severa tienen mayor probabilidad (RM = 3.28; IC95% 1.01-10.6, p< 0.048) de presentar lesiones cavitadas de caries. La presencia de HIM en niveles leves no se asoció con la presencia de lesiones cavitadas de caries. Se observó una alta prevalencia de HIM. Los niveles moderados y severos de HIM se asociaron con lesiones cavitadas de caries. Para prevenir la caries dental, es importante identificar la HIM en los niños, particularmente en las categorías moderada y severa.

Palabras clave: *Hipoplasia del esmalte dental, indicador de severidad, caries, higiene bucal.*

INTRODUCTION

Molar Incisor Hypomineralization (MIH) is a developmental defect in the structure of the dental enamel, affecting the mineralization of one to four first permanent molars, frequently associated with affected mineralization in one to eight permanent incisors[1].

Histologically, the hypomineralized enamel shows areas of increased porosity[2] and the enamel structure itself is disorganized, depending on the degree of hypomineralization[3]. Clinically, MIH is characterized by creamy white, yellow or brown demarcated opacities on the enamel surfaces, while in severe presentations, the tooth structure is lost[4].

The etiology of MIH has not been entirely elucidated. MIH is usually caused by systemic alterations occurring during the prenatal period or during the first three years of life, thus affecting enamel development[5, 6]. Furthermore, it has been proposed that the etiology of this condition is

associated with the gene variations involved in amelogenesis[7]. Teeth affected by MIH, due to the hypomineralization and their morphological characteristics may cause sensitivity and pain, particularly in children. Moreover, affected molars are more susceptible to the accumulation of biofilm, due to the loss of enamel structure, which occurs after tooth eruption and is usually caused by mastication forces[8]. Epidemiological studies show a wide range in the prevalence of MIH, from 2.8% in children in Hong Kong, [9] to 9.7% in children in India [10] and 40.2% in children in Brazil[11]. The literature provides information on the association between dental caries and MIH, and studies in Spain show that children with severe MIH are more likely to present dental caries[12]. However, a study conducted on German children found no association between MIH and dental caries[13]. While a systematic review of published research suggested an association between these conditions, the results should, however, be interpreted with caution due to the lack of high-quality studies[14].

The limitations to comparing the results found for the association between MIH and dental caries include the different criteria used in the studies to evaluate the presence and severity of MIH and dental caries. In recent years, new caries indices have been used, one such being ICDAS (International Caries Detection and Assessment System). ICDAS considers the degree of progress of the carious lesion, and includes the identification of incipient lesions, microcavities, and lesions that involve the destruction of more than half the tooth surface. Furthermore, the criteria developed by the European Academy of Paediatric Dentistry for the evaluation of MIH in index teeth considers the extent of the affected area, the loss of tooth structure and color of the lesion, which are characteristics associated with the severity of MIH[15, 16]. The use of these indices would enable a more detailed analysis of the association between MIH and dental caries. Therefore, the aim of this study was to identify the relationship between molar incisor hypomineralization (MIH) severity and cavitated carious lesions in schoolchildren in Mexico.

MATERIAL AND METHODS

Study group

This study comprised a cross-sectional design. The area selected had a population of 833,779 inhabitants (5.4% of the total population of the State of Mexico), in which 55.5% of people over 15 years of age did not have higher education, and 55.8% had access to health services. Government statistics indicated a very low socioeconomic level in study area[17]. For convenience, three schools were selected, one each from the northern, central and southern sections of the study area.

The sample size was calculated to detect an Odds Ratio (OR) = 2.5 with 80% power and an alpha of 0.05, considering a probability of 0.18 and MIH= 0.30 = dental caries. The study group included schoolchildren aged 8 to 12 years presenting the eruption of at least one first permanent molar. The exclusion criterion was presence of orthodontic attachments that prevented the examination of the tooth surface. A total 650 parents were asked to provide consent to the participation of their children in the study, with 600 accepting and signing an informed consent form (response rate 92.3%). Ninety-four of the potentially eligible 600 schoolchildren were excluded: 1 due to the presence of an orthodontic appliance, 83 because they did not attend school on the days of examination, and 10 who did not present the eruption of any first permanent molars when the oral evaluation was performed. Thus, 506 schoolchildren were included in the study. The study was approved by the Ethics Committee of the Faculty of Dentistry at the National Autonomous University of Mexico (Protocol 20180515).

The research was conducted in full accordance with the World Medical Association Declaration of Helsinki.

The variables included in the study were: age (in years); sex (boy/girl); toothbrushing frequency (number of times a day); and, the Simplified Oral Hygiene Index (OHI-S) dichotomized into poor (OHI-S≥2 score) and good hygiene (OHI-S<2 score). Dental caries were evaluated by applying ICDAS II criteria, forming the categories ICDAS II=0, ICDAS II= 1-3, and ICDAS II= 4-6. MIH was evaluated using the criteria of the European Academy of Paediatric Dentistry (EAPD) and was classified in terms of presence/absence and severity of the lesion and in three categories: mild, moderate and severe[18]. The MIH level in each child was individually defined according to the first permanent molar or permanent incisor most severely affected by MIH.

Clinical oral examination

The evaluation of MIH included the inspection of permanent occlusal /incisal buccal and palatal surfaces of all erupted molars and incisors, which were classified according to the criteria for EAPD[19]. This index classified MIH based on visual clinical presentation, the extension of the surface affected by the lesion (I - less than one third of the affected tooth, II - at least one third but less than two surfaces, and III - post-eruptive presence of structure loss) and the color of the lesion (white or creamy opacities and yellow or brown opacities). Based on the index, the following MIH severity categories / criteria were constructed:

Severity criteria

Mild: One white or creamy demarcated opacity with a diameter >1 mm and affecting less than one third of the tooth surface.

Moderate: One yellow or brown demarcated opacity with a diameter >1 mm and affecting less than one third of the tooth surface; two or more white or creamy demarcated opacities >1 mm affecting at least one third but less than two thirds of the tooth surface, on which rough enamel is frequently found; post-eruptive enamel breakdown ≤2 mm; and, atypical restorations involving at least one third but less than two thirds of the affected tooth surface.

Severe: Two or more yellow or brown demarcated opacities with a diameter>1 mm affecting at least one third or more of the tooth surface; two or more white or creamy demarcated opacities with a diameter >1 mm, affecting at least two thirds of the tooth surface; post-eruptive enamel breakdown >2 mm; and, atypical restoration involving more than two thirds of the affected tooth surface.

The assessment of dental caries was performed by applying the ICDAS II criteria, which include the identification of incipient lesions (white spots and microcavities on the enamel) and cavitated carious lesions, including in the highest category the destruction of more than half of the tooth surface. This index classified caries with scores ranging from 0 to 6, with higher values indicating greater severity of the carious lesions[20].

Clinical oral evaluations were conducted by one dentist using dental mirrors (# 5), WHO probe and type artificial light, with the teeth brushed prior to the procedure. The measurements taken by this dentist had previously been standardized with gold standard, with Cohen's kappa coefficient for intra-examiner calibration of 0.81 and 0.84 for MIH and dental caries, respectively. Infection control standards for the examination of the children's oral cavity were followed.

Statistical analysis

Comparisons were made for age, sex, toothbrushing, oral hygiene (OHI-S) and MIH between schoolchildren with and without caries, using the Pearson Xi square test for categorical variables and the Kruskal-Wallis test for continuous variables. The association between the independent MIH variable was classified as mild, moderate or severe at subject level, while the dependent caries variable for incipient lesions (ICDAS II = 0 and ICDAS II = 1-3) and cavitated lesions (ICDAS II ≥4) was assessed via multinomial logistic regression models, adjusting for the covariates age, sex, toothbrushing and oral hygiene. Logistic regression analysis was also performed to determine the association between MIH and cavitated caries lesions (ICDAS II ≥4) at tooth level. The construction of the logistic regression models considered the correlation between teeth within each individual via the cluster option. The Odds Ratio (OR) and a 95% confidence interval (95%) were obtained. Values of $p \leq 0.05$ were considered statistically significant. Theoretically plausible interactions, such as oral hygiene and MIH and age and MIH, were also explored. The analysis was performed using the Stata 14 program (Stata Corp, College Station, TX, USA).

RESULTS

A total 506 schoolchildren were included in the study, with mean age 9.74 (± 1.36) years. The percentage of girls and boys examined was 49.4% and 50.6%, respectively. It was ascertained that 61.1% brushed their teeth twice a day or more frequently, and 38.9% less than twice a day, with 98.9% using toothpaste and, according to the OHI-S evaluation, 44.9% of schoolchildren having good oral hygiene and 55.1% having poor oral hygiene. By sex, a higher proportion of girls reported that they brushed their teeth twice a day or more frequently, which was more frequent compared to the boys (54.0% vs. 45.9%, p = 0.009).

Molar incisor hypomineralization

The prevalence of MIH was 42.4% (215/506), distributed by sex as 51.6% boys and 48.4% girls (p=0.390). Of the schoolchildren who presented MIH; 29.5% presented sensitivity and 8.7% pain.

When only children with four erupted permanent first molars (PFMs) were considered (n=496), the prevalence of MIH was 42.9%, comprising 40.9% in boys and 45.1% in girls (p=0.359). The results, according to the MIH followed by showed that 57.5% of the schoolchildren did not present enamel defects; 21.7% presented mild, 7.7 % moderate and 13.0% severe enamel defects.

While the frequency of toothbrushing reported by the children was not associated with MIH (61.9% MIH vs. 60.5% non-MIH, p=0.753), poor oral hygiene (OHI-S≥2) was not associated with the presence of MIH in children with and without MIH, 58.6% and 52.6%, respectively (p=0.178).

Dental caries

The prevalence of caries (ICDAS II 1-6) was 95.6%, which, by category (ICDAS II 1-3 and ICDAS II ≥4) translated as 61.6% and 34.0%, respectively. The mean number of carious lesions was 3.80 (±3.53) for ICDAS II 1-3 and 1.53 (±2.72) for ICDAS II ≥4. Six point six percent (6.6%) of schoolchildren had restorations in permanent teeth and 7.4% had pit and fissure sealants.

Dental caries and MIH

The prevalence of caries in primary dentition (ICDAS II ≥4) was higher in schoolchildren with MIH compared to children without MIH (56.3% vs. 46.0%, p=0.023). In the logistic regression model an association was found between MIH and caries (ICDAS II ≥4) in primary dentition [OR=1.60 (1.09-2.34; p=0.015)].

The prevalence of caries in permanent dentition (ICDAS II ≥4) was higher in schoolchildren with MIH compared to children without MIH (44.7% vs. 26.1%, p<0.001). Teeth with MIH showed a higher percentage of cavitated carious lesions compared to teeth without MIH. Table 1 presents the distribution of MIH severity scores by ICDAS index and the association observed between these conditions, demonstrating that most of the children with severe forms of MIH showed cavitated caries lesions (p< 0.001).

In the multinomial logistic regression model undertaken at subject level for incipient caries lesions (ICDAS II= 1-3) and MIH, controlling for age, sex, toothbrushing frequency and oral hygiene, no significant association was found between MIH and initial caries lesions in the mild [OR=0.63 (CI95% 0.19 – 2.09) p=0.457], or moderate/severe categories [OR=1.11 (CI95% 0.34 – 3.55) p=0.854 (Table 2). On the other hand, for cavitated caries lesions (ICDAS II ≥4), the schoolchildren with presence of moderate/severe MIH were more likely to have lesions, ICDAS II ≥4 lesions [OR=3.28 (CI95% 1.01 – 10.6); p=0.048], compared to the group without MIH. In the category of mild MIH and cavitated lesions, no significant association was found (p=0.131). Poor oral hygiene was associated with cavitated caries lesions (ICDAS II ≥4) in schoolchildren with MIH (OR=3.62 (CI95% 1.36 – 9.61); p=0.010) (Table 2).

Table 1: Characteristics in schoolchildren aged 8 to 12 years with and without caries from the State of Mexico.

	ICDAS II=0 n=22	ICDAS II=2-3 n=312	ICDAS II=4-6 n=172	Value p*
Age	8.95 (±1.29)	9.86 (±1.34)	9.62 (±1.35)	0.003
Sex				
Male	11 (50.0)	175 (56.1)	70 (40.7)	0.005
Female	11 (50.0)	137 (43.9)	102 (59.3)	
Toothbrushing frequency				
< 2 times a day	12 (54.6)	176 (56.4)	121 (70.4)	0.009
≥ 2 times a day	10 (45.4)	136 (43.6)	51 (29.6)	
Oral hygiene (OHI-S)				
Poor hygiene	7 (31.8)	162 (51.9)	110 (63.9)	0.003
Good hygiene	15 (68.2)	150 (48.1)	62 (36.1)	
MIH				
Normal	14 (63.6)	201 (64.4)	76 (44.2)	<0.001
Mild	6 (27.3)	79 (25.3)	25 (14.5)	
Moderate	2 (9.1)	18 (5.8)	19 (11.1)	
Severe	0 (0.0)	14 (4.5)	52 (30.2)	

*Chi-square test, **Kruskal Wallis Test.

The results of the regression model for dental caries and the number of permanent first molars affected (Table 3) showed a significant association with the number of teeth with MIH-associated cavitated carious lesions (ICDAS II ≥4). Children presenting three or four permanent first molars with MIH were more likely (OR=4.30; CI95% 1.17 – 15.8, p=0.028) to present cavitated caries lesions (ICDAS II ≥4), while for one or two molars with MIH, the association was OR=3.69 [(CI95% 1.00 – 13.5), p=0.048] compared to non-MIH children. Finally, an association between enamel hypomineralization and dental caries in PFMs was detected when individual teeth were considered as the units of analysis (Table 4). The PFMs with MIH were more likely to have cavitated lesions (ICDAS II ≥4) compared to molars without MIH (OR=2.24; CI95% 1.52 – 3.28, p<0.001). No interaction was identified in the models.

Table 2: Adjusted odds ratios from the multinomial logistic regression model for dental caries and Molar Incisor Hypomineralization (MIH) in schoolchildren 8 -12 years of age.

	ICDAS II codes 1-3[b]		ICDAS II codes 4-6[b]	
Variables	Odds Ratio (95%CI)[a]	p	Odds Ratio (95%CI)[a]	p
Age	1.70 (1.15 – 2.51)	0.008	1.39 (0.93 – 2.07)	0.107
Sex[c]	0.70 (0.23 – 1.71)	0.447	1.29 (0.52 – 3.22)	0.582
Oral Hygiene (OHI-S)[d]	1.96 (0.76 – 5.06)	0.163	3.62 (1.36 – 9.61)	0.010
Toothbrushing frequency	1.04 (0.42 – 2.54)	0.934	2.00 (0.79 – 5.09)	0.149
Severity MIH[e]				
Mild	0.63 (0.19 – 2.09)	0.457	0.82 (0.23 – 2.84)	0.131
Moderate/Severe	1.11 (0.34 – 3.55)	0.854	3.28 (1.01 – 10.6)	0.048

[a]OR= Odds ratio; CI= confidence interval. Reference group: ICDAS IIb =0, Sex[c]= Male, OHI-S[d]= Good, Severity MIH[e]= non MIH, Toothbrushing frequency ≥ 2 times a day.

Table 3: Adjusted odds ratios from the multinomial logistic regression model for the association between enamel hypomineralization and dental caries in permanent first molars in schoolchildren 8 -12 years of age.

	ICDAS II codes 1-3[b]		ICDAS II codes 4-6[b]	
Variables	Odds Ratio (95%CI)[a]	p	Odds Ratio (95%CI)[a]	p
Age	1.74 (1.17 – 2.58)	0.006	1.32 (0.88 – 1.98)	0.172
Sex[c]	1.08 (0.44 – 2.67)	0.856	1.87 (0.74 – 4.68)	0.181
Oral Hygiene (OHI-S)[d]	2.26 (0.85 – 6.05)	0.102	3.88 (1.43 – 10.5)	0.008
Molar MIH[e]				
1-2 molars	0.72 (0.19 – 2.67)	0.627	3.69 (1.00 – 13.5)	0.048
3-4 molars	0.79 (0.21 – 2.90)	0.721	4.30 (1.17 – 15.8)	0.028

[a]OR= Odds ratio; CI= confidence interval. Reference group: ICDAS II[b] =0, Sex[c]= Male, OHI-S[d]= Good, molar MIH[e]= non molar MIH

Table 4: Association between enamel hypomineralization and caries (ICDAS II ≥4) in permanent first molars among schoolchildren with molar incisor hypomineralization: tooth level analysis.

Variables	ORa Crude (95%CI)	p	ORa adjusted (95%CI)	p
Age	0.90 (0.79 – 1.04)	0.165	0.85 (0.74 – 0.99)	0.037
Sex[b]	1.83 (1.26 – 2.65)	0.001	1.87 (1.27 – 2.74)	0.001
Oral Hygiene (OHI-S)[c]	1.73 (1.18 – 2.52)	0.004	1.82 (1.22 – 2.70)	0.003
MIH[d]	2.28 (1.56 – 3.32)	<0.001	2.24 (1.52 – 3.28)	<0.001

Logistic Regression: aOR= Odds ratio; CI= confidence interval. Reference group: Sexb= Male, OHI-Sc= Good, MIHd= non MIH.

DISCUSSION

In this study, the presence of MIH was associated with cavitated carious lesions assessed using ICDAS II. Furthermore, the severity of MIH showed an impact on dental caries, with schoolchildren with moderate/severe categories of MIH being more likely to have cavitated lesions compared to schoolchildren without MIH. The association found between cavitated carious lesions and MIH is consistent with the results of studies conducted in other population groups[14, 21, 22]. Children in Thailand with MIH were 4.6 times as likely to have caries in permanent teeth than children without MIH[6]. Similarly, an association between MIH and caries experience was found in 6 to 12-year-old Brazilian schoolchildren[23]. It is likely that the association between MIH and dental caries is easier to recognize in groups with high risk of caries and high severity of MIH.

The association between MIH and caries could be explained by the morphological changes observed in teeth with MIH because the hypomineralized enamel microstructure is disorganized, with the interprismatic spaces being less compact than in healthy enamel[24]. Moreover, mechanical properties such as hardness, elasticity and chemical composition are deteriorated, compared to healthy enamel[3]. As the enamel in teeth presenting MIH becomes brittle, it is clinically possible to detect loss of continuity, fractures or even loss of tooth structure, which favor the accumulation of biofilm on the porous enamel and the exposed dentin.

Accordingly, children with MIH showed a greater level of biofilm accumulation. Biofilm accumulation is favored by the porous surface of the affected teeth, leading to penetration by cariogenic bacteria, which destroys hypomineralized enamel more quickly than sound enamel[14]. Additionally, schoolchildren with teeth affected by MIH may suffer from dentinal sensitivity and are more likely to find it difficult to brush their teeth properly[25]. While this study revealed an MIH prevalence of 42.4%, Gurrusquieta BJ *et al*., found that prevalence in Mexican children aged 6 to 12 years was 15.8%, i.e., lower than that found in the present study[26]. This differences in prevalence of MIH among school children could be due to the differences in etiological factors, lifestyle and genetic characteristics in the populations studied. The percentage of children affected by MIH in the study group was higher than that identified in studies undertaken in populations in India (9.7%) [10] and Thailand (20.0%) [6], but similar to MIH prevalence reported in the study undertaken on schoolchildren in Brazil, (40%)[11]. It may be difficult to compare MIH prevalence among different studies, considering the different indices used for MIH assessment, the age of the participants and the caries risk for the specific study group, among other factors.

In the Mexican children included in this study, most of the MIH cases detected presented a moderate level of severity. While the MIH index of the European Academy of Pediatric Dentistry is widely used in epidemiological and clinical studies, it does not directly classify MIH severity; nevertheless, it does assess tooth characteristics that facilitate the construction of a severity score.

Advanced carious lesions (ICDAS II 4-6) and incipient carious lesions (ICDAS II 1-3) were evaluated in this study. The small lesions were not associated with the presence or severity of MIH. Ismail *et al.,* in a study conducted on infants and their caregivers in low-income families in Detroit, Michigan, identified that the risk factors for incipient lesions were not necessarily the same as those for cavitated lesions[27].

In this study, incipient and cavitated lesions were assessed by applying ICDAS II criteria, which provide a broad perspective on the caries process by considering initial lesions previously excluded by both clinical and epidemiological studies. In this research, incipient lesions (ICDAS II 1-3) were found in more than half of the study group, and cavitated lesions (ICDAS II ≥4) in approximately one third of the group. Similarly, studies conducted in Spain on 12-year-old children using the same cutoff value (ICDAS II ≥4) found a 37.7% caries prevalence in permanent dentition[28].

Some of the limitations of this study are related to its cross-sectional design, as some teeth affected by MIH were likely not to have been detected due to the presence of restorations that could mask the occurrence of MIH. However, only 6.6% of participants in the study group presented dental fillings in permanent teeth. The extrapolation of the results of the study to other groups from different areas of Mexico has limitations due to the heterogeneity of children in the city; however, the schools featured in this study are located in low income neighborhoods, the type of neighborhood where more than 50% of the families featured in this study live.

The identification of an association between MIH and dental caries is considered important, particularly in children presenting various dental problems, such as poor oral hygiene, hypersensitivity, high caries risk and high treatment needs. The results suggest that when MIH is mild, the dentist may select a conservative approach based on general preventive measures such as brushing with fluoridated toothpaste. However, when MIH is moderate/severe, a proactive approach is important, as is the use of additional measures for preventing caries, such as the application of glass ionomer sealants. A cohort study showed that it is possible to maintain the tooth structure of the areas with MIH opacities, with the authors of said study recommending a conservative approach in mild MIH cases[16].

In conclusion, this study shows that more than one third of the children had MIH, identifying an association between MIH and dental caries evaluated through ICDAS II. It is important that children with MIH are diagnosed early in order that they receive preventive measures and timely treatment to protect the tooth structure affected and prevent the deterioration of oral health and, thereby, quality of life.

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Effects of bleaching using 10% carbamide peroxide with calcium or amorphous calcium phosphate on enamel mineral content and hardness

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ABSTRACT

This study evaluated enamel mineral content and surface microhardness before and after bleaching treatment using 10% carbamide peroxide (CP) containing calcium (Ca) or amorphous calcium phosphate (ACP). Thirty-six bovine slabs were randomly allocated into 3 groups (n = 12) according to bleaching treatment: G1 - Opalescence PF 10% (CP), G2 - NiteWhite ACP (CP+ACP), and G3 - Opalescence PF (10%) with calcium (CP+CA). The bleaching agent was applied on enamel surface for 6 h/day over a period of 21 days. Enamel surface was evaluated by Knoop microhardness (KNH) and micro energy-dispersive X-ray fluorescence spectrometry (μ-EDXRF) at baseline and at after bleaching treatment. Data were statistically analyzed by repeated measures ANOVA and Tukey's test (α = 0.05). There was a significant decrease in microhardness after bleaching treatments for all study groups, but no difference between bleaching gels. There was no difference in the Ca/P ratio measured by μ-EDXRF for all groups at the study times, but the mean value was lower in group CP+CA than in group CP+ACP. Group CP was similar to both CP+ACP and CP+CA. It can be concluded that enamel microhardness decreased after the bleaching process, regardless of the presence of calcium or ACP, but there was no significant change in the Ca/P ratio of enamel after bleaching for each tested gel. This indicates that the bleaching gels have erosive potential, causing softening of enamel without promoting surface loss, regardless of the presence of calcium of ACP ions.

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Efeitos do tratamento clareador utilizando peróxido de carbamida a 10% com adição de cálcio ou fosfato de cálcio amorfo no conteúdo mineral do esmalte e microdureza

RESUMO

Este estudo avaliou o conteúdo mineral do esmalte e a microdureza superficial antes e após o tratamento clareador, utilizando peróxido de carbamida 10% (PC) contendo cálcio (Ca) ou fosfato de cálcio amorfo (ACP) em sua composição. Trinta e seis espécimes de esmalte bovino foram alocados aleatoriamente em 3 grupos (n = 12) de acordo com os tratamentos clareadores: G1 - Opalescence PF 10% (CP), G2 - NiteWhite (CP+ACP); e G3 - Opalescence PF (10%) com cálcio (CP + CA). O agente clareador foi aplicado na superfície do esmalte por 6 h/dia por um período de 21 dias. A superfície do esmalte foi avaliada por microdureza Knoop (KNH) e espectrometria de fluorescência de raios X micro-dispersiva (μ-EDXRF) no início e após o tratamento clareador. Os dados foram analisados estatisticamente pelo teste ANOVA de medidas repetidas e Tukey (α = 0,05). Houve uma diminuição significativa da microdureza após os tratamentos clareadores para todos os grupos estudados, mas não houve diferença entre os diferentes géis. Não houve diferença da relação Ca/P mensurada por μ-EDXRF para todos os grupos nos tempos estudados; no entanto, o grupo CP+CA apresentou menor valor comparado ao grupo CP+ACP. O grupo CP foi similar aos grupos CP+ACP e CP+CA. Portanto, pode-se concluir que houve redução significativa da microdureza do esmalte após o clareamento, independente da presença de cálcio ou APC na composição dos géis, embora não tenha havido alteração significando na relação Ca / P do esmalte após o clareamento. Isto indica um potencial erosivo dos géis clareadores, causando o amolecimento sem perda da estrutura do esmalte, independente da presença dos íons cálcio e ACP.

Palavras-chave: *Esmalte, clareamento, peróxido de hidrogênio.*

INTRODUCTION

As a result of modern western aesthetic parameters, which consider white teeth to be more attractive, there is current concern regarding oral appearance and tooth discoloration. Dental bleaching – a simple, effective treatment for removing dental pigments – is one of the resources for improving the appearance of discolored teeth [1].

The active agent in dental bleaching is hydrogen peroxide (HP), which penetrates the tooth structure, breaking down the chromophore molecules that cause dental pigmentation into smaller, lighter colored substances, as has been frequently reported over the years [1-4]. However, several studies have also described adverse effects of HP on tooth structure, such as microhardness reduction, mineral content changes and decrease in calcium and phosphate [4-10], mainly after longer exposure of dental tissues to highly concentrated HP [11-13].

These deleterious effects are attributed to the erosive potential of some bleaching gels, which damage enamel, reducing its integrity and increasing its porosity, mainly due to the loss of inter-rod spaces[14-15]. Attempts have therefore been made to develop bleaching gels that are less deleterious to enamel, by incorporating remineralizing agents such as calcium, fluoride, CCP-ACP and others, with contradictory results. The addition of calcium, for example, can increase bleaching gel saturation and decrease mineral loss, overcoming the undesirable effects of the treatment [16]. However, a recent report showed that the addition of calcium alone to a 35% HP bleaching gel was not efficient in preventing enamel microhardness reduction[17], and its desirable effects remain uncertain.

Despite microhardness and roughness analyses, the loss of calcium and phosphate on the tooth surface during bleaching are not fully understood, and research has been conducted on the molecular constituents of dental structures. Hydroxyapatite calcium/phosphate quantification is an indicator of the degree of enamel demineralization,[9] and some studies have reported that the amounts of calcium and phosphate are lower in whitened teeth[18,19].

The aim of this study was to evaluate the enamel mineral content after bleaching using carbamide peroxide gels with calcium or amorphous calcium phosphate (ACP) added to their formulation.

MATERIALS AND METHODS

Thirty-six bovine incisors were cleaned with periodontal curettes (HU-Friedy, Chicago, IL, USA), brushed with pumice and stored in 0.1% thymol solution (Byoformula, São José dos Campos, SP, Brazil) until use. Enamel samples were obtained from the coronal portion using a diamond disc (KG Sorensen, Barueri, SP, Brazil) with a low-speed handpiece under constant water irrigation. Four dental blocks (4 mm x 4 mm) 3 mm thick were cut from the buccal surface of each tooth and embedded in crystal polyester resin, keeping the enamel surface exposed. The samples were polished with 600-, 800-, 1500-, and 2500-grit water-cooled aluminum oxide papers (Arotec, Cotia, São Paulo, Brazil). Samples were immersed in distilled water and cleaned in ultrasound for 30 min to remove any debris left by the polishing.

Initial Knoop Microhardness

Initial microhardness was obtained for each sample using a Knoop indenter (Future Tech-FM-1e, Tokyo, Japan). Three indentations were made 100 µm apart with a 50 g load for 15 s, to prevent cracks on the enamel surface during the experiment. The microhardness value of each sample was defined by the arithmetic mean of the three measurements.

Initial micro energy-dispersive X-ray fluorescence spectrometry (µ-EDXRF)

Semi-quantitative elemental analyses of calcium (Ca) and phosphorus (P) levels in the enamel were carried out before treatments, using micro energy-dispersive X-ray fluorescence spectrometry (µ-EDXRF). Ca and P weight percentages (wt%) from enamel samples were evaluated by collecting three spectra from each sample before and after the bleaching treatments. Measurements were performed with a count rate of 100 s per point (live time). Voltage in the tube was set at 15 kV, with automatic current adjustment and a beam diameter of 50 µm. The equipment was adjusted using a certified commercial reagent (SIGMA, 2008) of stoichiometric hydroxyapatite (Sigma-Aldrich, Poole, UK) synthetic $Ca_{10}(PO_4)_6(OH)_2$, grade 99.999%) as a reference[19]. The measurements were collected under fundamental parameters of characteristic X-ray emission of the elements Ca and P, and the element oxygen (O) was used as chemical balance.

The samples were previously evaluated by a micro-energy-dispersive X-ray fluorescence spectrometer (m-EDXRF-1300, Shimadzu, Japan).

Groups and treatment

After the initial microhardness and m-EDXRF measurements, samples were divided into 3 groups (n=12) according to bleaching treatment: **CP** – 10% carbamide peroxide (Opalescence PF - Ultradent Inc., South Jordan, UT, USA), **CP+ACP** - 10% carbamide peroxide with amorphous calcium phosphate (NiteWhite ACP - Discus Dental, Culver City, CA, USA), and **CP+CA** – 10% carbamide peroxide with calcium (Opalescence PF + 2000 ppm calcium). For group CP+CA, the gel was obtained by mixing Opalescence PF 10% with 2000 ppm of calcium chloride ($CaCl_2$). The pH was measured before and after calcium addition and found to remain unchanged (pH = 6.8).

For all experimental groups, the bleaching agent was applied on the enamel surface (1 mm thickness) for 6 h/day at 37°C ± 1°C, after which the samples were rinsed with distilled water and individually stored in artificial saliva (1.5 mM Ca, 0.9 mM P, 0.1 Tris buffering solution, pH 7.0) until the next bleaching session. The bleaching treatments were performed for 21 consecutive days.

Final microhardness and micro energy-dispersive X-ray fluorescence spectrometry

Final microhardness and micro energy-dispersive X-ray fluorescence spectrometry were measured following the same steps as described above, after 7 days.

Statistical analysis

Data were statistically analyzed by repeated measures analysis of variance (RM ANOVA) and Tukey post-hoc test for multiple pairwise comparisons. The main variables were "bleaching agent" and "time". Statistical analysis was carried out in the statistical software SAS 9.1 (SAS Institute, Cary, NC, USA) with a confidence interval of 95%.

RESULTS

Data showed homogeneity of experimental variances and errors with a normal distribution of both variables studied (Knoop Microhardness and μ-EDXRF). Table 1 shows the microhardness results. Two-way ANOVA shows a significant interaction only for the time factor ($p = 0.0009$), with lower values after the treatment for all groups when compared to the initial values. For the bleaching agent, there was no difference between groups.

Table 2 shows the mean values for μ-EDXRF. There was no significant difference between times ($p = 0.95$), but there were significant differences between bleaching agents ($p = 0.04$). The Ca/P ratio was higher for group CP+ACP than for group CP+CA. Group CP presented similar results to groups CP+ACP and CP+CA.

DISCUSSION

Changes in the mechanical properties of enamel after bleaching indicate surface alterations due the action of peroxide on hydroxyapatite and its organic components[20-23]. Several studies have shown that enamel treated with 10% carbamide peroxide might exhibit porosity and morphological surface alterations after a bleaching process[24, 25]. In the present study, the microhardness analysis (Table 1) showed significant mineral loss for all groups after bleaching treatment, as has been observed previously[24]. This significant

Table 1: Knoop microhardness means (SD) for experimental groups.

Bleaching agent	Time	
	Baseline	After bleaching
CP	223.0 (8.2) Aa	113.5 (7.7) Ba
CP+ACP	217.6 (7.3) Aa	109.2 (12.5) Ba
CP+CA	221.7 (11.4) Aa	116.6 (6.9) Ba

Mean followed by different letters indicates statistical differences ($p < 0.05$). Capital letters compare the different times for each bleaching agent and lowercase letters compare bleaching agents at each time.

Table 2: Mean values (SD) of Ca/P ratio for experimental groups.

Bleaching agent	Time	
	Baseline	After bleaching
CP	1.95 (0.10) Aab	1.96 (0.04) Aab
CP+ACP	1.97 (0.11) Aa	1.97 (0.09) Aa
CP+CA	1.92 (0.06) Ab	1.91 (0.06) Ab

Mean followed by different letters indicates statistical differences ($p < 0.05$). Capital letters compare different times and lowercase letters compare bleaching agents.

decrease in microhardness after bleaching with 10% carbamide peroxide was similar to that found in other studies[26-29] and may be related to bleaching gel composition, hydrogen peroxide concentration, activators, pH, and bleaching agent thickener[19,28].

Carbamide peroxide gels contain urea, which degrades proteins in the organic matrix of the enamel, leading to a structural alteration of its surface[30-32]. The Carbopol and glycerin could also act as demineralizing agents[31]. Such changes in enamel organic and inorganic parts after bleaching can lead to a decrease in microhardness[33-34].

The interaction between the bleaching agent and hydroxyapatite results in the following reaction: $Ca_{10}(PO_4)_6(OH)_2 + 8H^+ \rightarrow 10\ Ca^{2+} + 6\ HPO_4^{2-} + 2H_2O$, where the calcium (Ca) element and phosphate (P) group analysis are good indicators of enamel demineralization[35]. In the present study, there was no significant difference in the Ca/P ratio before and after bleaching treatments for all groups (Table 2). This is similar to the results reported by Duschner *et al*.[36], who found no significant difference in Ca and P components after dental bleaching.

After bleaching, there were significant differences among groups in the Ca/P ratio. Changes in the enamel were probably caused by the other components in the formula, such as fluoride and calcium, which can promote surface remineralization, although we did not observe better behavior in relation to the enamel Ca/P ratio prior to whitening.

For the μ-EDXRF analysis (Table 2), the results showed that was no statistical difference in the ratio of calcium and phosphate, which are the chemical constituents of enamel mineral content. This is in agreement with other studies that have reported no difference in enamel micro-chemical analysis and no change in calcium and phosphate concentrations[36-37]. However, different studies have reported mineral loss after bleaching treatment, with changes in calcium and phosphate concentrations[19,26,38]. This can be explained by methodological differences such as the bleaching gel used, the protocol followed, the remineralizing agent in its composition, presence of calcium, and pH; in addition to the byproducts that could affect the oxidation reaction and even the storage solutions. In the present study, the samples were stored in artificial saliva, which is considered a remineralizing agent. In addition, the bleaching gels contained fluoride or calcium, which would make enamel mineral loss less likely.

Cochrane *et al*.[39] claim that fluoride ions can promote net remineralization of the dental surface if calcium and phosphate ions are bioavailable. All the bleaching agents used in the present study contain fluoride. In addition, NiteWhite ACP contains calcium and phosphate, and calcium was added to the bleaching agent 10% CP + Ca. The best result for Ca/P ratio observed for 10% CP + Ca, when compared to Nite White ACP, can be explained by the greater amount of calcium (2000 ppm) available for enamel remineralization during the bleaching process. Although the ACP gel was also a source of bioavailable calcium and phosphate for the same purpose, results are better when amorphous calcium phosphate (ACP) is stabilized by casein phosphopeptide (CPP), known as CPP-ACP, which becomes a higher calcium and phosphate reservoir under these conditions[40].

The microhardness and μ-EDXRF analyses provided different results. We believe that these two tests evaluate different enamel structures. Microhardness indirectly assesses loss of structure, including inorganic and organic contents, due to the possible interactions between the matrix and the oxidation reaction from the bleaching agent and its byproducts, as described previously. The μ-EDXRF test evaluates the quantity of calcium and phosphorus according to the stoichiometric balance of elemental oxygen. Thus, our hypothesis is that the microhardness surface analysis and Energy Dispersive X-Ray Fluorescence Spectrometric evaluate the enamel structure in different ways. Although there is no positive correlation between the tests, they can be complementary, in search of better understanding of the chemical reaction at molecular level. Further studies are required to confirm this hypothesis and correlate new technologies such as μ-EDXRF, FT Raman Spectroscopy and Fourier Transformed Infrared Spectroscopy with current surface analysis to achieve a better evaluation of mineral loss after dental bleaching treatment.

Based on the methodologies employed in this study, we conclude that regardless of the bleaching agents used, enamel microhardness decreased after the treatments. However, there was no difference in the proportion of calcium and phosphorous on the surface. This suggests that the bleaching gels have erosive potential which softens the enamel without promoting surface loss, regardless the presence of calcium of ACP ions.

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Microbiological study of the subgingival biofilm in HIV+/HAART patients at a specialized dental service

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ABSTRACT

The aim of this study was to describe the microbiological profile of HIV patients under highly active antiretroviral treatment (HAART). This cross-sectional study comprised 32 HIV patients with periodontal disease (PD) who had been under HAART for more than 6 months. Information about the patients' medical history was obtained from clinical records. Clinical dental examination was performed by a calibrated researcher using standard dental instruments to determine probing depth (PD), clinical attachment level (CAL), and bleeding on probing (BOP). A total 4,765 periodontal sites were evaluated, 125 of which were also studied microbiologically. Subgingival biofilm samples were obtained using sterile paper points; one set was used for microbiological culture studies and the other for end-point PCR. Statistical analysis was performed using Kruskal-Wallis and post-hoc Dunn-Bonferroni contrast tests. All participants were on HAART at the time of the study, and 90.6% had a viral load below 50 copies / mm³. Prevalence of periodontally active sites was low in the study population. Microbiological studies: Black pigmented anaerobic bacteria and fusiform CFU counts were significantly higher in samples from sites with BOP and PD ≥4mm (p 0.020 and p 0.005, respectively). Molecular Assays: Detection of Porphyromonas gingivalis (p 0.002), Tannerella forsythia (p 0.023) and Treponema denticola (p 0.015) was significantly more frequent at sites with BOP and PD ≥4mm. Conclusions: The patients living with HIV/AIDS under HAART studied here had low prevalence of clinical periodontal disease signs. However, significant detection of P. gingivalis, T. denticola, and T. forsythia in periodontal active sites, and the involvement of these microorganisms as potential HIV reactivators, show the importance of creating awareness among dental health professionals of the need for close dental and periodontal monitoring in HIV patients.

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Keywords: *HIV/AIDS, periodontal diseases, periodontitis, molecular biology, biofilm, risk factors.*

Estudio microbiológico del biofilm subgingival de pacientes VIH+ bajo TARGA en un servicio dental especializado

RESUMEN

El objetivo de este estudio fue describir el perfil microbiológico del biofilm subgingival de los pacientes con VIH bajo tratamiento antirretroviral de alta actividad (TARGA). El estudio comprendió a 32 pacientes VIH seropositivos con enfermedad periodontal (EP) que se encontraran en tratamiento con TARGA por más de 6 meses. Los antecedentes médicos de los pacientes se obtuvieron de las historias clínicas. El examen clínico instrumental (profundidad de sondaje (PS), nivel de inserción clínico (NIC) y sangrado al sondaje (SS)) fue realizado con instrumental odontológico estándar por un investigador calibrado. De este modo, se evaluaron un total de 4.765 sitios periodontales de los cuales 125 fueron estudiados microbiológicamente. Las muestras de biopelícula subgingival se obtuvieron empleando conos de papel estéril. Las muestras se emplearon en estudios microbiológicos y moleculares por PCR de punto final. El análisis estadístico se realizó según Kruskal-Wallis y pruebas de contrastes post-hoc de Dunn-Bonferroni. El 90,6% de la población en estudio presentó carga viral inferior a 50 copias/mm³. La prevalencia de sitios periodontales activos fue baja (1%). Los recuentos de bacterias anaerobias estrictas pigmentadas de negro y fusiformes fueron significativamente más altos en muestras de sitios periodontales con SS positivo y PS ≥4 mm (p 0.020 y p 0.005). La detección molecular de Porphyromonas gingivalis (p 0.002), Tannerella forsythia (p 0.023) y Treponema denticola (p 0.015) fue significativamente mayor en los sitios con SS y PS ≥4mm.

La prevalencia del 1% de enfermedad periodontal en el grupo de pacientes estudiados fue menor a la esperada, sin embargo; la detección significativa de P. gingivalis, T. denticola y T. forsythia en sitios periodontales activos y su potencial participación como agentes reactivadores del VIH, nos alerta de la importancia de crear conciencia en los profesionales de la salud (médicos y odontólogos) acerca de la necesidad de un monitoreo minucioso del estado periodontal de pacientes con características semejantes a las descriptas en la muestra poblacional estudiada.

Palabras clave: *VIH/SIDA, enfermedad periodontal, periodontitis, biología molecular, biopelícula, factores de riesgo.*

INTRODUCTION

Acquired Immunodeficiency Syndrome (AIDS) is characterized by severe immunologic alterations mainly affecting $CD4^+$ T cells, a subpopulation of T lymphocytes, and by subsequent development of potentially fatal opportunistic infections.

According to the World Health Organization, approximately 34 million people are infected with human immunodeficiency virus (HIV), and although there has been much progress in HIV research in recent years, including therapies such as highly active antiretroviral therapy (HAART), this disease still constitutes one of the most significant public health problems in the world[1]. The National Ministry of Health estimates that in Argentina there are 120,000 people living with human immunodeficiency virus, 30% of whom are unaware of their HIV status[2].

Since the introduction of HAART (highly active antiretroviral therapy), the epidemiological profiles of the link between infectious disease and HIV have changed[3,4]. The factors involved in these changes include partial restoration of the immune system, control of viral load and stability of the immune system over time[5]. HIV infection has turned into a chronic disease. Global mortality rates decreased from 97.4% in 1993 to 19.8% in 2001[6]. The current guidelines for antiretroviral therapy recommend initiating treatment earlier[7].

However, the disease has not yet been completely eradicated, probably due to the ability of the virus to remain latent in host immune cells (T lymphocytes, macrophages and dendritic cells), as well as to its high mutation rate[8].

Periodontitis, as a multifactorial ecological disease whose primary aetiology is bacterial biofilm, causes a chronic inflammatory response by the release of bacterial and host cell products[9]. Bacteria from subgingival periodontopathic biofilm have previously been shown to be a significant risk factor for many systemic diseases (heart disease, diabetes and low birth-weight, among others)[10].

Epidemiological studies that used continuous measures of probing depth and clinical attachment loss have shown that advanced forms of periodontitis that resulting in severe loss of supporting structures and substantial tooth loss affect 10-15% of the population globally[11]. This estimated prevalence range includes severe periodontitis, which primarily affects adolescents, young adults and adults, and whose prevalence increases with age in all populations[12-14].

Longitudinal studies have shown that prolonged use of HAART in HIV-infected periodontal patients was associated with a decrease in the prevalence of putative periodontal pathogens as compared to seronegative patients. However, seropositive patients had species not usually associated with periodontitis, regardless of their periodontal status[15]. Establishing comparisons among studies conducted in the pre- and post HAART eras is complex given the need for appropriate and consistent characterization of HIV-associated periodontal disease to avoid underestimating the prevalence and severity of the infection[16]. The impact of HAART on periodontal disease in HIV patients in developing countries such as Argentina remains unclear, and reports on periodontal microbiota in this population are not conclusive.

Recent studies suggest that bacteria found in the oral cavity, including *P. gingivalis*, are able to reactivate the latent HIV virus within infected cells. In 2009, Huang et al. evaluated the capacity of bacteria found in the oral cavity to stimulate HIV promoter activation[17].

Research has been conducted in which cellular T-lymphocyte, macrophage and fibroblast lines transfected with the HIV long terminal repeat promoter were stimulated with oral bacterial sonicates, and both the Gram-negative and Gram-positive species were able to elicit positive stimulatory activity[18]. Another assay suggests that *P. gingivalis* produces a fatty acid called butyric acid, which may induce reactivation of the latent HIV-1 virus[9]. González et al. looked specifically at the ability of supernatants (cytokines/chemokines) produced by human gingival fibroblasts and oral epithelial cells to modulate HIV promoter activation in macrophages when challenged with periodontal pathogens. Stimulation with *P. gingivalis* and extract of *F. nucleatum* showed a significant increase in cytokines/chemokines, especially Interleukin-6 and Interleukin-8, which have the ability to modulate the activation of HIV-1 promoters[18].

The superposition of events such as these, with reactivation of the HIV virus due to the coexistence of active periodontal disease, increase the systemic inflammatory state of the host. This worsens the effector response and subsequent clinical outcome[19]. The aim of this study was to describe the microbiological profile of periodontal pathogens in a population of local patients living with HIV/AIDS on highly active antiretroviral therapy.

MATERIALS AND METHODS

The population study comprised male and female HIV-infected patients treated with HAART who received care the High-Risk Patients Dental Care Unit (CLAPAR I), School of Dentistry, University of Buenos Aires, Argentina. The patients were informed of the study objectives, risks and benefits. All participants agreed to participate voluntarily and signed an informed consent form approved by the Ethics Committee of the School of Dentistry, University of Buenos Aires. This study fulfils the requirements of STROBE rules for human observational studies.

This descriptive study focused on people between 21 and 65 years old living with HIV / AIDS who had been under treatment with HAART for at least six months prior to the dental examination. A non-probabilistic sequential sample of 50 subjects seeking care at the CLAPAR I was evaluated. Information on the patients' medical history was obtained from clinical records (antiretroviral treatment history, viral load and $CD4^+$ levels). Viral load was detected by real-time quantitative polymerase chain reaction (qPCR) to quantify plasma HIV-1 RNA, using ABBOTT REAL TIME HIV-1 RNA VERSION 3.0 with a sensitivity of 40 HIV-1RNA copies/ml (a viral load below 50 copies / ml was considered undetectable). The missing data from the clinical histories were traced in each of the hospital's services.

Dental clinical examination was performed by a calibrated researcher (intra-examiner kappa >0.80) using standard dental instruments (Marquis periodontal probe) to determine probing depth (PD), clinical attachment level (CAL) and bleeding on probing (BOP). Clinical parameters were evaluated at 4,765 periodontal sites, 8 sites per tooth, in order to provide more accuracy for the description of periodontal status.

A total 50 persons were given appointments for screening for eligibility. Exclusion criteria were: less than 6 teeth (n=3), antibiotic or antifungal treatment within the past 6 months (n=7), incomplete clinical histories, $CD4^+$ lymphocyte level count lower than 250 cells / ml and absence of viral load data (n = 5), concomitant systemic or infectious diseases (n = 3). As a result of the selection, 32 patients were included in the study protocol. All evaluated patients received their dental treatment and follow-up in CLAPAR 1 (Figs.1 and 2).

Based on the most severe gingival inflammatory signs, one site per quadrant was selected to be studied microbiologically (n=128 sites). Samples of subgingival biofilm were collected by absorption using 4 sterile Nº35 paper points. They were placed one at a time into the depth of the pocket and removed after 20 seconds. The 4 paper points from each site were placed in 1ml of refrigerated transport fluid (RTF)[20]. Smears were prepared with

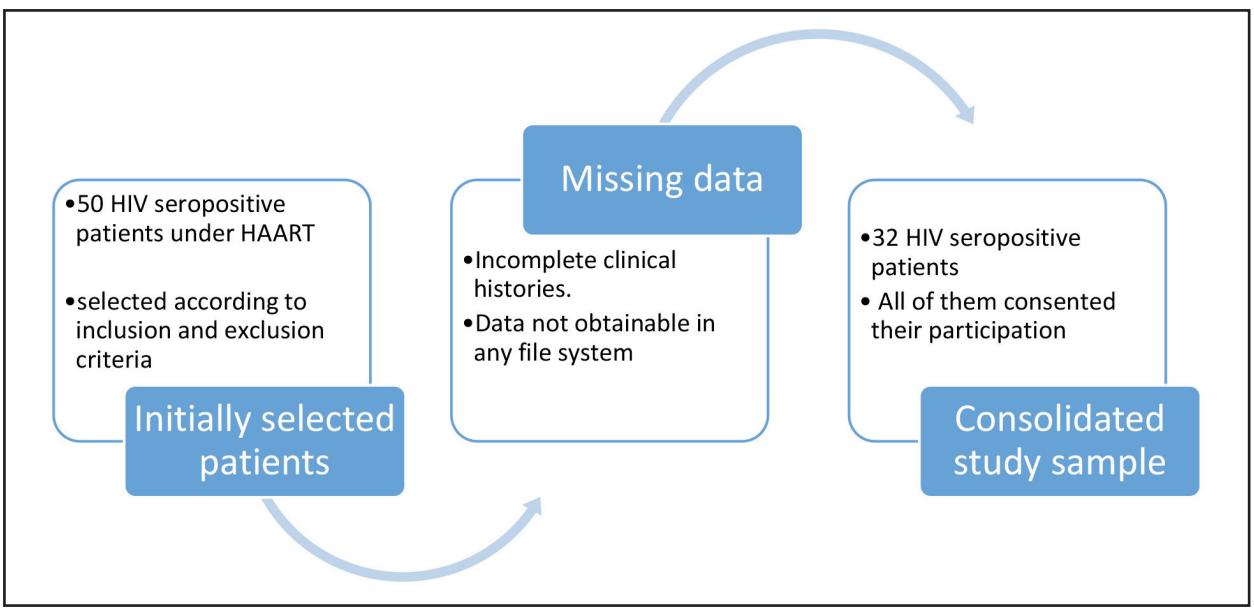

Fig. 1: Flowchart. Number of individuals at each stage of study.

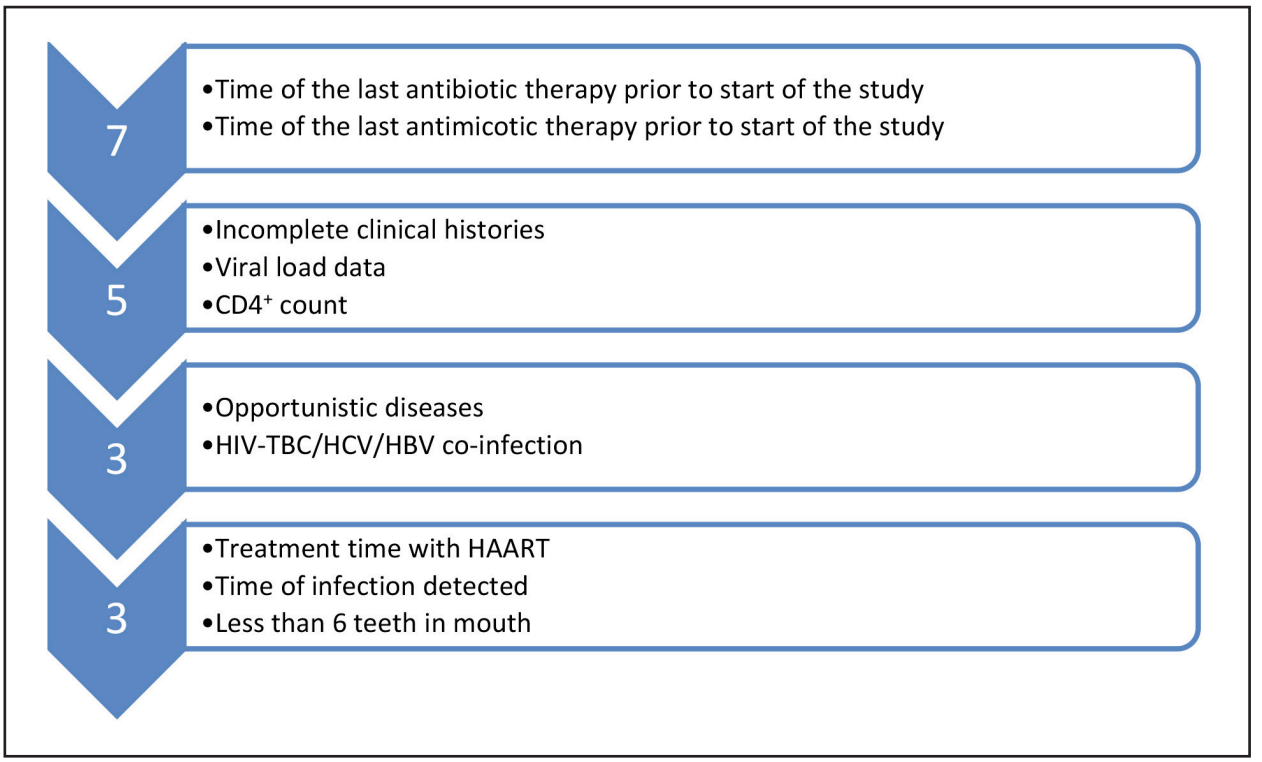

Fig. 2: Flowchart. Number of participants with missing data for each variable of interest.

the material obtained from the soft wall of each periodontal site.

For the microbiological assay, the periodontal pockets studied were assigned to four groups (Groups I to IV) according to their periodontal parameters such as bleeding on probing (BOP) and probing depth (PD). Group I: BOP/PD ≥ 4mm n=21 (16.4 %); Group II: BOP/PD < 4mm n=30 (23.4%); Group III: NO BOP/PD ≥ 4mm n=12 (9.4%); Group IV: NO BOP/PD <4mm n=62 (49.6%). Details are provided in Table 3.

Microbiological Processing

To isolate strict anaerobic microorganisms, 100 µl of the homogenized diluted (10^{-2}, 10^{-3}) samples were cultured in Anaerobic Blood Agar (ABA-BHI Agar) supplemented with 5% lacked sheep blood (Laboratorio Gutiérrez, Argentina), 5 µg/ml of hemin (Sigma-Aldrich®, Argentina),and 1 µg/ml of menadione (Sigma-Aldrich®, Saint Louis, MO, USA). The plates were incubated under strict anaerobic conditions, and analysed at 7 and 15 days (GasPakTM, Mitsubishi, Medica-tec®). For detection of *Aggregatibacter actinomycetem comitans* (Aa), enteric bacteria, non-fermenting gram negative bacilli (NFGNB), *Staphylococcus aureus* (Sa), coagulase-negative staphylococci (CoNS), and *Candida* spp, 100 µl of the samples were cultured directly on selective and differential agar medium as follows: AASM[21] for *Aa,* Levine (Oxoid®, UK) and CLDE (Oxoid®, UK) for enteric bacteria, bile esculin agar (Britania Laboratory, Argentina) supplemented with 0.04% potassium tellurite for enterococci, Chapman's mannitol salt agar (Oxoid®, UK) for Sa, and CHROMagar® Candida for Candida spp. The plates were incubated under aerobic or capnophilic conditions according to bacterial atmospheric requirements, for 24h, 48h and 5 days. In all cases, the species were biotyped using conventional methods (Rosco® Diatabs) and a commercially available kit (Remel® enteric bacteria).

Molecular processing

For molecular processing, 200ul of each sample were stored at -20ºC, and processed using a commercially available kit (Bioneer® Genbiotech) for extraction of genomic DNA. Seven "in house" end point polymerase chain reactions (PCR) were run for each sample (n=896), using specific primers

(Ashimoto et al. 1996) for detecting periodontopathic bacteria of the red complex (RCB) i.e. *Porphyromonas gingivalis* (*Pg*), *Treponema denticola* (*Td*), and *Tannerella forsythia* (*Tf*), and of the orange complex (OCB), i.e. *Fusobacterium nucleatum* (*Fn*), *Prevotella intermedia* (*Pi*) and *Aggregatibacter actinomycetem comitans* (*Aa*).

The amplification products were separated by electrophoresis in agarose gels (2%) in TAE buffer[22], stained with Gold View (Montebio), and analysed using the Gel DocTM XR+ Imaging System (Bio-rad). Negative and positive controls were used in all cases (*Porphyromonas gingivalis* ATCC 33277, *Prevotella intermedia* ATCC 25611, *Fusobacterium nucleatum* ATCC 10953, *Aggregatibacter actinomycetamcomitans* ATCC 29522, *Tannerella forsythia* ATCC 43037), and DNA of wild-type strains, which were characterized, biotyped, and stored in the Collection of Microbial Cultures (Laboratory for Microbiological Diagnosis of the School of Dentistry, University of Buenos Aires, Argentina). Ubiquitous (Ubi) primers were also used to detect genomic bacterial DNA.

Statistical Analysis

Quantitative variables are expressed as average, standard deviation, median, and percentiles. Comparison of quantitative variables and factors or groups was performed using Student's t-test for independent samples. In all cases, homoscedasticity was evaluated using Levene's Test, and data normality was analysed using Kolmogorov Smirnov and Shapiro Wilks tests, as well as graphic normality tests. The data that did not meet parametric test assumptions were analysed using Kruskal Wallis test and post hoc Dunn-Bonferroni contrast test. Categorical variables were analysed and compared using Chi-square test and Yates continuity corrections.

When distribution of data was binomial, percentages were compared using the proportions test (difference of percentages) and a normal approximation. Comparisons among more than two groups were performed applying a Bonferroni correction to the level of significance.

In all cases, the level of significance was set at 5% to rule out the null hypothesis. All statistical analyses were performed using statistical software SPSS (version 25), STATA 14, MS Excel 2016, Epidat 4.2.

RESULTS

The study comprised 32 patients (9 female and 23 male). All participants fulfilled the inclusion criteria, as shown in Flowcharts 1 and 2. Of the study patients, who were mostly Argentinians, 90.6% had less than 50 copies / mm^3 viral load; data shown in Tables 1 and 2. The proportion of active periodontal sites and their distribution according to the analysis groups are shown in Table 3.

Isolates of conventional microorganisms were headed by *Candida* spp. (32.8%; *C.albicans*: 15.2%; *C.dubliniensis*: 16%) closely followed by Gram-negative bacilli (18.4%; NFGNB: 16%; enteric bacteria: 2.4%), *Staphylococcus* spp. 17.6% (CoNS 12.8%; *S. aureus*: 4.8%), and *Enterococcus faecalis* (0.8%) in the last position. Recovery rates of microorganisms using conventional cultures, and comparison of recovery rates among groups are shown in Table 4.

The mean anaerobic bacterial count (CFU) was 1.45 x 10^6 CFU/ml, and there were significant differences in total counts of pigmented anaerobic and fusiform bacteria between Groups I and IV, as expected.

Table 1: Profile of HIV study population.

Population aspects		Frequency	Percentage
Sex	Female	9	28.1%
	Male	23	71.9%
Nationality	Argentine	30	93.7%
	Other	2	6.3%
Place of residence	Buenos Aires City	23	71.9%
	Buenos Aires Province	8	25.0%
	Other province in Argentina	1	3.1%

Conventional anaerobic culture and biotyping methods were unable to establish significant differences in the prevalence of *P.intermedia* and *P.gingivalis* in any of the study groups. Similarly, different *Fusobacterium* species were observed but could not be identified at the species/subspecies level in any of the cases except as *F.nucleatum.*

Microbiological results for opportunistic bacteria such as *S.aureus* showed statistically significant differences in Group I versus the others ($p<0.01$). Enteric bacteria were only isolated from Groups III and IV. No statistically significant difference was found for recovery rates for *E. faecalis*, NFGNB or total yeasts between the study groups.

Endpoint PCR results established statistically significant differences between groups according to periodontal clinical parameters (GI/GIV). *P.gingivalis, T.denticola* and *T.forsythia,* as perio-

Table 2: Relevant clinical data for study population health status.

Health status	Affected individuals (Total n=32)	Percentage
Initial CD4+ <350 cells/mm^3	12	37.5%
Initial CD4+ ≥350 cells/mm^3	20	62.5%
Initial VL< 50 copies/mm^3	29	90.6%

CD4+ (Lymphocytes cell cluster); VL (Virus load)

Table 3: Percentage of total study sites corresponding to each group.

Group	BOP	PD	Percentage of total sites corresponding to each group	
			Total sites included in periodontal study only (n=4765)	Total sites also studied microbiologically (n=125/4765)
I	Positive	≥ 4mm	3.7%	16.8%
II	Positive	< 4mm	3.3%	9.6%
III	Negative	≥ 4mm	14.1%	24.0%
IV	Negative	< 4mm	78.9%	49.6%

BOP: bleeding on probing; PD: probing depth.

Table 4: Microorganism recovery rates using conventional methods.

Microorganism	Group I %	Group II %	Group III %	Group IV %	*p.*
Total anaerobic bacteria	61.90	56.67	41.67	43.86	NS
Total pigmented bacteria	71.43*	53.33	25.00*	37.50	0.020
Prevotella spp	38.10	20.00	16.70	12.10	NS
P. intermedia	4.80	3.30	8.30	6.90	NS
Porphyromonas spp	19.00	13.30	0.00	5.20	NS
P. gingivalis	0.00	0.00	0.00	1.70	NS
Fusobacterium spp	71.40*	56.70	41.70	16.40*	0.001
F. nucleatum	9.50	16.70	25.00	10.90	NS
Enterobacteria	0.00	0.00	16.70*	1.60*	0.008
E. faecalis	0.00	3.30	0.00	0.00	NS
S. aureus	19.00*	0.00	0.00	3.20*	0.008
NFGNB	19.00	13.30	8.30	17.70	NS
Total yeasts	33.30	23.30	33.30	37.10	NS
C. albicans	31.60	11.50	8.30	15.00	NS
C. dubliniensis	5.30	11.50	16.70	23.30	NS

NS: No statistically significant difference
Kruskal Wallis test and post hoc Dunn-Bonferroni contrast test

Table 5: Percentage of microorganisms detected by PCR.

Microorganism	Group I %	Group II %	Group III %	Group IV %	*p.*
A. actinomycetemcomitans	4.80	6.70	0.00	1.60	NS
P. intermedia	0.00	3.30	0.00	4.80	NS
P. gingivalis	66.70*	30.00	41.70	21.00*	0.002
T. denticola	52.40*	40.00	33.30	19.40*	0.023
T. forsythia	66.70*	36.70	41.70	27.40*	0.015
F. nucleatum	52.40	63.30	50.00	33.90	NS

NS: No statistically significant difference
Kruskal Wallis test and post hoc Dunn-Bonferroni contrast test

dontopathic species, were the most prevalent (66.7% *Pg, Tf*; 52.4% *Td*) in the group of patients with active periodontal parameters (GI) (Table 5).

DISCUSSION

Studies reported in the literature have shown a similar composition of subgingival microbiota in seronegative and seropositive HIV patients[23,24,15]. More recently, in 2019, a study on the subgingival periodontopathic microbiome showed that although *P.gingivalis* is the marker species of the "red complex", *Treponema denticola* and *Tanerella forsythia* as well as *Filifactor alocis, Mitsuokella dentalis, Slackia exigua, Dialister pneumosintes, Selenomonas sputigena* and *Treponema lecithinolyticum* are among the ten best biomarkers for this pathology[25]. However, other reports[26,27] have found seropositive HIV patients to show a higher prevalence of putative periodontal pathogens such as *A. actinomycetemcomitans* spp., *P.intermedia* and *T.denticola*, as well as combinations of species. In addition, microbiological studies have reported isolation of species not usually associated with chronic periodontitis, including *Staphylococcus epidermidis, C.albicans, E.faecalis, Clostridium difficile, Clostridium clostridiforme, Klebsiella pneumoniae* and *Mycoplasma salivarius*, in subgingival biofilm samples from HIV-infected patients[28-31]. Aas *et al*. studied the subgingival biofilm of seropositive patients with different immune status[32]. The authors found microorganisms such as *Saccharomyces cerevisiae* in subjects with higher viral load and low lymphocyte counts, lending support to the opportunistic nature of periodontal infections in these patients. Gonçalves et al. found higher prevalence of *Enterococcus faecalis* and *Acinetobacter baumanii* in subgingival biofilm of HIV^+ patients on HAART with $CD4^+$ T-lymphocyte counts < 200 cells/mm^3 and viral loads in the range of 105 copies/ml compared to seronegative patients, regardless of their periodontal status[15]. Conversely, recovery of opportunistic microorganisms was not significant in our study population, in which 90.6% of the participants showed undetectable viral loads, and 62.5% had $CD4^+$ T lymphocyte counts above 350 cells / mm^3. These discrepancies may be due to differences in the immune status of the study populations.

Gonçalves *et al*. observed a lower incidence of periodontal lesions in patients under HAART[33]. Despite the lower frequency of clinical determinants of periodontal disease in our study population, more than 50% of the study sites in Group 1 were positive for periodontopathic bacteria belonging to Socransky's red complex, which were detected using endpoint PCR[34]. The primers designed by Ashimoto enable detection of *A. actinomycetemcomitans, P. gingivalis, P. intermedia, T. denticola* and *T. forsythia* DNA in subgingival biofilm samples with high sensitivity and specificity (more than 99.9%). These primers have been used successfully by different authors[35] and therefore we consider them reliable for use in our study.

Brenchley *et al.* and Collman *et al*. posited that infections by Gram-negative bacteria and bacterial translocation associated with the mucosa in HIV-1-infected patients might directly or indirectly contribute to virus reactivation via pro-inflammatory cytokines released from stimulated cells[36,37]. Periodontal disease, seen as a chronic polymicrobial, ecological and immune-inflammatory

disease, could play a role in viral reactivation in HIV seropositive patients[38].

Because HIV infection affects the host's immune response[39], gaining better understanding of the role of gingival-periodontal disease in immune activation in HIV seropositive patients would seem to be of the utmost importance for the development of therapeutic strategies aimed at controlling virus reactivation, patient immune status, and progression of AIDS.

Patients living with HIV / AIDS on HAART that were studied together in the High-Risk Patients Dental Care Unit (CLAPAR I) and the Microbiological Diagnostic Laboratory at School of Dentistry, University of Buenos Aires, Argentina showed low prevalence (1%) of periodontal lesions. Yeast and non-fermenting Gram-negative bacilli were distributed homogeneously, regardless of the depth of the periodontal pocket and in quantities that resemble a colonizing function.

Despite the low prevalence of periodontal disease and the moderate level of activity recorded in this study, *P. gingivalis, T. denticola* and *T fosythia* are within the framework of the microbiological profile of the subgingival biofilm of the eligible HIV-seropositive population. They were detected more frequently in periodontal active pockets (GI). As known, these pathogens and their products can contribute to systemic inflammation, so periodontal treatment could contribute to overcoming, and there by improving the general condition of seropositive HIV patients.

This is the first local study to record the prevalence of periodontopathic microorganisms in periodontal pockets of HIV patients. The results justify further in-depth longitudinal studies of the subgingival microbiome of local patients living with HIV / AIDS who are on HAART, which might clarify many of the interactions of these and other still unknown periodontal bacteria, describe new pathogenicity factors and contribute to understanding the particular dynamics of these subgingival microbiomes and HIV-1 virus homeostasis.

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Self-reported dentin hypersensitivity in south brazilian adolescents: occurrence and risk indicators

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ABSTRACT

The aim of this study was to assess the occurrence of self-reported dentin hypersensitivity (DH) and its risk indicators in adolescents from a southern Brazilian city. 736 students (15-19 years old) were randomly selected from 20 public and private schools in the city of Passo Fundo, Brazil. DH was assessed through the answers on a Likert scale to the question: "Do you have tooth sensitivity?".Participants underwent a clinical examination in which present teeth were counted, and answered an interview based on a structured questionnaire on sociodemographic information, history of dental bleaching, orthodontic treatment and oral health habits. The dependent variable (DH) was dichotomized at the point where hypersensitivity occurs fairly often or always. Data were analyzed by multivariable logistic regression, including demographic, health history, socioeconomic and behavioral variables. Results: 556 (75.5%) subjects reported having sensitive teeth never, rarely or sometimes, while 180 (24.5%) reported having sensitive teeth fairly often or always. In the final model, number of present teeth, age, skin color, flossing, smoking, bleaching and orthodontic history were not associated with self-reported DH. Females showed significantly greater chance of having DH than males [odds ratio (OR)=1.91; 95% confidence interval (95%CI) 1.34-2.72]. The likelihood of DH in students at public schools was 63% higher than in those at private schools (OR=1.63 95%, CI 1.01-2.75). DH is a common perception among adolescents and is associated with female gender and studying at public schools.

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Keywords: *dentin sensitivity, risk factors, adolescent, hypersensitivity.*

Hipersensibilidade dentinária autorreportada em adolescentes do sul do Brasil: ocorrência e indicadores de risco

RESUMO

O objetivo deste estudo foi determinar a ocorrência de hipersensibilidade dentinária (HD) autorreportada e seus indicadores de risco de uma cidade do sul do Brasil. 736 estudantes (15-19 anos) foram selecionados aleatoriamente em 20 escolas públicas e privadas da cidade de Passo Fundo, Brasil. HD foi aferida pela resposta em escala Likert à pergunta: "Você tem sensibilidade nos dentes?". O exame clínico incluía a contagem de dentes presentes e um questionário estruturado foi aplicado incluindo dados sociodemográficos, história de clareamento dental, tratamento ortodôntico e hábitos de saúde bucal.A variável de pendentes (HD) foi dicotomizada no ponto que a hipersensibilidade ocorria repetidamente ou sempre. Os dados foram analisados por meio de regressão logística multivariada, incluindo variáveis demográficas, histórico de saúde, dados socioeconômicos e comportamentais. Nos resultados 556 (75.5%) indivíduos reportaram experiência de sensibilidade dentinária, nunca raramente ou às vezes, enquanto 180 (24.5%) reportaram ter sensibilidade repetidamente ou sempre. No modelo final, número de dentes presentes, idade, raça, uso do fio dental, tabagismo, histórico de clareamento dental e tratamento ortodôntico não foram associados com HD autorreportada. Mulheres mostraram uma chance significativamente maior de ter HD comparado aos homens [odds ratio (OR)=1,90; 95% intervalo de confiança (95%CI) 1.33-2.71]. Frequentar escolas públicas aumentou a chance de HD em 63% comparado com escolas particulares (OR=1,63 95%IC 1.01-2.75). HD é uma percepção comum entre adolescentes e está associada com gênero feminino e estudar em escolas públicas.

Palavras chave: *sensibilidade dentinária, Brasil, fatores de risco, adolescente, hipersensibilidade.*

INTRODUCTION

Dentin hypersensitivity (DH) is an important clinical problem. It can be defined as an acute pain of short duration resulting from the exposure of dentin to thermal, evaporative, tactile, osmotic or chemical stimulation. It cannot be attributed to any other oral disease. DH is a common problem in many adult populations. Estimates of its occurrence are highly variable and range from less than 10% to more than 50% of the population[1-5]. Such disparity

in the occurrence rate of DH may be related to the target populations, the methods used to measure it, and lack of representativeness in most studies.

The condition tends to be most evident in patients aged 20-40 years, and occurs about equally in males and females. It may affect any tooth, though the most common (50%) are canines and first premolars. It occurs most frequently (95%) on buccal surfaces at the cervical margin[4]. The most widely accepted explanation of its cause is the hydrodynamic theory, which states that after an external stimulation on the exposed root surface, the fluid inside dentinal tubules moves, stimulating dental pulp neural fibers and causing pain[1].

It is well known that all kinds of pain ultimately affect quality of life, and DH is no exception. DH is considered chronic pain, since it is present practically all the time. People who suffer from DH are known to have worse oral health-related quality of life (OHRQoL) [5-7], which directly influences overall health, especially in adolescents. Representative studies assessing the occurrence of DH are not available from most countries, though there are studies on adults from China, India and, more recently, Brazil[8]. Costa et[8] reported the occurrence of DH in a representative sample of adults and elderly people in Brazil, with prevalence higher than 30%. DH was associated with gender, age, smoking, gingival recession and periodontal therapy.

However, no representative studies have reported the occurrence of DH in adolescents. It is important to emphasize that during adolescence, some of the reported associated factors might not have acted for long enough, with other factors potentially being responsible in the etiological chain. It is essential to know the prevalence of DH and its associated factors in order to establish strategies for its adequate prevention and control. The aim of this study is to determine the occurrence of self-reported dentin hypersensitivity and its risk indicators in adolescents from southern Brazil.

MATERIALS AND METHODS

Study design and target population

The present study is a cross-sectional observational survey, carried out in the city of Passo Fundo, located in the south of Brazil. The target population for the study comprised adolescents aged 16-19 years, enrolled at public or private high schools in the city in 2012. Passo Fundo has a population of about 190,000, and is considered to be a medium-sized city[9]. In 2012, there were a total 23 high schools, including 7 private and 16 public, and a total 7,558 students enrolled, of whom 6,256 (82.78%) attended public schools and 1,302 (17.22%) attended private schools.

Ethical Aspects

The study was approved by The Institutional Review Board of the University of Passo Fundo (protocol 066/2012), and all selected students provided informed consent signed by parents or guardian.

Sampling strategy

The study coordinator visited and invited all high schools to participate in the survey, of which 20 (87%) agreed to participate. The student population was 6,122. It was arbitrarily determined that 30% of the students from each school would comprise the study sample. These students were randomly selected by draw from the lists of students enrolled at each school.

After the selection, the research team visited the classrooms to explain the study and its objectives. In case of absence of the selected individual, an extra contact was made.

Interview and clinical examinations

Students were interviewed and examined by a trained team. A structured questionnaire, including demographic information, socioeconomic data, history of dental bleaching, orthodontic treatment, and oral hygiene habits was applied to all respondents. DH was assessed through the answers to the question: *"Do you have tooth sensitivity?"* indicated by selecting an option on a Likert scale (never, rarely, sometimes, fairly often and always). These answer options were constantly visually available on a card.

Clinical examination to determine the number of present teeth (excluding third molars) was performed after the interview with the aid of a wooden spatula, under standard room illumination. Teeth that could be rehabilitated in some way were considered present in the count. Teeth or roots suitable for extraction were considered absent.

Training was conducted among team members and with non-selected students, using the field conditions.

Reproducibility of the clinical examination was conducted in 10% of the examined individuals, randomly selected, revealing an agreement rate of 98%, which implies adequate reproducibility of the method for counting present teeth.

Statistical analysis

Occurrence of DH, the primary outcome of this study, was analyzed as a dichotomous variable. Respondents were categorized as having DH if they answered farly often or always to the question applied in the questionnaire.

Independent variables included age, gender, skin color, use of dental floss, private/public school, smoking, past and current orthodontic treatment, tooth bleaching and number of present teeth. Age was analyzed using three categories (15, 16, and ≥17 years of age). Skin color was dichotomized into white or non-white (black, yellow, brown or indigenous). Self-reported use of dental floss was analyzed as a dichotomous variable (yes/no). Smoking habit was defined using two groups: adolescents without any history of smoking and adolescents who were either current or former smokers at the time of the interview. Orthodontic treatment was dichotomized into never and past/current treatment. Respondents were divided into those that reported having undergone any type of tooth bleaching (toothpaste, dental office or home-bleaching) and those that had never been exposed. They were classified according to whether they attended private or public schools, as a proxy for socioeconomic status. Number of present teeth was dichotomized into 28 and less than 28 teeth.

Univariable associations between occurrence of DH and other variables were assessed by either the chi-square test or Fisher's exact test when appropriate. Binary logistic regression models were fitted to assess risk indicators for DH. Models were built using a purposeful selection of variables[10]. Univariable regression models were fitted and all variables with p-values <0.25 were included in a multivariable model. Variables that did not significantly contribute to the multivariable model (p≥0.05) were assessed for confounding before being eliminated. Confounding was defined as a >20% change on other variable coefficients. Odds ratios (OR) and 95% confidence intervals (95%CI) were reported.

The significance level was set at 5%. Data analyses were performed using statistical package Stata 10 for Macintosh (STATA, College Station, TX, USA).

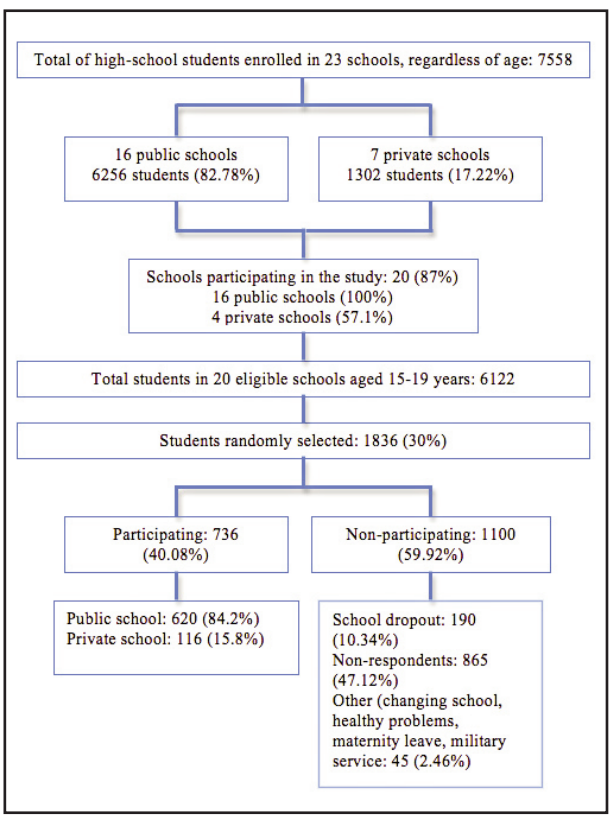

Fig. 1: Flowchart of the study.

RESULTS

A total 1,836 students were randomly selected and invited to participate in the study, of whom 736 accepted, yielding a response rate of 40.1%. Of these, 323 (43.9%) were male and 413 (56.1%) were female. Non-participation in the study was due to either not signing the consent form, school dropout of selected individuals or non-acceptance. Of the 736 participants, 620 (84.2%) were from public schools and 116 (15.8%) from private schools (Fig. 1).

Table 1 presents the characteristics of the study sample. Data parallel the distribution of demographics in the schools and in the city, including the 84.2% of adolescents enrolled in public schools. Most of the students had never smoked, had not undergone or were not undergoing orthodontic treatment and had not undergone tooth bleaching procedures. Approximately half the students reported using dental floss and the majority (approximately 80%) had 28 teeth.

Table 2 presents the occurrence of DH and its association with independent variables. Overall, 556 (75.5%) subjects reported sensitive teeth never, rarely or sometimes, while 180 (24.5%) reported having sensitive teeth often or always. Of all risk indicators assessed, gender was significantly associated to greater dentin hypersensitivity occurrence in females. Students from public schools tended to present higher occurrence of DH.

Table 3 shows the univariable and multivariable logistic regression models. In the univariable models, gender was significantly associated with the occurrence of DH. In the final multivariable model, gender and type of school had statistically significant association. Females showed a significantly greater chance of having sensitivity than males (OR=1.90 95%CI 1.33-2.71), and attending public schools increased the chance of sensitive teeth by 63% compared to students from private schools (OR=1.63 95%CI 1.01-2.75).

Table 1: Characteristics of the study sample.

	Total	
	N	%*
Age		
15 years	236	32.1
16 years	251	34.1
≥17 years	249	33.8
Gender		
Male	323	43.9
Female	413	56.1
Ethnicity		
White	511	69.4
Non-white	225	30.6
Use of dental floss		
Yes	390	53.0
No	346	47.0
Type of school		
Public	620	84.2
Private	116	15.8
Smoking		
Current smoker	17	2.31
Former smoker	23	3.53
Never smoked	693	94.2
Past orthodontic treatment		
Yes	405	55.0
No	331	44.9
Current orthodontic treatment		
Yes	241	32.7
No	495	67.3
Tooth bleaching		
Never	433	58.8
Whitening toothpaste	179	24.3
In-office tooth bleaching	107	14.5
Home-performed tooth bleaching	17	2.3
Number of teeth		
28	581	78.9
< 28	155	21.1
Total	736	100

Table 2: Occurrence of DH according to independent variables.

	Without sensitivity		With sensitivity		
	n	%	N	%	p
Age					
15 years	180	76.3	56	23.7	
16 years	188	74.9	63	25.1	
≥17 years	188	75.5	61	24.5	0.94*
Gender					
Male	265	82.0	58	17.9	
Female	291	70.5	122	29.5	<0.001**
Ethnicity					
White	391	76.5	120	23.5	
Non-white	165	73.3	60	26.7	0.35**
Use of dental floss					
Yes	293	75.1	97	24.9	
No	263	76.0	83	23.9	0.79**
Type of school					
Public	460	74.2	160	25.8	
Private	96	82.8	20	17.2	0.05**
Smoking					
Never smoked	528	76.2	165	15	
Current or former smoker	28	65.1	23.81	34.9	0.10**
Orthodontic treatment					
Never	227	72.8	85	27.2	
Past or current	329	77.6	95	22.4	0.14**
Tooth bleaching					
Never	332	76.7	101	23.3	
Yes	224	73.9	79	26.1	0.43**
Number of teeth					
28	445	76.6	136	23.4	
< 28	111	71.6	44	28.4	0.20**
Total	556	75.5	180	24.5	

*Chi-square test
** Fisher exact test

Table 3: Univariable and multivariable logistic regression models of the association between DH and risk indicators.

	Univariable			Multivariable		
Age						
15 years	1					
16 years	1.07	0.71-1.63	0.72			
≥17 years	1.04	0.69-1.58	0.84			
Gender						
Male	1			1		
Female	1.91	1.34-2.72	<0.001	1.90	1.33-2.71	<0.001
Ethnicity						
White	1					
Non-white	1.18	0.82-1.69	0.35			
Use of dental floss						
Yes	1					
No	0.95	0.68-1.33	0.78			
Type of school						
Public	1			1		
Private	1.67	0.99-2.79	0.05*	1.63	1.01-2.75	0.04
Smoking						
Never smoker	1					
Current/former smoker	1.71	0.89-3.28	0.10			
Orthodontic treatment						
Never	1					
Past or current	0.77	0.54-1.08	0.13			
Tooth bleaching						
Never	1					
Yes	1.15	0.82-1.62	0.39			
Number of teeth						
28	1					
< 28	1.29	0.87-1.93	0.20			

Number of present teeth, age, skin color, flossing, smoking, bleaching and orthodontic history were not associated with self-reported sensitivity.

DISCUSSION

The aim of present study was to assess the occurrence and risk indicators of DH in Brazilian adolescents. Epidemiological studies that assess prevalence of DH are extremely heterogeneous, so the methodology used in each of them needs to be taken into account in order to understand the estimates. The uniqueness of the present study is that it assesses the condition in adolescents. To the best of our knowledge, there are few studies on prevalence of DH in adolescents. The only study conducted with adolescents found a prevalence of 19%, but it was conducted in Piaui, where the climate and socioeconomic situation differ from those in Rio Grande do Sul[11].

The study was performed in a medium-sized city in southern Brazil. All the schools in the city were invited to participate, and 86% of them agreed to do so. In these schools, 30% of the enrolled students were randomly selected to be invited to participate in the survey, of whom 40% accepted. The response rate is similar to that found in other epidemiological studies in the literature[12]. Non-participants did not differ from participants in terms of demographic and socioeconomic characteristics and the reasons for non-participation were related to school drop-out or parents/guardians not signing the informed consent. These reasons are relatively common in studies with adolescents[13]. Considering that the percentages of participants in terms of gender and type of school resemble the percentages of students in the city, some degree of representativeness can be claimed in the present study.

DH was assessed by means of self-report. There is ongoing profound discussion about the strengths and limitations of using self-reported pain in different studies. There is no doubt that self-reported pain is a true outcome variable[1] that has to be understood differently from surrogates. Even though there is some degree of subjectivity involved in assessing self-reported pain, there is no uncertainty that it is considered impacting by the study volunteer. Studies with DH have been performed worldwide and used different measurements. Air stimulation, probing and self-reported DH are the most commonly used outcomes in published studies[14,16]. Falta la referencia 15. Studies that use self-reported DH and another type of stimulation in the assessment of DH suggest that self-reported DH tends to overestimate the occurrence of the disease[17, 18].

The present study shows occurrence of DH in 25% of high school adolescents, i.e., 1 out of 4, 15 to 19-year-olds. The answers “fairly often” or “always” on the Likert scale were considered to indicate presence of DH. This cutoff point infers more impact and has been widely used in the literature[10]. In terms of prevalence of different diseases/conditions, 25% is considered a relatively high occurrence[3, 20].Falta referencia 19 In the same state in Brazil, Costa et al. (2014)[8] recently analyzed the prevalence of DH in adults and the elderly by means

of an air blast and probing, reporting estimates of 33% and 34%, respectively. Considering that the region of the country is the same and that gingival recession increases with age[21,7], the figures reported in the present study are probably not overestimated. It should also be noted that there is a cycle in DH, related to biological factors. Even though gingival recession increases with age, dentin also forms over the years. A study by Costa et al. found that the elderly tend to present lower estimates of DH than do younger adults[8].This should be taken into account when analyzing data from adolescents. Although gingival recession in adolescents may not be as extensive as in adults, nevertheless the pulp chamber is larger, and therefore closer to external stimulation[22].

In addition to determining prevalence, it is important to understand the associated factors that can be considered risk indicators for DH. The present study conducted univariable and multivariable analyses to assess which factor(s) may be associated to self-reported DH in adolescents. Age, ethnicity, oral hygiene habits, smoking, orthodontic and bleaching experiences were not associated to DH in the present study. Although these variables have been shown to be risk indicators for DH in different studies[20, 22-25], it should be highlighted that the age range in this survey is 15-19 years, a relatively short period of the lifespan. In addition, the low occurrence of some of the possible risk indicators –smoking, dental treatment and tooth loss – might have contributed to the lack of association. These variables were not associated with DH in this age range. It should be kept in mind that the information in the literature from other age ranges should not be dismissed, and that these adolescents will grow older, exposed to these risk indicators, which could lead to an increase in DH.

The results of the present study showed that female gender and studying in public schools are associated with DH. Other studies with different populations have also reported higher rates in women. There are various possible explanations for this. All kinds of self-perceptions have greater impact in women than in men[20, 26]. In addition, women present both higher quality of oral hygiene and more gingival recession. This leads to higher chances of DH among females. A study by Costa et al. sh owed an odds ratio of more than 2 for female:male adults for DH[8]. In the present study, females had a 90% greater chance of reporting DH than males. This will probably increase over time. Especially in adolescence, there is an opportunity for informing females of these chances in order to limit the consequences of DH.

The type of school that the adolescent attended was also associated with higher chances of DH. Studying in a public school increases the chances of reporting DH by 67%. Although this was a borderline significance, it should not be disregarded as an opportunity for implementing preventive strategies. In the context of this study, the type of school and ethnicity were used as a proxy of socioeconomic status[27]. Skin color was not associated with DH. One of the reasons for this is the low percentage of non-whites in the study. However, students from public schools tended to present higher degrees of self-reported DH. Other studies that assessed gingival recession found higher occurrence in subjects from lower socioeconomic levels. This should be taken into account to focus on educational strategies at public schools, where students could benefit.

The strengths and limitations of this study should be highlighted. In terms of strengths, studying the epidemiology of DH in adolescents is important in view of the lack of literature on the subject and the opportunity it provides for creating preventive strategies. Moreover, the representativeness of the study increases its external validity, providing information on disease occurrence, which is limited in non-representative studies. One of the possible limitations of the study is the lack of a second DH measurement, which could increase the internal validity of the study –probably confirming the self-reported outcomes–, and might enable the extension of DH to be determined, providing interesting information. The present study, understanding its methods, shows that DH is a condition that impacts subjects as from adolescence, so the opportunity to apply preventive strategies as from this age should not be missed.

DH has direct impact on both oral and systemic health, affecting quality of life. It is important for adolescents to learn health-related attitudes and behaviors that will last through adulthood[28]. One of the World Health Organization (WHO) strategies to improve oral health for the twenty-first century, in addition to the effective use of fluoride and a healthier diet, is to improve oral health for children

and adolescents through health promotion in schools[29]. Because adolescents are establishing their independence from the influence of their parents in relation to various aspects of their development[30], they are a challenging group in terms of oral health. The vulnerability of their permanent teeth often exposes them to major chronic oral diseases, such as caries and periodontal diseases, with early onset and progressive, cumulative effect, manifesting in childhood, adolescence and in all age groups of adult life[31].

In conclusion, DH is a prevalent condition in adolescence. It is associated with female gender and lower socioeconomic status. Preventive strategies for DH need to begin in adolescence; taking into consideration these risk indicators.

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H_2S in periodontal immune-inflammatory response and bone loss: a study in rats

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ABSTRACT

Halitosis is highly prevalent in periodontitis and attributed mainly to the presence of volatile sulfur compounds (VSC), where hydrogen sulfide (H_2S) is the chief culprit in the characteristic malodor of periodontitis and thus may play an active role in its pathogenesis. The aim of this study was to evaluate the effect of H_2S in the acute, intermediate and chronic immune-inflammatory host response and alveolar bone loss in vivo by using an animal model of induced periodontal disease. Thirty-six rats were divided into 2 groups: test group (n = 18), rats exposed to H_2S (NaHS - H_2S donor molecule) and control group (n = 18), rats treated with saline only (Ctrl). All animals had one of their lower second molars ligated to induce periodontal disease (PD). The sound contralateral molar was used as control (H). Each group was subdivided into 3 (n = 6), according to follow-up time (3h, 5 days and 14 days). The gingival tissue was used for mRNA expression analysis (IL-1, IL-6, RANKL, OPG and SOFAT) by real-time PCR and the mandibles were analyzed morphometrically. Data analysis showed that the ligature promoted alveolar bone loss, observed mainly at 14 days, both in the group exposed to H_2S and in the Ctrl group. H_2S administration did not result in additional bone loss. Gene expression showed a significant increase in IL-1, IL-6, RANKL and SOFAT only in the Ctrl-PD group ($p<0.05$). A significant downregulation in OPG expression was observed over time in the Ctrl-PD group ($p<0.05$). In conclusion, H_2S had no effect on alveolar bone loss in the absence of a ligature. In the presence of a ligature, however, exposure to H_2S had an immunoregulatory effect on the expression of pro-inflammatory and pro-resorptive cytokines.

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***Key words**: Hydrogen sulfide, periodontal disease, alveolar bone loss, halitosis.*

Influência do H_2S na resposta imunoinflamatória e perda óssea periodontal: um estudo em ratos

RESUMO

A halitose é altamente prevalente na periodontite e é atribuída principalmente à presença de compostos sulforosos voláteis (CSV), sendo o sulfeto de hidrogênio (H2S) o principal gás relacionado ao mau odor e que pode estar envolvido na patogênese da doença periodontal. O objetivo deste estudo foi avaliar o efeito agudo, intermediário e crônico do H_2S na resposta imuno-inflamatória e na perda óssea alveolar em ratos, com e sem doença periodontal induzida. Trinta e seis ratos foram divididos em 2 grupos: teste (n = 18), ratos expostos ao H2S (NaHS - molécula doadora de H2S) e grupo controle (n = 18), ratos tratados apenas com solução salina (Ctrl). Todos os animais tiveram um dos seus segundos molares inferiores submetidos à colocação de uma ligadura para o desenvolvimento da doença periodontal (DP), em comparação com o dente contralateral saudável (H). Cada grupo foi subdividido em 3 (n = 6), de acordo com o tempo de eutanásia (3h, 5 dias e 14 dias). Os tecidos gengivais foram utilizados para a análise da expressão gênica (IL-1, IL-6, RANKL, OPG e SOFAT) por PCR em tempo real e as mandíbulas foram analisadas morfometricamente. Análise dos dados demonstrou que a ligadura promoveu perda óssea alveolar, observada principalmente aos 14 dias, tanto no grupo exposto ao H2S quanto no grupo Ctrl. A administração de H2S não resultou em perda óssea adicional. A expressão gênica demonstrou aumento significativo de IL-1, IL-6, RANKL e SOFAT apenas no grupo Ctrl-PD ($p <0,05$). Uma significativa regulação negativa na expressão de OPG foi observada ao longo do tempo no grupo Ctrl-PD ($p <0,05$). Pode-se concluir que o H_2S não teve efeito adicional na perda óssea alveolar, na ausência de ligadura. Entretanto, na presença de ligadura, a exposição ao H_2S teve um efeito imunorregulatório na expressão de citocinas pró-inflamatórias e pró-reabsortivas.

***Palavras-chave:** Sulfeto de hidrogênio. Doença periodontal. Perda óssea alveolar. Halitose.*

INTRODUCTION

Periodontal disease may be defined as an infectious condition resulting from the accumulation of bacterial dental biofilm, in which the immune-inflammatory response plays a crucial role in bone destruction patterns[1]. Several molecules and products are involved in this process, accelerating or preventing bone loss dynamically. The disease

process begins with dysbiosis between the host's immune response and the microbial challenge, where the latter is regarded as a primary etiological factor[2]. Clinically, periodontitis may present as gingival inflammation, decreased tissue resistance to probing, attachment loss, alveolar bone loss, presence of local irritants associated with disease progression[1] as well as a characteristic malodor[3].

Persistent oral malodor, also known as bad breath or halitosis, is composed of a series of volatile chemicals, mostly sulfur compounds. Sulfur-derived volatile substances, designated by their generic name of volatile sulfur compounds (VSC), include hydrogen sulfide (H_2S), methyl mercaptan (CH_3SH) and dimethyl sulfide ($(CH_3)_2S$)[4]. These compounds are frequently produced in the oral environment as a result of the metabolism of proteins, mainly by anaerobic bacteria[3].

Evidence has suggested that VSC may be directly involved in the pathogenesis of periodontal disease by increasing epithelial permeability and consequent penetration of lipopolysaccharides and prostaglandins[5]. The presence of VSC in the oral cavity may increase IL-1 and PGE_2 levels, activate collagenase[6], reduce collagen synthesis and cause apoptosis of gingival fibroblasts[7]. In addition, VSC may act to reduce basement membrane synthesis and proliferation of gingival epithelial cells[8] and osteoblasts[9].

H_2S is a gas with multiple tissue-dependent roles, e.g. vasodilation, inflammation, cardiac reaction to ischemic injuries, and is regarded as a Janus-faced molecule because at lower concentrations it causes antioxidant and cytoprotective effects, whereas at higher concentrations it becomes cytotoxic and stimulates oxidative stress[10]. It has been shown that H_2S accelerates healing in diabetic rats[11] and endogenous levels of H_2S can preserve the proliferative capacity of stem cells from the periodontal ligament[12]. H_2S may also lead human gingival fibroblasts to apoptosis and DNA damage with increased levels of reactive oxygen species[7].

Regarding periodontal disease, H_2S, which is primarily responsible for halitosis, is a byproduct of bacterial metabolism and is excreted from within the periodontal pocket as well as from other surfaces such as the dorsum of a coated tongue. Therefore, due to its pro-inflammatory properties, H_2S might play an important role in bacteria-induced inflammatory response in periodontal diseases[13]. Studies *in vitro* have shown that H_2S may be involved with the initiation and development of periodontal disease by inhibiting keratinocyte proliferation, reducing protein synthesis by fibroblasts and inhibiting collagen synthesis in the basement membrane[14]. Additionally, a study *in vivo* reported a transient increase in osteoclast differentiation in rats, with up-regulation of RANKL expression in osteoblasts from the periodontal tissues after 3 days of topical application of H_2S[15].

Clinically, several studies (cited by Basic & Dahlén[16]) have been performed in an attempt to develop methods to measure H_2S in gingival crevices and correlate its presence with periodontal destruction. Although the studies have shown a positive correlation, the complexity of such methods may have hindered further testing in clinical settings. The fact that the gas is volatile and easily converted to polysulfides has further hampered such endeavors. There is therefore a lack of comprehensive knowledge on the role of H_2S in bacterial biofilm, the possible effect on local environment and on the host response, as well as on its relationship to clinical parameters in health and disease. In the present study, we hypothesized that H_2S may have different effects on immunomodulation depending on whether the inflammatory response is acute or chronic with potential consequences to alveolar bone loss.

Taking into consideration the evidence on the supposedly positive correlation between H_2S and periodontal disease as well as the controversial findings reported in the literature, the aim of this study was to investigate the effect of H_2S on the immunoinflammatory host response and alveolar bone loss in rats with induced periodontal disease at acute (3h), intermediate (5 days) and chronic stages (14 days).

MATERIALS AND METHODS

Ethical statement

This study was approved by the research ethics committee for animal use - CEUA / SLMANDIC, protocol #2017/011. This study was designed and executed according to the ARRIVE guidelines.

Experimental animals

Thirty-six male mice (Rattus Norvegicus, Albinus, Wistar, SPF) weighing between 200 and 300g were used. Prior to the experimental procedures, the

animals were acclimatized for 30 days in plastic cages with access to food and water *ad libitum* and with light-dark cycles of 12 hours, under controlled humidity and temperature.

Study Design

The experimental units (rats) were submitted to treatments for a maximum of 14 days. They were divided into 2 groups:

- NaHS group: 18 rats submitted to daily exposure to H_2S via topical applications of NaHS (H_2S donor molecule).
- Control group (Ctrl): 18 rats, treated with saline solution and no exposure to NaHS.

In each group, the animals were randomly divided into 3 experimental subgroups according to follow-up period: 6 animals from each group (NaHS and Ctrl) were euthanized 3 hours after exposure to H_2S (acute response); 6 animals were euthanized after 5 days of exposure to H_2S (intermediate response) and 6 animals were euthanized after 14 days of exposure to H_2S (chronic response), as shown in Fig. 1.

Experimental procedures

a) Inducing Periodontal Disease

The animals were weighed and anesthetized accordingly via intramuscular injection in the external region of the thigh using ketamine solution (0.8 ml / kg / IM) (Francotar®; *Virbac do Brasil Industria e Comércio LTDA, Roseira, SP*, Brazil) and xylazine hydrochloride (0.3 ml / kg / IM) (Virbaxil®; *Virbac do Brasil Industria e Comércio LTDA, Roseira, SP*, Brazil). The animals were positioned on a modified Doku apparatus and a silk thread ligature was placed around one of the lower second molars, randomly selected, at the level of the gingival sulcus, in order to favor accumulation of bacterial biofilm and development of periodontal disease (PD). Sham-operated animals had the ligature immediately removed after the procedure, serving as control (Healthy-H).

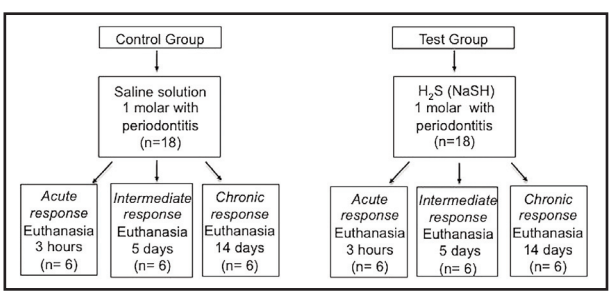

Fig. 1: Experimental design.

b) Exposure to H_2S

Exposure to H_2S consisted of topical application of NaHS (H_2S donor) at 5.6mg NaHS (0.9% saline solution) to the gingival sulcus region of the second molars (with PD and H) using a micropipette. In total, 6 applications were performed within 1 hour at 10-minute intervals. The animals from the Ctrl group underwent the same procedures, except NaHS application, which was replaced with 0.9% saline solution.

c) Euthanasia

All animals were euthanized according to the follow-up periods for each group via intraperitoneal injection of barbiturate-based anesthetic [Sodium Thiopenal (71-73-8) 150mg / kg and Lidocaine (137-58-6) 10mg / ml)]. The mandibles were removed following excision of the gingival tissue from around the second molars.

d) Sample collection for analysis

Gingiva: the marginal portion of the gingiva from the ligated teeth was collected along with their respective contralateral controls, using a scalpel and a micro chisel. The tissue samples were stored in Eppendorf tubes containing RNA Later solution (Ambion Inc., Austin, TX, USA). The samples were frozen for subsequent quantitative evaluation of the expression patterns of the following genes related to the immune-inflammatory response and bone metabolism: Interleukin-1β (IL-1β), Interleukin-6 (IL-6), receptor activator of nuclear factor kappa-B ligand (RANKL), osteoprotegerin (OPG) and secreted osteoclastogenic factor by activated T cells (SOFAT).

Mandibles: After collecting the gingival tissue, the mandibles were completely removed and stored in 3% hydrogen peroxide overnight followed by staining with 1% methylene blue. Horizontal bone loss was quantified morphometrically by measuring the distance between the cementoenamel junction (CEJ) and the alveolar bone crest as described by Casati et al.[17]. Measurements were performed by a trained, calibrated (Intraclass correlation = 93%) and treatment-blind examiner (A.J.S.N.), using the public domain software ImageJ (NIH).

e) Polymerase chain reaction

Total RNA was isolated from the gingival tissue using Trizol reagent (Life Technologies). Briefly, the tissue was collected and homogenized with 1 ml of Trizol. and the aqueous and organic phases were separated by addition of 0.2 ml of chloroform followed by centrifugation (12000 g, 15 minutes, 4°C). RNA was precipitated from the aqueous phase with 0.5 ml of isopropanol (12000 g, 15 minutes, 4° C), washed with 75% ethanol and resuspended in water. One µg samples of RNA were treated with 1U of DNAse I and reverse transcriptase cDNA synthesis was performed using the RevertAid H Minus First Strand cDNA Synthesis Kit (Thermo Scientific) reagent according to the manufacturer's instructions. Briefly, reactions occurred from 1 µg of RNA, 0.5 µg of oligo (dT) 18, 1 mM of the dNTP mix, 200 U RevertAid H Minus M-MuLV transcriptase and 20 U of RiboLockRNAse Inhibitor at 42° C for 60 minutes. The reactions were then terminated by heating at 70° C for 5 minutes. The amplification reactions occurred from 40 ng of cDNA and 0.3 µM primer pairs (Table 1), added to the Maxima SYBR Green qPCR Master Mix (Thermo Scientific). Reaction conditions were as follows: 10 minutes at 95° C, followed by 40 cycles at 95° C, 15 seconds at 60° C, 1 minute. The 7500 Fast Real Time PCR System (Life Technologies) was used. Expression levels were quantified using the SDS System program (Life Technologies), and the relative expression between the samples was calculated according to the Ct (threshold cycle) comparison method, based on the formula $2^{-\Delta\Delta Ct}$. Normalization of expression levels was carried out based on the endogenous GAPDH gene.

Statistical analysis

Sample size calculation was based on the findings from a pilot study. It was based on the equation for a finite population[18] and indicated a sample size of 6 animals per group, providing 80% power with $\alpha = .05$. A bifactorial experimental design was considered for statistical calculations, in which the study factors were treatment (NaHS and Ctrl) and time (3h, 5d and 14d). The response variables were alveolar bone loss and mRNA expression of interleukins. Statistical tests were applied to study the significance of the factors as well as their interaction on the independent variables, namely two-way ANOVA) followed by the *post-hoc* Tukey test for multiple comparisons., The paired Student t-test was used for the intragroup comparisons between ligated and non-ligated teeth. All calculations were performed at a significance level of 5% on GraphPad Prism program, version 6.0.

RESULTS

Morphometric evaluation

The morphometric readings revealed that the ligature alone promoted significant bone loss from day 5, both in the Ctrl group and in the H_2S group

Table 1: Primers for real-time q-PCR analyses.

Gene name	Gene Symbol	Accession number (NM)	Sequences
Interleukin 1 beta	*IL1B*	031512.2	F 5'- CGACAAAATCCCTGTGGCCT -3' R 5'- TGTTTGGGATCCACACTCTCC -3'
Interleukin 6	*IL6*	012589.2	F 5'- CTGGTCTTCTGGAGTTCCGT -3' R 5'- TGCTCTGAATGACTCTGGCT -3'
TNF superfamily member 11**	*TNFSF11*	057149.1	F 5'- GAAACCTCAGGGAGCGTACC -3' R 5'- ACCAGTTCTTAGTGCTCCCC -3'
TNF receptor superfamily member 11B†	*TNFRSF11B*	012870.2	F 5'- GTATCAGGTGCACGAGCCTT -3' R 5'- AGCCAAGTCTGCAACTCGAA -3'
Threonine synthase-like 2‡	*THNSL2*	001009658.1	F 5'- GCAGCCCAGTAGCATCCC -3' R 5'- CATTGGGGTACAGCGTGTCT -3'
Glyceraldehyde-3-phosphate dehydrogenase	*GAPDH*	017008.4	F 5'- CGCCTGCTTCACCACCTTC -3' R 5'- GACTGTGGATGGCCCCTC -3'

* Also known as RANK
** Also known as RANKL
† Also known as OPG
‡ Also known as SOFAT

(Table 2). No significant difference ($p > 0.05$) was observed between the groups with PD, i.e., H_2S neither increased nor prevented bone loss ($p > 0.05$).

mRNA expression

Regarding the proinflammatory cytokines IL-1β and IL-6, no significant difference was observed between groups at 3h ($p<0.05$). Significant upregulation was observed at days 5 and 14 ($p<0.05$) for the Ctrl-PD group only, as described in Fig. 2, A-B. The two-way ANOVA test detected a significant influence of "time" on the expression of IL-1β and IL-6, which increased in the Ctrl-PD group from day 5 to day 14 ($p<0.05$). No such pattern was detected in the other groups ($p>0.05$).

In terms of bone metabolism markers (Fig. 3, A-C), RANKL was upregulated in the Ctrl-PD group ($p<0.05$) at 3h, 5 days and 14 days ($p<0,05$), with no significant difference between the remaining treatments ($p>0.05$). The "time" factor had no influence on the remaining treatment groups (two-way ANOVA $p>0.05$). OPG expression did not differ between treatments at any given time ($p>0.05$). The two-way ANOVA test, however, detected an influence of the factor "time" in OPG expression only for the Ctrl-PD group ($p<0.05$). SOFAT was upregulated in the Ctrl-PD group ($p<0.05$) at 3h, 5 days and 14 days. The comparisons over time within the same treatment group revealed no influence of the factor "time" in the expression of SOFAT (two-way ANOVA $p>0.05$).

DISCUSSION

In addition to the evidence that halitosis is a characteristic sign of periodontal disease[3], it is reasonable to suggest that because VSCs are produced by proteolytic activity of periodontopathic bacteria[19], H_2S could contribute to periodontal destruction, as reported in several studies[5-7,15,20]. The aim of the present study was to investigate the effect of exogenous H_2S on periodontal tissues with and without ligature-induced periodontitis. In general, the results demonstrated that the presence of a ligature induced alveolar bone loss and that exogenous exposure to H_2S, despite having an impact on tissue immunomodulation, had no effect on periodontal bone destruction.

Regarding the relationship between H_2S and periodontitis, studies show discrepant results depending on the H_2S-donor used, the administration route and dosage[20]. Since H_2S is a highly volatile gas, H_2S-donor molecules should be used to allow for optimized contact between the periodontal tissues and the substance, in order to simulate the situation observed within the oral cavity. Several H_2S donor molecules have been tested, such as Na_2S, NaHS or saturated solutions of H_2S gas[21]. Following methods used in other studies[15,22], NaHS administration was chosen to deliver H_2S to the target tissues. In order to interpret the findings thereof, it is necessary to consider the limitations involved in making exogenous H_2S available, since bacteria within the periodontal pocket produce the gas continuously and at lower concentrations compared to exogenous topical application. Such differences could be crucial for the outcomes, so there is a need to develop novel H_2S donors or even slow, sustained delivery methods in order to approach true likeness with *in vivo* conditions as well as reproducibility across studies.

The morphometric findings from the present study demonstrated that at 14 days, alveolar bone loss was significantly higher in the ligature groups than in the control groups, though no significant difference in alveolar bone loss was observed between the treatment groups (with or without NaHS). These findings are comparable to those reported by Toker et al.[23], who evaluated the effect of systemic administration of three different doses of NaHS to

Table 2: Mean ± standard deviation of the alveolar bone loss measurements in micrometers (μm), for the groups exposed to H_2S (NaHS), control (Ctrl), with and without periodontal disease.

Ligature	Ctrl			NaHS		
	3 h	5 d	14 d	3h	5 d	14 d
No (H)	370±62 Aa	302 ± 51 Aa	383 ± 49 Aa	340 ± 39 Aa	324 ± 29 Aa	415 ± 82 Aa
Yes (PD)	368 ± 48 Aa	610 ± 86 *Ab	660 ± 85 *Ab	356 ± 86 Aa	564 ± 69 *Ab	672 ± 134 *Ab

(*) indicates significant difference between the groups with and without PD (unpaired t-test). Different uppercase letters indicate significant intergroup differences (Ctrl x NaHS). Different lowercase letters indicate significant intra-group statistical differences (different times). (two-way ANOVA, Tukey's post hoc test, p<5%).

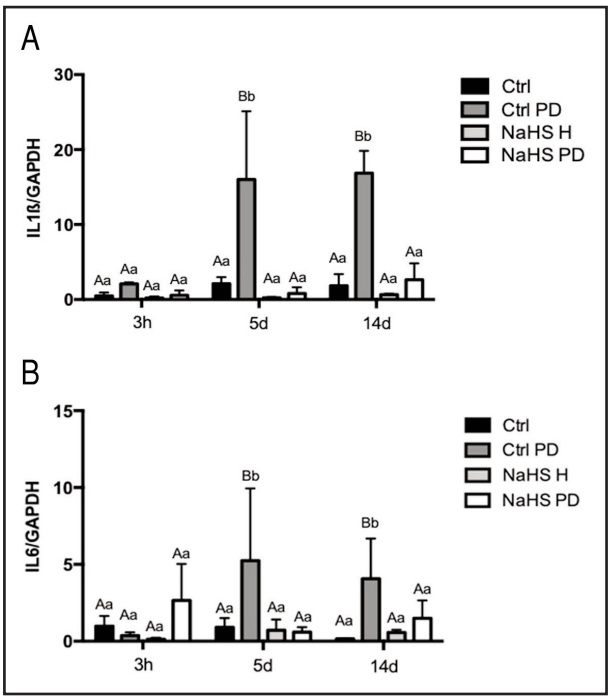

Fig. 2: Mean (± standard deviation) of mRNA expression of the proinflammatory cytokines IL-1β (A) and IL-6 (B) for the different treatments and times evaluated.
Different capital letters indicate significant intra-group differences (different times). Different lowercase letters indicate significant intergroup differences (different treatment, within a same period) (two-way ANOVA criteria, Tukey's post hoc test, 5% alpha).
Ctrl - control group: no exposure (NaHS) and no periodontitis; Ctrl PD: no drug exposure, with periodontitis (PD); NaHS H: drug exposure and no periodontitis (H); NaHS PD: drug exposure and periodontitis.

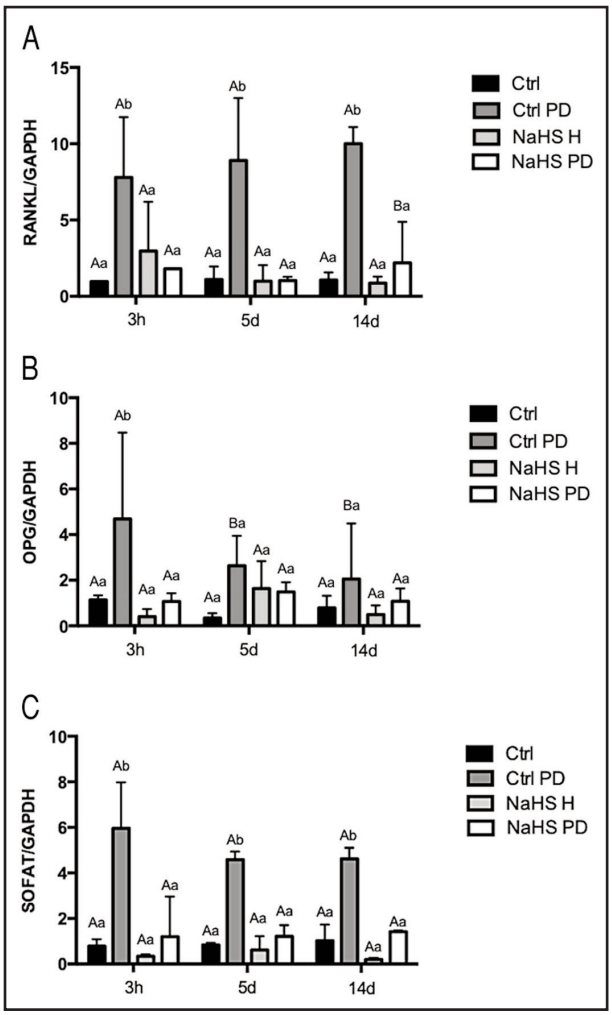

Fig. 3: Mean (± standard deviation) of RANKL (A), OPG (B) and SOFAT (C) mRNA expression in the different groups and at different times.
Different capital letters indicate significant intra-group differences (different times). Different lowercase letters indicate significant intergroup differences (different treatment, within a same period) (two-way ANOVA criteria, Tukey's post hoc test, 5% alpha).
Ctrl - control group: no exposure (NaHS) and no periodontitis; Ctrl PD: no drug exposure, with periodontitis (PD); NaHS H: drug exposure and no periodontitis (H); NaHS PD: drug exposure and periodontitis.

rats (with and without ligature-induced periodontal disease), suggesting that systemic NaHS neither prevented nor increased alveolar bone loss. The present study intended to simulate a clinical scenario in which the periodontal pockets of individuals with periodontitis and halitosis experience high H_2S concentration locally. Although the ligature-induced periodontitis groups showed greater bone loss than the controls, topical administration of H_2S did not influence such tissue loss. This might be explained by the fact that periodontitis is a chronic disease characterized by several episodic cycles of acute-chronic inflammation over a long period of time[2]. Therefore, in such a context, it may be speculated that a relatively short, high-dose exposure to H_2S in a single episode of acute-chronic cycle might be insufficient to draw definitive conclusions regarding alveolar bone loss.

Periodontal bone loss is a result of osteoclastic activity, which is orchestrated by a series of cytokines, where pro-inflammatory cytokines may be inactivated by anti-inflammatory cytokines to achieve balance[2]. It is likely that an imbalance in favor of inflammation would therefore determine the level of periodontal loss[2,24]. In the present study, H_2S seems to have interfered with such mechanism, based on mRNA expression of several cytokines for which exposure to H_2S cancelled out their

upregulation, placing them back at basal levels, as observed for IL-1, IL-6, RANKL and SOFAT, which were significantly upregulated in the groups with periodontitis (PD) but not in those exposed to H_2S (Ctrl). The findings from the present study corroborate the association between overexpression of proinflammatory cytokines and osteoclastogenic factors, with consequent periodontal bone loss[2,24-27]. mRNA expression was analyzed in an attempt to elucidate some of the mechanisms potentially involved in periodontal bone loss and its relationship with H_2S and, interestingly, downregulation of proinflammatory and pro-resorptive cytokines did not translate into less bone loss.

Although the mechanism of bone destruction is not yet well understood, evidence suggests that the RANKL/OPG axis plays an important role in it[28]. In the present study, RANKL expression was significantly higher in the groups with periodontitis throughout the 14 days, except for the H_2S group, as previously discussed, whereas OPG was upregulated only at 3h. According to Chun-Mei et al.[22], the H_2S-related mechanism of tissue damage and repair may vary considerably from tissue health through to acute and chronic inflammation. Furthermore, periodontitis is a chronic disease with peaks and troughs as it alternates between periods of aggressiveness and calm[1,2,24]. This is why we performed evaluations at three times: at an acute phase of bacterial accumulation (3 hours), at an intermediate phase (5 days) and at a chronic phase (14 days), where a cumulative effect of bacterial biofilm was observed, although no clear role of H_2S could be established. Morphometric evaluation is a validated and widely used method of analysis[17,29], though its detection requires bone loss to have occurred already, which is an important limiting factor. Further studies with more sensitive techniques and longer follow-up may aid in understanding the process between intracellular signaling following H_2S exposure and the expression phenotype of bone loss.

Halitosis may be regarded as a public health problem because it has high prevalence in the population – approximately 32%, according to a recent systematic review with meta-regression[30]. Additionally, individuals with periodontitis were 3.16 times more likely (OR 3.16; 95% CI: 1.12-8.95) to have halitosis[3]. Despite the widespread incidence in the general population, it is a subject with little scientific research and somewhat neglected by health professionals. Considering that halitosis may exert a negative impact on social interactions and quality of life, a better understanding of its complex etiology is of utmost importance, especially as it may be associated with health issues such as diabetes, depression, lupus erythematosus, Sjogren's syndrome and periodontitis[21]. In the present study, we sought to investigate the effects of H_2S because it is the main halitosis-related gas, has dubious role in the inflammatory response and may affect bone loss in periodontitis[10,21]. Understanding such effects both locally and systemically might assist in the development of preventive and therapeutic approaches, not only for halitosis but also for other related systemic problems.

In view of the different effects attributed to H_2S regarding cytoprotection and/or cytotoxicity[10-13,20,23], it is assumed that some factors may contribute to the paradoxical findings reported in the literature, such as the different concentrations and delivery methods of the drug, the presence or absence of biofilm accumulation as well as tissue sensitivity at varying degrees of inflammation. There may be a concentration threshold for topical application of H_2S that distinguishes between beneficial and harmful effects. The findings from the present study suggest that in an infectious condition such as periodontitis, there are self-sufficient factors to regulate bone loss, either by direct bacterial aggression, or indirectly by host defense mechanisms. Within the morphometric parameters investigated, exposure to H_2S did not influence periodontal bone loss during the 14 days of the experiment. Regarding the cytokines studied, however, the presence of H_2S caused an immunomodulatory effect on the mRNA expression of important pro-inflammatory and pro-resorption proteins. Extrapolating such findings to a clinical scenario, individuals with periodontitis and halitosis (CSV, especially H_2S) would not appear to have greater short-term bone loss compared to individuals with periodontitis alone.

CONCLUSION

H_2S had no synergistic effect to ligature (biofilm accumulation) on alveolar bone loss, though exposure to appears to have an immunoregulatory effect on the expression of pro-inflammatory and pro-resorptive cytokines.

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Tooth loss and associated factors in the elderly in Cruz Alta, Brazil: a cross-sectional study

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ABSTRACT

The aim of this study was to evaluate severe tooth loss and associated factors among the elderly. A home-based cross-sectional study, using random probabilistic sampling, was conducted with elderly persons from Cruz Alta, Rio Grande do Sul, Brazil. Oral health was examined and a structured questionnaire was answered. Associations between severe tooth loss and independent variables were determined using Poisson regression with robust variance. The level of significance adopted was 5%. Overall, 287 elderly persons were included. Among the elderly, 86 (29.9%) were edentulous, and 282 (98.3%) had lost at least one tooth. In this sample, median tooth loss was 21 (mean±standard deviation: 19.69±8.21). The sample was dichotomized into two groups according to the definition of severe tooth loss: less than nine remaining teeth or ≥9 remaining teeth. The prevalence of severe tooth loss was 60.3% (n=173). Females were associated with higher prevalence ratio (PR) of severe tooth loss (PR; 95% CI: 1.77; 1.39 – 2.24). Higher level of education was associated with lower PR of severe tooth loss (PR; 95% CI: 0.48; 0.30 – 0.77). The lack of access to dental care was associated with severe tooth loss (PR; 95% CI: 1.38; 1.13 – 1.67). Conversely, frequency of toothbrushing was not significantly associated with severe tooth loss (PR; 95% CI: 0.88; 0.73 – 1.06). High prevalence of severe tooth loss among the elderly was observed and found to be associated with sex, level of education, and access to dental care.

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Key words: *Tooth loss, aging, epidemiology, public health.*

Perda dentária e fatores associados em idosos em Cruz Alta, Brazil: um estudo transversal

RESUMO

Esse estudo objetivou avaliar a perda dentária severa e seus fatores associadas em idosos. Um estudo transversal, de base populacional, usando uma amostra probabilística, foi conduzido com os idosos de Cruz Alta, Rio Grande do Sul, Brasil. Exames de saúde bucal e um questionário estruturado foram realizados. Associações entre perda dentária severa e as variáveis independentes foram feitas, utilizando regressão de Poisson com variância robusta. O nível de significância adotado foi de 5%. No total, 287 idosos foram incluídos. Entre os idosos, 86 (29,9%) eram edêntulos, e 282 (98,3%) apresentavam pelo menos uma perda dentária. Nessa amostra, a mediana de perda dentária foi 21 (média ± desvio padrão: 19,69±8,21). A amostra foi dividida em dois grupos de acordo com a definição de perda dentária severa: menos de nove dentes remanescentes ou ≥9 dentes presentes. A prevalência de perda dentária severa foi de 60,3% (n=173). As mulheres estiveram associadas com maior razão de prevalência (RP) de terem perda dentária severa (RP; IC95%: 1,77; 1,39 – 2,24). Alto nível educacional esteve associados com menor RP de ter perda dentária severa (RP; IC95% 0,48; 0,30 – 0,77). A falta de acesso ao dentista esteve associada com maiores taxas de perda de dentária severa (RP; IC95%: 1,38; 1,13 – 1,67). Por outro lado, frequência de escovação não esteve significativamente associada perda dentária severa (RP; IC95%: 0,88; 0,73 – 1,06). Alta prevalência de perda dentária severa foi detectada entre os idosos e ela foi associada com sexo, nível educacional, e acesso ao dentista.

Palavras-chave: *Perda de dente, envelhecimento, epidemiologia, saúde pública.*

INTRODUCTION

One of the biggest challenges in the aging population, which is also observed in Brazil, is oral health. Overall, most elderly people present poor oral health, as shown by higher rates of periodontitis and dental caries[1,2]. These conditions are chronic, irreversible and present a cumulative effect, often leading to tooth extraction as the only treatment option[3].

The literature reports that tooth loss may negatively impact quality of life of the elderly[4]. Its extension and severity over life may cause major deleterious effects on functional, esthetic, nutritional, psychological and social aspects[5]. The higher prevalence of oral diseases may affect overall health, impairing the aging process *per se*. This condition also has major economic impact, as oral rehabilitation is very expensive[6].

Although mean tooth loss and prevalence of edentulism have been decreasing over the years, they are still major problems in several underdeveloped countries, especially among the elderly[7,8]. Tooth loss is considered severe when the individual has less than nine permanent teeth[9], and this condition is reported as being highly prevalent in most studies conducted with Brazilian elderly people during recent decades[2,10].

Tooth loss may also be considered as a marker of social inequalities, as in developed countries, the rate of tooth loss did not decrease among less economically privileged individuals as much as it did in other socioeconomic classes[11]. This scenario may be more alarming in undeveloped countries, such as Brazil, where social and educational inequalities are more evident. Tooth loss is also a true outcome in Dentistry and is probably the best proxy of overall oral health. The aim of the present study was to investigate severe tooth loss and its associated factors among the elderly in a city in southern Brazil. The study hypothesis is that demography, socioeconomics, access to care and health habits are associated with severe tooth loss.

MATERIAL AND METHODS

Study design, location and ethical aspects

This cross-sectional study interviewed and examined elderly persons aged 65 to 74 years living in the urban area of Cruz Alta, a city located in the State of Rio Grande do Sul, Brazil. According to the latest census, city population was 62,821[12], with more than 95% living in the urban area. Of these, 3,730 are in the 65- to 74-year age stratum, 42% are male and 58% are female. In 2010, the Gini Index was 0.5419[13].

The present study was reviewed and approved by the Institutional Review Board of the University of Passo Fundo (UPF) under protocol #1.531.862. All participants signed informed consent before their inclusion in the study.

Sample size calculation and sampling strategy

The formula used for sample size calculation was: sample size = standard normal variate^2 x outcome prevalence x (1 – outcome prevalence) / absolute error^2. We assumed a type error of 5% (standard normal variate of 1.96) and an absolute error of 0.1%. The estimated sample size calculation used the prevalence of tooth loss in the elderly, as previously reported, as 94.72%[14]. Therefore, the total number of individuals considered necessary was 205. Due to the expected attrition rate, we added 20% to the final sample, estimating a total 246.

A probabilistic per conglomerate sampling was performed by visiting 300 households. Further details of the sampling strategy may be found elsewhere[15]. Briefly, the study area consists of 68 neighborhoods and districts, of which 17 (25%) were randomly selected, according to the proportion of elderly people in each area. The blocks and corners were also randomly selected. After the first interview, the visits continued in clockwise direction until sufficient individuals in the sector were included. If necessary, new blocks were selected until the necessary number of households had been visited.

Inclusion and exclusion criteria

Only home-dwelling elderly persons aged 65 to 74 years, from the selected households were included. Elderly persons with physical, medical and mental conditions that enabled this study to be conducted were included. In order to be included, participants also needed to understand the questions in the questionnaire and allow examinations. When there was more than one elderly person who met the inclusion criteria in one household, they were all were invited to participate. Assisted living facilities, commercial living facilities, visitors, and uninhabited households were excluded. A household was excluded only after two unsuccessful visits.

Clinical examination and interview

A structured questionnaire was applied, which included the following variables: demographic data, socioeconomic condition, marital status, oral hygiene habits, behavioral, and health history. All questions were taken from the block of questions of the PCA Tool-SB Brasil, adult version, validated in Brazil[16]. Oral examination was performed by counting teeth and assessing use of and need for dental prosthesis.

Oral examination was performed according to the World Health Organization recommendations[17].

Oral examination was performed after the interview, using a wooden spatula, without artificial illumination or dental mirror. All teeth were considered, except for the third molars. Teeth that could be somehow rehabilitated were considered in the count. Teeth or roots that would not possible to be retain in the oral cavity were considered absent.

Participants were interviewed and examined between July and August 2016 by two teams of researchers. Each team consisted of an interviewer and an oral health examiner. They were all trained by the study coordinator in order to standardize fieldwork. The training consisted of theoretical lectures about the study, discussion of each question in the questionnaire, and explanation of how the oral examination should be performed. Prior to the beginning of the study, two examiners were calibrated with elderly persons who sought treatment at the School of Dentistry of the University of Passo Fundo. The examinations were performed twice, with a two-day interval. For intra-rater reliability, the kappa coefficient was 1.00 for need for dental prosthesis and 0.85 for tooth count. Inter-rater reliability had a minimum kappa coefficient of 0.70 for all the previously reported variables.

Statistical analyses

Severe tooth loss was defined as the outcome of the present study. It was not possible to perform a linear regression because tooth loss presented a non-symmetrical distribution (Shapiro-Wilk test showed a p-value <0.001). We used severe tooth loss to dichotomize the sample. Two groups were created, one with individuals with severe tooth loss (0 to 8 remaining teeth) and another with individuals without severe tooth loss (≥9 remaining teeth). The independent variables were: age, sex, ethnicity/skin color, level of education, marital status, retirement, oral hygiene habits, use and need of dental prosthesis, access to dental care in the past 12 months, smoking and alcohol exposures, general health problems, and use of medication.

Median age in this sample was 70, so we categorized the sample in two groups: one for age <70 years and another for age ≥70 years. Ethnicity/skin color was dichotomized into white or non-white. The non-white group included participants who reported they were black, yellow, brown or indigenous. Level of education was categorized into low for those that had up to complete elementary level of education, including the illiterate; medium, for those with incomplete or complete high school; and high for those with at least incomplete higher education.

Health problems were categorized into two groups, one with participants who reported having at least one health problem, and another with participants who did not know or did not report any health problems. Similarly, the use of medications was also dichotomized into participants who did not use any medication and participants who used at least one medication per day. Toothbrushing frequency was categorized in two groups: <3 times/day or ≥3 times/day.

All analyses were performed in the software SPSS, version 21.0 (SPSS, version 21.0, IBM Corp., Armonk, NY, USA). Associations between the dependent and independent variables were tested by chi-square of Mann-Whitney tests, and are presented by frequency distribution. Univariate and multivariate analyses were performed using Poisson regression with robust variance. The variables included multivariate model were those that presented a p-value <0.25 in the univariate analysis. A combination of p-value <0.05 and modification of effect analyses determined the maintenance of these variables in the final multivariate model. Multicollinearity analyses were performed among the independent variables, and none was observed. The cutoff point for multicollinearity was determined as variance inflation factor <5 and tolerance >0.2.

RESULTS

A total 287 elderly persons were interviewed and examined. This number provided the minimum required for the sample to be representative. The response rate was 89.04%, as shown in Fig. 1. Mean age was 69.30 (standard deviation – SD – ±3.52); 102 participants (35.5%) were male and 185 (64.5%) female. Regarding ethnicity/skin color and level of education, 196 (68.3%) were white and 190 (62.6%) had low level of education. Approximately 40% of the participants were single, divorced or widowed, and 76.3% were retired. History of smoking exposure was detected in 42%. Mean (±SD) tooth loss was 19.69(±8.21), and 86 (29.9%) of the participants were edentulous. Prevalence of at least one missing tooth was 98.3% (n=282), and prevalence of severe tooth loss was 60.3% (n=173).

Age (p=0.007), sex (p<0.001), level of education (p<0.001), marital status (p= 0.002), alcohol exposure (p=0.011), and access to dental care (p<0.001) were significantly associated with severe tooth loss (Table 1). Ethnicity (p=0.766) and smoking exposure (p=0.936) were not associated with severe tooth loss.

Table 2 shows the univariate analyses of the association between severe tooth loss and independent variables. The following variables were significantly associated with severe tooth loss: age, sex, level of education, marital status, alcohol exposure, and access to dental care. The prevalence ratio (PR) for severe tooth loss was 29.7% higher in participants aged ≥70 years than in participants aged <70 years (p=0.007); 66.7% higher in females than in males (p<0.001); lower in participants with higher level of education than in those with lower level of education (PR 0.422 – 95% CI: 0.264 – 0.675); and higher among widowed than among married participants (PR; 95% CI: 1.477; 1.220 – 1.788).

Participants who were not exposed to alcohol presented 29.8% higher PR of severe tooth loss than participants who drank regularly. Participants who had not visited a dentist in the past 12 month presented 55% higher PR of severe tooth loss (p<0.001) than those who had access to dental care. In the initial multivariate model, the following variables were included: age, sex, level of education, marital status, retirement, exposure to alcohol, toothbrushing frequency, and access to dental care. In the final multivariate model, sex, level of education, and access to dental care remained associated with tooth loss (Table 3). PR for severe tooth loss was 76.7% higher in females than males (p<0.001); lower in participants with high level of education than in participants with low level of education (PR; 95% CI: 0.479; 0.300 – 0.765), and 37.5% higher in participants who had not visited the dentist during the past 12 months (p<0.001). However, toothbrushing frequency did not show statistically significant association with severe tooth loss (p=0.164).

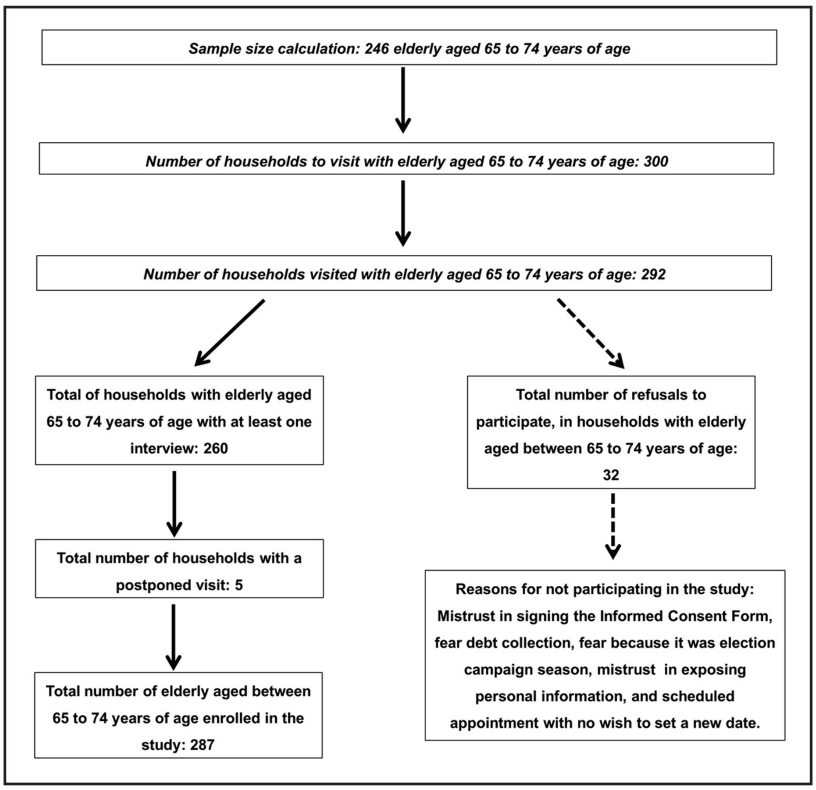

Fig. 1: Flowchart of the participants during the study.

DISCUSSION

The aim of the present study was to evaluate severe tooth loss and associated factors among the elderly in a city in southern Brazil. Prevalence of tooth loss and severe tooth loss were very high (98.3% and 60.3%, respectively). Mean tooth loss was 19.69 (±8.21), and severe tooth loss was associated with sex, level of education, and access to dental care. Analysis of tooth mortality is very important because it reflects the efficacy of oral care in a population[18]. In addition, it should be kept in mind that within the oral health field, tooth loss is the most important outcome, i.e. preventive and therapeutic approaches should ultimately aim to maintain functional teeth. Therefore, in this study, tooth loss is considered a true outcome and not a surrogate as it is in most studies in Dentistry[19]. The literature also shows that, among the elderly, oral health-related quality of life decreases in those that experience at least one tooth extraction within a period of six years[20]. These results may provide useful information for planning public health policies, including allocation of financial resources for oral rehabilitation of the elderly population.

The high mean tooth loss found in the present study was also reported in other home-based studies

Table 1: Association of demographic, economical and behavioral variables with severe tooth loss, Cruz Alta, Rio Grande do Sul, Brazil, 2016.

Variables		No severe tooth loss (n=114; 39.7%)	Severe tooth loss (n=173; 60.3%)	*p*-value
Number of lost teeth	Mean±SD	11.17±5.91	25.31±3.03	<0.001#
	(Median – min.; max.)	(12 – 0; 19)	(27 – 20; 28)	
Age (years)	<70 – n (%)	72 (63.2)	81 (46.8)	0.007*
	≥70 – n (%)	42 (36.8)	92 (53.2)	
Sex	Male – n (%)	59 (51.8)	43 (24.9)	<0.001*
	Female – n (%)	55 (48.2)	130 (75.1)	
Ethnicity/skin color	White – n (%)	79 (69.3)	117 (67.6)	0.766*
	Non-white – n (%)	35 (30.7)	56 (32.4)	
Level of education	Low – n (%)	60 (52.6)	130 (75.1)	<0.001*
	Medium – n (%)	22 (19.3)	30 (17.3)	
	High – n (%)	32 (28.1)	13 (7.5)	
Marital status	Married – n (%)	76 (66.7)	89 (51.4)	0.002*
	Single – n (%)	16 (14.0)	15 (8.7)	
	Divorced – n (%)	10 (8.8)	22 (12.7)	
	Widowed – n (%)	12 (10.5)	47 (27.2)	
Retirement	Yes – n (%)	92 (80.7)	127 (73.4)	0.155*
	No – n (%)	22 (19.3)	46 (26.6)	
Health problems	Yes – n (%)	97 (85.1)	151 (87.3)	0.595*
	No – n (%)	17 (14.9)	22 (12.7)	
Use of medication	Yes – n (%)	93 (81.6)	144 (83.2)	0.717*
	No – n (%)	21 (18.4)	29 (16.8)	
Smoking exposure	Smokers – n (%)	13 (11.4)	22 (12.7)	0.936*
	Former smokers – n (%)	35 (30.7)	51 (29.5)	
	Never smokers – n (%)	66 (57.9)	100 (57.8)	
Alcohol exposure	Yes – n (%)	54 (47.4)	56 (32.4)	0.011*
	No – n (%)	60 (52.6)	117 (67.6)	
Access to dental care	Yes – n (%)	72 (63.2)	63 (36.4)	<0.001*
	No – n (%)	42 (36.8)	110 (63.6)	
Toothbrushing frequency	<3/day – n (%)	28 (24.6)	54 (31.2)	0.222*
	≥3/day – n (%)	86 (75.4)	119 (68.8)	

Legend: SD: standard deviation; *Chi-square test; #Mann-Whitney test.

conducted in southern Brazil. One study with a representative sample of the metropolitan region of Porto Alegre, Rio Grande do Sul, reported mean tooth loss of 20.2[10]. Another two studies conducted in different cities in Brazil showed mean tooth loss ranging from 18.7 to 19.21 in the elderly[2,21]. National mean tooth loss for the aged is 25.29, according to the latest national oral health survey[22]. These results suggest that the oral health of the elderly has not improved as expected over the years. Similar high severity of tooth loss is also widespread in other parts of the world, including developed countries[7].

In the present study, female sex was associated with higher PR of severe tooth loss. This result is consistent with a previous report of higher mean

Table 2: Univariate analysis of the association between independent variables and severe tooth loss, Cruz Alta, Rio Grande do Sul, Brazil, 2016.

Variables		Prevalence ratio (95%CI)	*p*-value
Age	<70	Ref.	0.007
	≥70	1.297 (1.074 – 1.565)	
Sex	Male	Ref.	<0.001
	Female	1.667 (1.304 – 2.132)	
Ethnicity/skin color	White	Ref.	0.764
	Non-white	1.031 (0.845 – 1.258)	
Level of education	Low	Ref.	
	Medium	0.843 (0.655 – 1.085)	0.185
	High	0.422 (0.264 – 0.675)	<0.001
Marital status	Married	Ref.	
	Single	0.897 (0.607 – 1.325)	0.585
	Divorced	1.275 (0.970 – 1.674)	0.081
	Widowed	1.477 (1.220 – 1.788)	<0.001
Retirement	Yes	Ref.	0.130
	No	1.167 (0.956 – 1.424)	
Health problem	Yes	Ref.	0.610
	No	0.926 (0.691 – 1.242)	
Use of medication	Yes	Ref.	0.723
	No	0.955 (0.738 – 1.234)	
Smoking exposure	Smokers	Ref.	
	Former smokers	0.943 (0.693 – 1.285)	0.712
	Never smokers	0.958 (0.722 – 1.272)	0.768
Alcohol exposure	Yes	Ref.	0.016
	No	1.298 (1.051 – 1.605)	
Access to dental care	Yes	Ref.	<0.001
	No	1.551 (1.263 – 1.904)	
Toothbrushing frequency	<3/day	Ref.	0.204
	≥3/day	0.881 (0.726 – 1.071)	

tooth loss among elderly females than among elderly males[4]. Based on these results, it is important to highlight that tooth loss may reflect not only the burden of oral diseases, but also the attitudes of the patients and their dentists, the availability of dental services, and prevailing philosophies during oral treatment[1]. In fact, women have been found to be more careful about their oral health and also seek more health services than men do,[23] and are consequently more exposed to different treatment options, which may include more tooth extractions. In the present study, tooth loss was analyzed including both extracted teeth and teeth whose condition would not allow rehabilitation. Therefore, the question may arise as to whether females are more exposed to overtreatment in terms of extractions.

Similarly, low level of education was associated with higher tooth loss, which has already been reported in the literature, regardless of the socioeconomic characteristics of the country[10,24]. The level of education may be the most important factor associated with health problems, and it has been used as a proxy of socioeconomic status, including

Table 3: Multivariate analysis of the association between independent variables and severe tooth loss, Cruz Alta, Rio Grande do Sul, Brazil, 2016.

Variables		Prevalence ratio (95%CI)	*p*-value
Sex	Male	Ref.	<0.001
	Female	1.767 (1.393 – 2.241)	
Level of education	Low	Ref.	
	Medium	0.938 (0.741 – 1.187)	0.593
	High	0.479 (0.300 – 0.765)	0.002
Access to dental care	Yes	Ref.	0.001
	No	1.375 (1.131 – 1.671)	
Toothbrushing frequency	<3/day	Ref.	0.164
	≥3/day	0.877 (0.730 – 1.055)	

In the initial multivariate model, the following variables were included: age, sex, level of education, marital status, retirement, exposure to alcohol, access to dental care and toothbrushing frequency.

income[3]. The literature shows that higher level of education is associated with better health conditions and greater longevity[25]. According to the latest national census, the majority of older Brazilians have a lower level of education[12], which was also observed in the presented study. We found that 190 of the participants (66.2%) had up to complete elementary level of education, including the illiterate. It may thus be hypothesized that the lower level of education partially explains the results detected in the present study.

The poor oral health condition observed in the majority of the participants, mainly reflected by high tooth loss, represents the cumulative experience of dental caries and periodontitis, among several other oral diseases[26]. All those conditions are directly related to the poor health habits of the elderly. The higher frequency of toothbrushing is associated with lower chances of having periodontitis[27] and dental caries[28], which may impact directly in tooth survival. However, another study showed that the impact of toothbrushing frequency may be small[29], while the quality of the brushing technique may be more important. This may explain why the present study did not detect a significant association between toothbrushing frequency and severe tooth loss.

Access to oral health information, promotion of preventive aspects of oral health, and oral rehabilitation should be a priority in this age group. The lack of access to dental care during the past 12 months was associated with higher severe tooth loss in this population. It is important to consider that several aspects may be related to the absence of regular visits to the dentist. The cost of oral treatment, fear, and lack of availability are the most frequently cited problems among irregular users of dental services[30]. Strategies to overcome these problems are of the utmost importance in order to increase the regularity with which the elderly receive dental services.

One of the strengths of this study is that a representative sample of the elderly population in Cruz Alta, Brazil was achieved. When compared to the latest census in the city, the present study included similar proportions of sex, skin color and level of education. Another strength was that the examiners were trained and calibrated in order to ensure reproducibility of the data. However, the study also had some limitations. The cross-sectional design does not allow temporal associations between tooth loss and the independent variables. We did not analyze the reasons for tooth loss. Additionally, we did not perform a linear regression in our analysis, as the distribution of tooth loss in the sample was asymmetrical. Nevertheless, the study design enables data generalization and comparison to other home-based representative studies.

The main outcome of the present study was severe tooth loss. This enables an analytical approach in which different patterns of tooth loss can be compared by trying to assess what the associated factors are. Clearly, the selected cutoff point is high

(9 remaining teeth), but it is consistent with previous studies reported in the literature[2,9]. It should be highlighted that the participants still present a high experience of oral diseases, since the preventive strategies that are used currently did not cover them when they were younger. This distribution is in line with what has been observed in several places[31].

The continuous evaluation of tooth loss is very important in order to verify the effectivity of public oral health-related policies, especially among the elderly population. The present study demonstrated that severe tooth loss is very high among the elderly, and found that severe tooth loss is associated with the female sex, low level of education, and lack of access to dental care.

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INDEXES TO VOLUME 32 (2019)

SUBJECT INDEX

INDEXES TO VOLUME 32 (2019)

AUTHOR INDEX

INDEXES TO VOLUME 32 (2019)

AUTHOR INDEX

SAIO
SOCIEDAD ARGENTINA
DE INVESTIGACION ODONTOLOGICA
División Argentina de la International Association for Dental Research

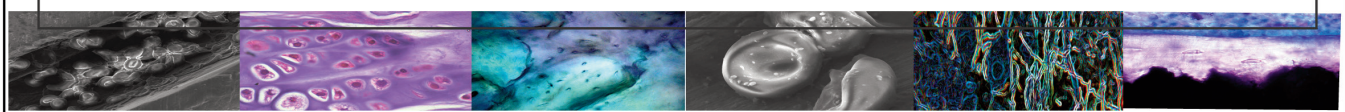
IADR
International Association for Dental Research

On November 14, 15 and 16, the 52nd Annual Meeting of the Argentine Society for Dental Research (SAIO) -the Argentine Division of the IADR- was held in Rosario, Santa Fe Province. Three hundred and four participants, including researchers, graduate students and alumni attended the meeting.
During the three-day meeting, we shared a number of activities, including lectures, round tables and group sessions, as well as oral and poster sessions in which 237 research works were presented.
We were honored by the presence of Jaime Castellanos and Andrés Duque. Special features of the 52nd Anniversary meeting included the following lectures:
- "Proactive approach to the prevention of caries and periodontal disease. Implications for practice and research " by Andrés Duque
- "Biomodification of dentine by flavinoids, a strategy for the improvement of plastic fillings" by Jaime Castellanos
The executive meetings of the following Research Groups were held:
-Periodontal & Implantology Research
-Dental Materials
-Orthodontics
-Education Research
-Oral Medicine & Pathology
-Cariology & Public Health Research
Fourteen proposals for research projects were presented during the executive meeting of the Research Group related to the subject of the project in order to encourage discussion and closer collaboration among experts and peers in each dental discipline.
The following prizes and fellowships were awarded:
Unilever Division Travel Award, Colgate-Palmolive Award for Professionals, Colgate-Palmolive Award for Students, Federa Award, "Rodolfo Erausquin" Award, "Rodolfo Erausquin" Subsidy, "María Inés Egozcue" Award, "Omar Tumilasci" Award.
The Editorial Committee of the Acta Odontológica Latinoamericana met with SAIO members during the event.

Thanks to the 304 registered participants and their research presentations, the LII Annual Scientific Meeting of SAIO 2019 in Rosario have had an excellent scientific level.

Thanks to Dr. Gustavo Feser, President of the Organizing Committee, and his great team for having been very attentive in all the details so that this scientific meeting was developed properly.

We look forward to seeing you at the LIII Annual Scientific Meeting 2020 to be held in the province of Tucumán. We wish Dr. Luis Wuscovi, President of the Meeting the greatest success!



Picture Authors -in order- Daniel Olmedo, Natalia Escudero, Maria Julia Piloni, Daniel Olmedo, Patricia Mandalunis, and Daniel Olmedo

LII REUNIÓN CIENTÍFICA ANUAL SAIO

14 al 16 de noviembre 2019

ROSARIO, SANTA FE - ARGENTINA

HOTEL ARISTON

Opening ceremony.

Opening ceremony. From left to right: Gabriel Sánchez (President of the SAIO), Jaime Castellanos (President of the LAR), Gustavo Feser (President of the 52nd Annual Scientific Meeting).

Opening ceremony.
From left to right: Jaime Castellanos (President of the LAR), Gabriel Sánchez (President of the SAIO), Gustavo Feser (President of the 52nd Annual Scientific Meeting).

President of LAR and SAIO Board of Directors.
From left to right: Drs. Ángela Argentieri, Mariana Picca, Susana Molgatini, Jaime Castellanos, Gabriel Sánchez, Analía Garrofé, Daniel Di Croce, Noemí Bordoni, Luis Wuscovi, Raquel Gallara and Carlos Rozas.

Organizing Committee of the 52nd Annual Meeting.
From left to right: Drs. María Virginia Antuña, Natalia Escudero, Gustavo Feser, Anabella Quintero and Andrés Barros.

Organizing Committee of the 52nd Annual Meeting and President of SAIO. From left to right: Drs. Santiago Pedrol, Gabriel Sánchez, Gustavo Feser, Carla Grimoldi and Nazaret Basaldella.

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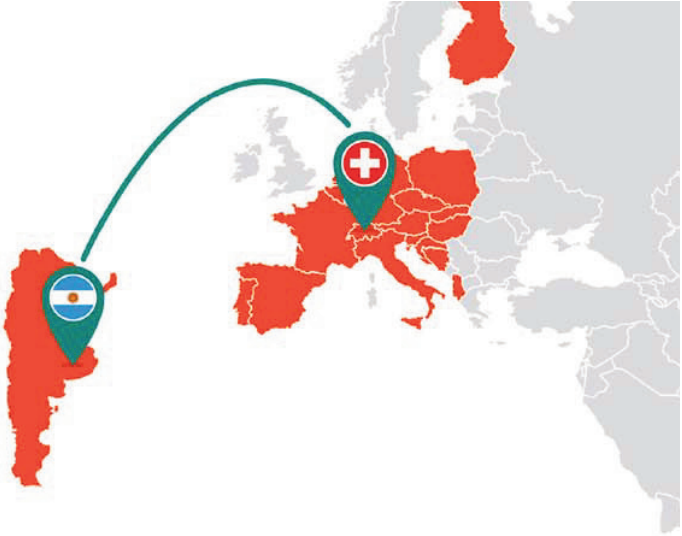
MARCA SUIZA



Con más de 50 años de tradición y liderazgo en Suiza, **elmex** trae a Argentina una nueva línea de protección y fortalecimiento del esmalte dental, con beneficios científicamente comprobados.

La pérdida de minerales o esmalte ocurre cada vez más frecuentemente en la población debido a los hábitos de la vida moderna. Este desgaste prematuro o envejecimiento de los dientes es causado por una combinación de factores extrínsecos e intrínsecos como corrosión, erosión, estrés, bruxismo, uso de medicamentos y reflujo gastroesofágico, entre otros.

La principal causa de la biocorrosión dental en la actualidad proviene de la dieta rica en bebidas y alimentos ácidos. En estos casos, el flujo salival cambia y afecta directamente la función remineralizante en el esmalte, haciéndolo más vulnerable. Debido a esto, todos somos susceptibles al daño del esmalte. Por lo tanto, el secreto es protegerlo y fortalecerlo diariamente, evitando la degradación temprana de los dientes y manteniéndolos saludables durante toda la vida.

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