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El objetivo de **Acta Odontológica Latinoamericana** (AOL) es ofrecer a la comunidad científica un medio adecuado para la difusión internacional de los trabajos de investigación, realizados preferentemente en Latinoamérica, dentro del campo odontológico y áreas estrechamente relacionadas. Publicará trabajos originales de investigación básica, clínica y epidemiológica, tanto del campo biológico como del área de materiales dentales y técnicas especiales. La publicación de trabajos clínicos será considerada siempre que tengan contenido original y no sean meras presentaciones de casos o series. En principio, no se aceptarán trabajos de revisión bibliográfica, si bien los editores podrán solicitar revisiones de temas de particular interés. Las Comunicaciones Breves, dentro del área de interés de AOL, serán consideradas para su publicación. Solamente se aceptarán trabajos no publicados anteriormente, los cuales no podrán ser luego publicados en otro medio sin expreso consentimiento de los editores.

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Maturation of cervical vertebrae and chronological age in children and adolescents

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ABSTRACT

In maxillary orthopedics and related areas, it is essential to determine patient growth peak in order to provide timely diagnosis and treatments. This requires the use of biological indicators that enable children and adolescents to be assigned to maturation stages. The aim of this study was to determine the correlation between cervical vertebrae maturation stages and chronological age in children and adolescents. In this study were evaluated 93 lateral cranium radiographs of 6- to 17-year-old patients who visited the Postgraduate Maxillary Orthopedics Clinic at the School of Dentistry at Universidad del Zulia. Two examiners made independent assessments of cervical vertebrae maturation stage using the method described by Baccetti et al. For each stage, descriptive statistics for chronological age were evaluated, classified according to sex. In addition, parametric and non-parametric tests were performed in which p <0.05 was considered significant. Mean age of the children and adolescents studied was 9.6 years, with standard deviation 2.5 years. The correlation coefficient (r=0.771) certified a high positive correlation between bone maturation and chronological age. This correlation coefficient was highly positive for girls (r=0.858) and moderately positive for boys (r=0.688). The model obtained explains 59.4 % of the variation between bone maturation and chronological age, evidencing an average age increase of three years when maturation stage increases by approximately 1 year. The results suggest that although the degree of covariance between chronological age and maturation stages was highly positive in this study, chronological age does not allow bone maturation to be determined precisely, since it may be influenced by genetic and/or environmental factors.

Key words: *Cervical vertebrae, age, bone age.*

Maduración de vértebras cervicales y edad cronológica en niños y adolescentes

RESUMEN

En ortopedia maxilar y áreas afines resulta esencial determinar el pico de crecimiento de los pacientes para establecer diagnósticos y tratamientos oportunos para lo cual es necesario utilizar indicadores biológicos, que permiten ubicar a los niños y adolescentes en estadios de maduración. El objetivo de este estudio fue determinar la correlación de los estadios de maduración de las vértebras cervicales según la edad cronológica en niños y adolescentes. Se evaluaron 93 imágenes de radiografías lateral de cráneo, de pacientes entre 6 y 17 años de edad que asistieron a la clínica del Posgrado de Ortopedia Maxilar de la Facultad de Odontología de La Universidad del Zulia, dos examinadores estimaron de forma independiente el estadio de maduración de las vértebras cervicales, utilizando el método descrito por Baccetti et al. y para cada estadio se evaluaron los estadísticos descriptivos de la edad cronológica categorizando según sexo, además se realizaron pruebas paramétricas y no paramétricas donde un p <0,05 fue considerado como significativo. La edad media de los niños y adolescentes estudiados resultó de 9,6 años y una desviación típica de 2,5 años. El coeficiente de correlación (r=0,771) certificó una correlación positiva alta entre maduración ósea y edad cronológica, igual producto se obtuvo en el caso de las niños y adolescentes del sexo femenino (r=0,858), mientras los del sexo masculino obtuvieron una correlación positiva moderada (r= 0,688). El modelo obtenido explica el 59,4 % de la variación entre maduración ósea y edad cronológica, lo cual evidencia el aumento de la edad promedio en tres años, cuando el estadio de maduración aumenta 1 año aproximadamente. Los resultados registrados sugieren que, aunque el grado de covarianza entre edad cronológica y estadios de maduración en esta investigación fue positiva alta, la edad cronológica no permite determinar con exactitud la maduración ósea, pudiendo estar influenciada por factores genéticos y/o ambientales.

Palabras clave: *Vértebras cervicales, edad, edad ósea.*

INTRODUCTION

Throughout human life, the different periods of growth and development involve biological transformations that characterize an individual and are influenced by genetic and environmental factors. The study of craniofacial growth is essential for diagnosis and therapeutic planning in orthodontics and maxillary orthopedics. Some authors[1-5]

highlight the need to identify the degree of skeletal maturation in order to begin treatment of certain craniofacial skeletal alterations in a timely manner. Treatments with removable appliances in functional or dentofacial orthopedics during the early developmental stage or during the pubertal phase increase the therapeutic efficacy of the treatment of class II dysgnathia, according to Perinnetti[6] et al. and at dentoalveolar level[7].

Bone maturation is evaluated by means of different biological indicators such as height, weight, chronological age, dental age, carpal x-rays or cervical vertebrae.[8,9]

When the degree of biological maturity is evaluated according to dental, skeletal and chronological age, there may be inconsistencies, and all three indicators are often studied for patient diagnosis. Nevertheless, skeletal age is the most reliable method for determining an individual's physical development.

The determination of growth periods through the evaluation of cervical vertebrae has been supported by Baccetti[10] et al., Haseel[11] et al. and McNamara[1] et al. Other studies criticize the subjective nature of such evaluations[12]. Gray et al.[13] claim that the analysis of cervical vertebrae may be useful for determining whether mandibular growth spurt has occurred.

Bone maturation can be studied in the cervical vertebrae viewed in lateral cephalic radiographs instead of by taking left-hand radiographs, thereby reducing patient exposure to radiation.[14,15]

The study of cervical vertebrae bone maturation is controversial, so further research is needed to contribute more in-depth knowledge to different areas. The aim of the current study was to determine the correlation between cervical vertebrae maturation stages and chronological age, categorized according to sex, in a group of children and adolescents aged 6 to 17 years.

MATERIALS AND METHODS

Patients

One hundred and three (103) patients of both sexes, 6 - to 17-year-old, who attended the clinic in the program "Atención integral del niño y adolescente" (Comprehensive child and adolescent care) of the Postgraduate Degree in Maxillary Orthopedics at the School of Dentistry of University del Zulia (FACOLUZ), Maracaibo City, Zulia State, Venezuela, between March 2012 and June 2016, were selected for this study. Inclusion criteria were: children and adolescents born in Venezuela, with lateral cephalic x-ray of cranium taken at beginning of orthopedic treatment and showing up to fourth cervical vertebra, and complete clinical history. Exclusion criteria were: patients undergoing orthodontic treatment, patients with craniofacial syndromes or malformations, patients with structural problems in cervical vertebrae or related diseases, patients with special healthcare needs, lateral cephalic radiographs with distortion. All parents and/or representatives of the children who participated in the study signed an informed consent after having the purpose of the study explained to them and in accordance with the Ethics Code for Life of the Ministry of Popular Power for Science, Technology and Intermediate Industries of the Bolivarian Republic of Venezuela and the principles of the Declaration of Helsinki.

Study Design

A cross-sectional correlational study was conducted. The selected images were assessed by two raters who made independent assessments of cervical vertebral maturation stage using the method described by Baccetti[10].

A lateral cephalic cranial x-ray is requested as part of the examinations required for the FACOLUZ maxillary orthopedics post-graduate diagnostic protocol. All radiographs were taken at the same radiological center.

Estimation of bone maturation

To estimate the cervical vertebrae maturation, the selected radiographs were digitalized using a SONY DSC-W220 digital camera. To obtain the images, the radiographs were placed on a conventional portable negatoscope, without flash. Subjects' sex and age in years were recorded. Digitalized images were evaluated using Adobe Photoshop software. Brightness, contrast and magnification features were used during assessment. The two raters were trained and calibrated following the method described by Baccetti[10], by evaluating 10 lateral cephalic radiographs of children and adolescents. The Kappa coefficient was calculated, finding intra-rater reliability 0.85 to 0.92 and inter-rater reliability 0.81.

Statistical analysis of results

After obtaining the database on an Excel file, statistical analysis was performed using the IBM SPSS Statistics 24 package. Descriptive statistics were used to obtain frequency tables, contingencies, central tendency measures and dispersion, which enabled adequate interpretation of the set of qualitative and quantitative variables included in the study. Normality assumptions for ages per comparison group were tested using Shapiro-Wilk's test, and equality of variances were tested by Levene's test. In both cases, rejection of the null hypothesis indicated the need to use non-parametric tests to compare age means (Kruskal-Wallis test). All tests used a statistical significance level of 0.05.

RESULTS

A total of 93 child and adolescent lateral cephalic radiographs were included in the study. Ten radiographs were excluded due to problems of image distortion. Of the cephalic radiographs included in the study, 51 were females (54.8%) and 42 males (45.2%). Subject age ranged from 6 to 17 years, with mean age 9.6 years and standard deviation 2.5 years, median and mode 9.0 years. Average age for females was 9.4 years with standard deviation 2.5 years, and average age for males was 9.9 years with standard deviation 2.5 years. Mann-Whitney's U test for comparison of means between girls and boys indicated no significant difference (p =0.332) (Table 1).

Analysis of cervical bone maturation showed that the percentages of children and adolescents in stages I, II, III, IV and V were 33.3%, 28.0%, 24.7%, 10.8% and 3.2%, respectively.

Bone maturation stages according to sex were similar to those described above. For stage I, percentage was 33.3% for both girls and boys, decreasing progressively up to stage V, where it was 3.9% for girls and 2.4% for boys (Table 1).

The chi-square test applied to determine the association between the variables, 'sex' and 'bone maturation stage' showed independence between these variables (p=0.489). This result needs to be interpreted with caution considering that in cross tabulation, more than 20% of the expected frequencies were lower than 5.

The average difference between the mean values for chronological ages was 1.5 years between stages I, II and III, and 2.4 years between stages III, IV and V (Table 2).

Considering that at each stage (I, II, III, IV, V) the number of subjects is approximately 30 (in stage I) or lower than 30 (Table 2), Shapiro-Wilk's test was used to test the normality assumption for ages in each group, finding that age distribution is not normal at all stages. Levene's test for the assumption of homogeneity of variances found p=0.010, therefore the null hypothesis was rejected and variances considered unequal.

The Kruskal-Wallis test to determine variability of mean chronological ages among the different stages showed that they differ significantly (p<0.001).

The dispersion diagram (Fig. 1) relating chronological age to the different stages of cervical maturation shows that bone age increases as chronological age increases in the children and adolescents of both sexes studied, reflecting a positive correlation between these variables. Pearson's correlation coefficient (r=0.771) shows a positive correlation between

Table 1: Mean values for ages and cervical bone maturation stages according to sex.

Girls		Boys		P-value
Mean	Standard deviation	Mean	Standard deviation	
9.4	2.5	9.9	2.5	0.332
STAGE	**N**	**%**	**N**	**%**
I	17	33.3	14	33.3
II	13	25.5	13	31.0
III	11	21.6	12	28.6
IV	8	15.7	2	4.7
V	2	3.9	1	2.4
TOTAL	51	100.0	42	100.0

Table 2: Maturation stages and chronological age in children and adolescents studied.

Stage	N	Mean chronological age	Standard deviation
I	31	7.71	1.296
II	26	9.04	1.865
III	23	10.61	1.699
IV	10	13.00	1.491
V	3	15.33	2.082

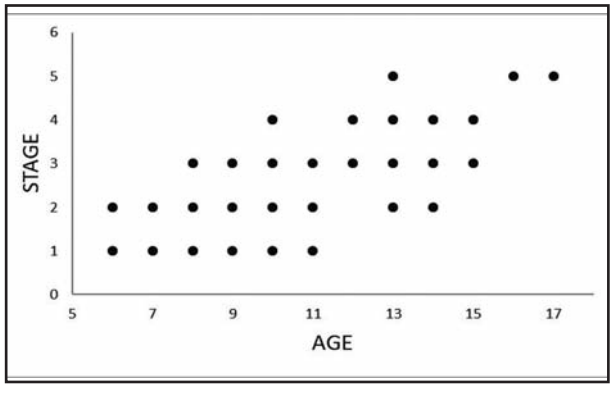

Fig. 1: Dispersion diagram for the variables chronological age and bone age.

these two variables, and was highly positive for girls (r=0.858), and moderately positive for boys (r= 0.688).

A simple linear correlation model was obtained between chronological age and bone age. The result of the ANOVA test ($p < 0.05$) indicated the possibility of constructing a linear regression model with these two variables, with the following predictive model:

$$\mathbf{STAGE = -1.07 + 0.34 \cdot AGE}$$

According to Pearson's correlation coefficient (r), the correlation between chronological age and cervical bone maturation stage in this model is moderate, while the coefficient of determination (R^2) indicates that the model can predict 59.4% of the variability of the stage through the variability of chronological age. Moreover, the equation indicates that when chronological age is increased by one year, stage will vary by 0.34 years.

For female subjects, the correlation between chronological age and bone age was highly positive, while for males it was moderately positive. The linear model obtained for females predicts 73.6% of the variability of bone maturation through chronological age, while for males it predicts 47.4%. The linear regression model for females and males can be seen in the following equations, respectively.

$$\mathbf{STAGE = -1.545 + 0.411 \cdot AGE}$$
$$\mathbf{STAGE = 0.277 \cdot AGE}$$

DISCUSSION

The results of this study on 93 children and adolescents show great variability regarding age and sex in the different maturation stages. The percentages for the whole sample decrease successively as bone maturation increases, as do the percentage of males and females independently. Similar results were reported in the study by Plazas[16].

The Chi square test applied to determine the association between the variables sex and bone maturation stage provided a non-statistically significant result (p=0.489), thereby demonstrating independence between these variables. Similar results were reported by Bedoya[17] (p= 0.120), who found no association between those variables.

The results of the current study show that bone age increases as in chronological age of children and adolescents of both sexes increases, with a highly positive correlation between the variables, expressed with the result of Pearson's correlation coefficient r=0.771. When the samples of both sexes were studied separately, the correlation was highly positive for females, r=0.858 and moderately positive for males, r= 0.688.

Considering the results reported by Bedoya[17], with a highly positive correlation (r= 0.69), the results reported by Seyed[18] in a study on 196 females aged 9 to 14 years, with a low correlation (r=0.62) between chronological age and cervical maturation

stage, and adding the results of the current study regarding Pearson's correlation coefficient, it can be inferred that even though the degree of covariance between the variables 'chronological age' and 'maturation stages' in the current study was highly positive, chronological age does not enable bone maturation to be determined precisely as reported by Bedoya[17].

The paper by Bedoya[17] highlights the fact that the values of maturation stage increase as chronological age increases in both sexes, in agreement with the current study. However, according to Bedoya[17], Tukey's post-hoc test indicates that this occurs only up to stage 3. In the current study, the non-parametric tests used did not allow this type of analysis to be performed.

The model obtained in the current study explains 59.4 % of the variation in maturation stage and chronological age, showing that when average age increases by three years, bone maturation stage increases by approximately 1 year, in contrast to other studies[17] which explain 50.4 % of the variability in chronological age in children and adolescents.

The results of the current study show that female children and adolescents attained higher maturation stages at earlier ages, in agreement with Bedoya[17].

CONCLUSIONS

There is no association between sex and bone maturation stage.

There is a positive correlation between chronological age and cervical bone maturation stages. Bone age increases as chronological age increases. For girls, the correlation was highly positive, while for boys it was moderately positive.

This study shows once again that girls attain bone maturation stages at earlier ages than boys do.

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Study of epithelial rests of Malassez in an experimental periodontitis model

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ABSTRACT

The aim of this study was to evaluate the morphological alterations of epithelial cell rests of Malassez (ERMs) and their relationship with root resorption, in an experimental periodontitis (EP) model at 4 and 11 days. EP was induced in 14 male Wistar rats by placing a cotton thread ligature around the neck of the first lower right molar, for 4 (n= 7) and 11 (n= 7) days. The contralateral molar (left) was used as control. Following euthanasia, jaws were extracted and processed histologically to provide mesio-distal sections which were subject to H&E stain and histochemical detection technique with tartrate-resistant acid phosphatase (TRAP). The following histomorphometric parameters were evaluated on micrographs: bone area (BAr./TAr)(%), number of ERMs/mm², number of cells/ERM, ERMs area (µm²), and percentage of root resorption surfaces (%RR). The results were analyzed statistically by ANOVA and Bonferroni post hoc (p≤ 0.05). Significant bone loss was observed in molars with EP compared to their controls. In the EP 4-Day group, no change was observed in the parameters with relation to the ERMs; however, in the EP 11-Day group, there was significant root resorption (%RR) (C: 3.21±3.07, EP-4D: 3.91±3.17, EP-11D: 23.67± 11.40; p≤ 0,05) and increase in ERMs area (µm²) (C: 455.87±145.42, EP-4D: 577.6±156.1, EP-11D: 1046.3± 582.9; p≤ 0,05). No TRAP+ ERM was found in either group. ERM hypertrophy may be related to ERM participation in mechanisms tending to establish periodontal homeostasis, inhibiting resorption and contributing to periodontal regeneration.

Key words: *periodontitis, root resorption, periodontal ligament, alveolar bone loss, epithelial rests of Malassez.*

Estudio de los restos epiteliales de Malassez en un modelo de periodontitis experimental

RESUMEN

El objetivo de este trabajo ha sido evaluar las alteraciones morfológicas de epithelial cell rests of Malassez (ERMs) y su relación con la reabsorción radicular, en un modelo de experimental periodontitis (EP) a 4 y 11 días. La EP fue inducida en 14 ratas Wistar macho mediante la colocación de una ligadura de hilo de algodón alrededor del cuello del primer molar inferior derecho, a 4 (n= 7) y 11 (n= 7) días. El molar contralateral (izquierdo) fue usado como control. Tras la eutanasia, se extrajeron los maxilares y se procesaron histológicamente para la obtención de cortes en sentido mesio-distal que se colorearon con H&E y técnica histoquímica de detección de tartrate-resistant acid phosphatase (TRAP). Se tomaron microfotografías y se evaluaron los siguientes parámetros histomorfométricos: Bone area (BAr./TAr)(%), Nº de ERMs/mm², Nº de células/ERM, área de ERMs (µm²), y porcentaje de superficies de reabsorción radicular (%RR). Los resultados se analizaron estadísticamente mediante Anova y Bonferroni post hoc (p≤ 0.05). En los molares con PE se observó una pérdida ósea significativa en relación a sus controles. En el grupo EP 4 días no se observaron cambios en los parámetros en relación a los ERMs, sin embargo, en el grupo PE de 11 días se registró reabsorción radicular (%RR) significativa (C: 3.21±3.07, EP-4D: 3.91±3.17, EP-11D: 23.67±11.40; p≤ 0,05) junto con un aumento del área de ERMs (µm²) (C: 455.87±145.42, EP-4D: 577.6±156.1, EP-11D: 1046.3±582.9; p≤ 0,05). No se observaron ERMs TRAP+ en ninguno de los dos grupos. La hipertrofia de los ERMs, podría estar relacionada a la participación de los mismos en mecanismos tendientes a la homeostasis periodontal, inhibiendo dicha reabsorción y contribuyendo a la regeneración periodontal.

Palabras clave: *periodontitis, reabsorción radicular, ligamento periodontal, pérdida ósea alveolar, restos epiteliares de Malassez.*

INTRODUCTION

The insertion periodontium consists of three highly specialized connective tissues: periodontal ligament, root cementum and alveolar bone. These three structures constitute a topographic and functional unit that keeps the teeth in their respective jaws and buffers the mechanical forces exerted on them. The periodontal ligament is a fibrous connective tissue with a large number and variety of cells, including fibroblasts (main cells responsible for remodeling

the ligament), osteoblasts, cementoblasts, osteoclasts, macrophages, mastocytes, epithelial cell rests of Malassez (ERMs) and undifferentiated ectomesenchymal stem cells**.** Of all the above, fibroblasts and perivascular ectomesenchymal stem cells play an important part during the development and homeostasis of periodontal tissues. Several signaling factors modulate the activity of these cells, which provide the machinery for tissue growth and regeneration[1]. The fibrillar organic component consists of two types of fibers: collagenous fibers, mainly type I, inserted in the cementum and the alveolar bone, and collagen types III and XII and elastic fibers, which include three types: elastin, oxytalan and elaunin[2].

Within the study of periodontal components, it is worth highlighting the presence of the epithelial rests of Malassez (ERMs), which are groups of cells that appear during the formation of root hard tissues when the Hertwig epithelial root sheath breaks down. They persist in the periodontal ligament space throughout the lifetime of the tooth. They are unique in that they are the only odontogenic cells of epithelial nature within the periodontal structure. Studies on human ERMs[3] have reported their characteristics: an irregular nucleus with dense heterochromatin and a halo of peripheral cytoplasm, small and scarcely distinguishable, and a high nucleus/cytoplasm ratio. A previous report[4] describes that in rat molars, ERMs undergo increase in size, signs of apoptosis and cell proliferation with age. The authors conclude that ERMs are maintained in the periodontal ligament by cell turnover throughout the lifetime of the tooth.

Regarding ultrastructure, different studies have shown that a basal membrane separates the islands of ERMs from the connective tissue[5]. The presence of tonofilaments, desmosomes and hemidesmosomes has also been demonstrated, all of which anchor ERMS in the basal membrane[6, 7]. These characteristics provide evidence of the epithelial nature of these cells.

Rincon et al.[8] reported the average distance from the cementum to the ERMs in three regions: apical 21 microns, middle radicular: 33 microns, and cervical: 41 microns, indicating a coronal migration of ERMs away from the root surface towards coronal in human teeth.

ERMs have been shown to express different types of proteins and macromolecules, including cytokeratins[9,10] and neuropeptides[11,12,13]. With regard to the expression of cytokeratins, CK 17 could be a marker to identify them[14]. Other studies[15,16] also report the expression of cell surface proteins, including epidermal growth factor receptors. Despite their ectodermal origin, ERMs can synthesize components frequently associated to cells of mesenchymal origin, such as glycosaminoglycans, hyaluronic acid, dermatan sulfate and chondroitin sulfate[17], as well as osteopontin (OPN), bone sialoprotein (BSP) and osteoprotegerin (OPG)[8,18], and can also degrade collagen by synthesis of collagenases and proteinases[19,20,21]. It has therefore been suggested that they may make a considerable contribution to periodontal regeneration by synthesizing a series of proteins related to bone and root cementum[22]. The importance of ERMs in the etiopathology of odontogenic cysts and tumors is well known. Yet there is still much to learn regarding their role in periodontal diseases, especially periodontitis, which is the most frequent oral inflammatory pathology.[23, 24]

Current scientific evidence suggests that the possible roles of ERMs in the adult periodontal ligament include maintenance of homeostasis of the periodontal medium, thereby preventing ankylosis; maintenance of the periodontal ligament space, thereby inhibiting root resorption; and contributing to repairing periodontal cementum[25, 26].

The aim of this study was to conduct morphological evaluation of the epithelial rests of Malassez and to evaluate their relationship with root resorption in an experimental periodontitis model.

MATERIALS AND METHODS

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

Experimental Periodontitis

Experimental periodontitis (EP) was induced in 14 male Wistar rats with body weight 280 g. On Day 1, all animals were anesthetized by intraperitoneal administration of 5% Ketamine (50 mg/Kg body weight) and 2% xylazine (5mg/kg body weight). Experimental periodontitis was induced by placing a cotton thread ligature around the cervical region of the

first lower right molar[27] (Fig. 1). Two experimental times were used: 4 days (n=7) and 11 days (n=7). In both groups, the contralateral molar was used as a control, thereby determining 3 groups: Control Group, EP Day 4 Group and EP Day 11 Group. The animals were euthanized by intraperitoneal injection of sodium thiopental solution (150 mg/kg body weight) (Pentovent, 49 Laboratorios Richmond, Buenos Aires, Argentina) and acepromazine maleate (3 mg/kg body weight) (Acedan, Holliday-Scott S.A., Buenos Aires, Argentina).

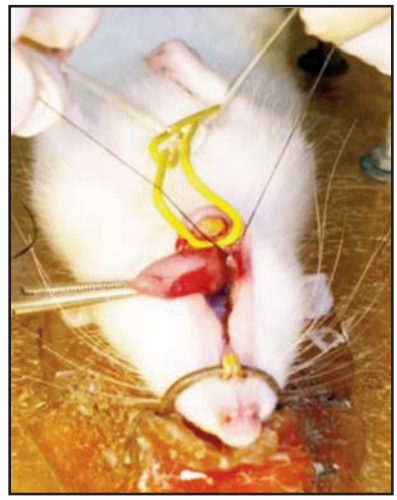

Fig. 1: Placing cotton thread ligature on first lower molar to induce experimental periodontitis (EP).

Histology and Histomorphometry

The section of each hemimandible corresponding to the three lower molars was fixed 10% buffered formalin (pH 7.4), decalcified in 10% EDTA solution, pH 7.0 for 25 days, and embedded in paraffin to prepare mesiodistally oriented histological sections of the first lower molar. The sections were a) stained with Hematoxylin-Eosin and b) subjected to histochemical determination with tartrate-resistant acid phosphatase (TRAP) to evaluate whether this enzyme, which is characteristic of cells that resorb mineralized tissues, is expressed in ERMs.

The following histomorphometric parameters were evaluated:

- Using 40x digital micrographs and Image Pro Plus software (Fig. 2):
  - **Bone Area (BAr/TAr)(%)**: Percentage of total interradicular area occupied by bone tissue.
  - **ERMs area (µm²)** of the ERMS in the interradicular space.
  - **Root resorption (% RR):** Percentage of surfaces in resorption**,** with or without presence of odontoclasts on the root surface**.**

- Using direct 100x bright field microscopy:
  - **ERMs number/mm²:** count of the number of ERMs in the interradicular space (Fig. 2).
  - **Cell number / ERM**: count of cell nuclei observable in each ERM.

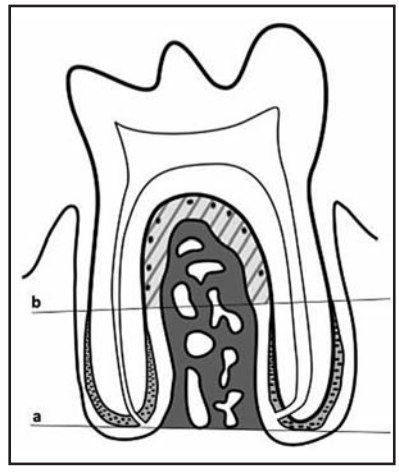

Fig. 2: Diagram of rat molar in situ. Above line a is the area used to calculate bone area (BAr/TAr)(%):. Above line b, marked at the beginning of acellular cementum, the periodontal area is shown (shaded and hatched) used for conducting ERM histomorphometric measurements.

Results were analyzed statistically using ANOVA and Bonferroni *post hoc*, considering p≤ 0.05 as significant.

RESULTS

Bone loss in the interradicular space was observed at 4 and 11 days after induction of periodontitis, in comparison to the contralateral molar (Fig. 3, Table 1). In 4-day experimental periodontitis, no statistically significant change was observed in the other study parameters. However, at 11 days of periodontal disease, there was a statistically significant increase in root resorption (%RR) and ERMs area (Fig.4). Table 2 shows the values recorded for histomorphometric measurements. No TRAP-positive ERM was found in either of the study groups.

DISCUSSION

The results of this study show that at both 4 and 11 days after periodontitis was installed, there was bone loss at the level of the root furcation. In addition, at 11 days there was marked root resorption accompanied by a significant increase in the size of the ERMs. This morphological change

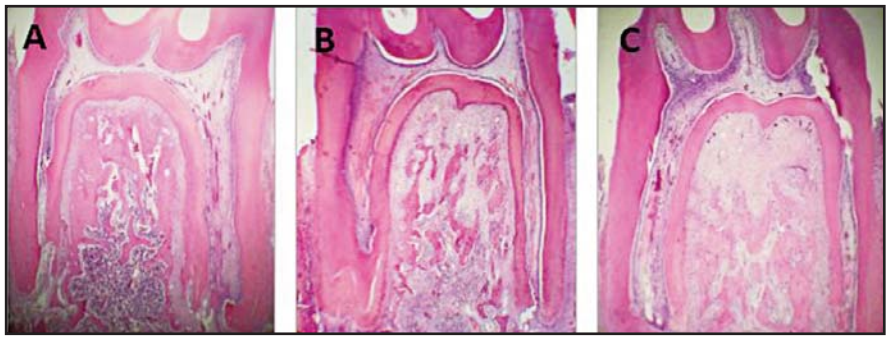

Fig. 3: A, B, C: Bright field micrographs. Decalcification technique. H&E Stain. A Lower molar in situ corresponding to control group; B Molar with 4-day experimental periodontitis. C Molar with 11-day experimental periodontitis. Note the reduction in bone area (BAr/TAr) in the groups with EP. 40X.

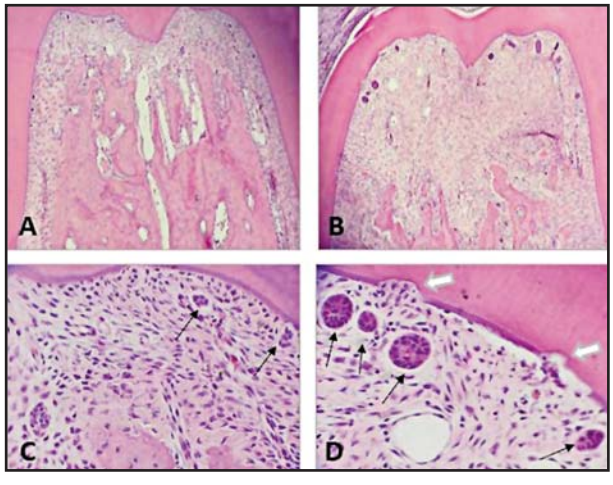

Fig. 4: Bright field micrographs. Decalcification technique. H&E Stain. Root furcation zone of first lower molar.
A. Control; B. Molar with 11-day experimental periodontitis. Note bone loss and ERMs. C. Control: ERMs visible (black arrows). D Molar with 11-day experimental periodontitis. Note enlarged ERMs (black arrows) and root resorption areas (white arrows). A and B: 100 X; C and D: 400 X.

Table 1.

	CONTROL	EP 4D	EP 11D
(BAr/TAr)(%)	40.44 ± 6.86	27.97 ± 7.61	27.26 ± 6.50

EP 4D: experimental periodontitis 4 DAYS; EP 11D: experimental periodontitis 11 DAYS
⌊⌋ p≤ 0.05. X ± SD of the parameters evaluated.

Table 2.

	CONTROL	EP 4D	EP 11D
ERMs area (μm²)	455.87 ± 145.42	577.6 ± 156.1	1046.3 ± 582.9
Root Resorption (%RR)	3.21 ± 3.07	3.91 ± 3.17	23.67 ± 11.40
ERMs number/mm²	7.15 ± 3.53	6.14 ± 2.19	7.50 ± 6.28
Cell number/ERM	9.70 ± 2.86	8.12 ± 3.64	10.43 ± 5.28

EP 4D: experimental periodontitis 4 DAYS; EP 11D: experimental periodontitis 11 DAYS
⌊⌋ p≤ 0.05 X ± SD of the parameters evaluated

occurred at a result of an increase in volume, not a greater number of cells. The data suggest an association between the onset of root resorption and the morphological changes detected in the ERMs. To date, there is no published paper reporting ERM behavior as a result of an infectious/inflammatory stimulus such as experimental periodontitis. The question is whether ERMs are involved in the induction of root resorption or whether their persistence and hypertrophy are related to the expression of signaling factors associated to their potential participation in the regulation/inhibition of said resorption, as part of the homeostasis in the periodontal ligament.

In this regard, the current study performed histochemical determination of tartrate-resistant acid phosphatase (TRAP) with the aim of ascertaining whether ERMs express that enzyme, which characterizes cells that resorb mineralized tissues. These ERMs were found to be negative to histochemical marking, suggesting that they do not participate directly in the root resorption observed in the group with experimental periodontitis at 11 days.

Another study on rats subjected to *in vivo* orthodontic forces found that ERMs responded to experimental orthodontics by proliferating and increasing in size[28]. These findings partially agree with the current study, which found ERM hypertrophy but no increase in number. Other

papers[28,29,30] have reported that orthodontics can stimulate ERMs to secrete different factors, contributing to maintaining normal periodontal structure and function. Consolaro[29] reported that ankylosis occurs when cementoblasts and ERMs are absent.

Studies on mechanical forces *in vitro* have reported that ERMs express heat shock proteins, vascular endothelial growth factor (VEGF), osteopontin (OPN)[26] and HSP 70[31]. The expression of these proteins may contribute to the maintenance of cementogenesis and osteogenesis, and in addition, particularly HSP 70 may play a part by protecting against different factors, including oxidants, inflammation, hypoxia, hyperthermia and mechanical stimuli such as orthodontic forces[32].

Hasegawa et al.[33] used Nakane's root resorption model (by mechanical injury) to study ERMs 7 days after injury, and observed ERMs adjacent to resorbed surfaces. Immunohistochemical tests revealed that the ERMs expressed BMP-2, osteopontin and ameloblastin, suggesting that they may participate in periodontal repair.

All of the aforesaid suggests that ERM hypertrophy in the current study at 11 days after induction of experimental periodontitis may be related to ERM participation in mechanisms that tend to maintain periodontal homeostasis.

As mentioned above, ERMs can express different proteins of epithelial nature[14] and typical of the enamel matrix. Nishio et al.[34] identified two novel proteins, apin (APIN) and amelotin (AMTN), produced by ameloblasts and junctional epithelium, and assessed whether ERMs express them under normal conditions and under disruption of periodontal integrity. They found that after a lesion, ERMs increased in size and they only obtained immunodetection of APIN, suggesting that its expression may lead to the activation of ERMs during periodontal healing and regeneration.

More recently, new findings suggest that within ERMs there is a cell population with stem cell-like characteristics and that in an adequate microenvironment, this cell population can differentiate into cells that produce mineralized matrices[35].

Takahashi et al.[36] examined the expression of amelogenin and ameloblastin, metallopeptidase (MMP 20) and kallikrein (KLK-4) in ERMs and fibroblasts in culture, from samples of human periodontal ligament. Immunohistochemical analysis revealed that those proteins were expressed weakly by ERMs and were not detected in periodontal fibroblasts. Previous studies suggest that amelogenin and ameloblastin may both have activity as growth factors, as well as participating in cell bonding, proliferation, migration and differentiation of fibroblasts of the periodontal ligamentt[37, 38]. Given the reported capacity of amelogenin[39] and its clinical use, it is important to conduct further studies on ERMs, considering their potential to induce regeneration of periodontal tissues by synthesizing proteins such as amelogenins.

CONCLUSIONS

This study found that the ERMs present in the periodontium of rat teeth with experimental periodontitis with 11 days' evolution reacted to root resorption in those teeth, with evident increase in size. Taking into account the information currently available in the literature, this behavior may be related to ERM participation in mechanisms that tend to maintain periodontal homeostasis by inhibiting the resorption process. Further studies are needed to learn about the behavior of these cell groups in response to stimuli of various origins, and their impact on periodontal regeneration.

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Evaluation of a protocol for reducing the microbial contamination of dental unit water

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ABSTRACT

Biofilm on dental unit waterlines can spread microbial contamination in the water. The aim of this study was to investigate microbial contamination of water from supplies and dental units before and after the implementation of a protocol for microbial quality improvement and maintenance of dental unit water. The microbial load was evaluated in water from 27 taps and dental units (reservoirs, air-water syringes and high-speed outputs without handpieces) using the Petrifilm™ system (total aerobic bacteria and fungi) and conventional culture media (enterobacteria and Legionella spp.). The bacterial load in water samples from taps and reservoirs was within the parameter established by Brazilian legislation (<500CFU/mL); but the bacterial load in samples from air-water syringes and high-speed outputs without handpieces was not. The implementation of the protocol for the maintenance of microbial quality in dental unit water reduced bacterial load in high-speed outputs without handpieces (p=0.004). Enterobacteria and Legionella spp. were not isolated from any of the water samples from taps and dental units.

Key words: *Biofilms, dental equipment, water microbiology.*

Avaliação de um protocolo para redução da contaminação microbiana da água de equipos odontológicos

RESUMO

Biofilme nas linhas d'água de equipos odontológicos pode propagar contaminação microbiana na água. O objetivo deste estudo foi investigar a contaminação microbiana da água de abastecimentos e equipos odontológicos antes e após a implementação de um protocolo para melhoria e manutenção da qualidade microbiológica da água de equipos odontológicos. Avaliou-se a carga microbiana da água de 27 torneiras e equipos (reservatórios, seringas tríplice e alta rotação sem as peças de mão) de uma clínica odontológica por meio do sistema Petrifilm™ (bactérias aeróbias totais e fungos) e meios de cultura convencionais (enterobactérias e Legionella spp.). A carga bacteriana em amostras de água das torneiras e reservatórios estava dentro do parâmetro estabelecido pela legislação brasileira (<500 UFC/mL), mas a carga bacteriana das seringas tríplices e das saídas dos alta rotação sem as peças de mão não estava. A implementação do protocolo para manutenção da qualidade da água dos equipos reduziu a carga bacteriana nas saídas dos alta rotação sem as peças de mão (p=0,004). Enterobactérias e Legionella spp. não foram isoladas de qualquer das amostras de água das torneiras e dos equipos odontológicos.

Palavras chave: *Biofilmes, equipamento odontológico, microbiologia da água.*

INTRODUCTION

Over the past century, dental units have evolved from the original pedal-powered pulley models to the current versions with technology that provides safety and reduces biological risk. The greatest transformation took place in the early 1950s, with the emergence of air-water syringes and high-speed handpieces. Because this kind of equipment generates heat that can cause thermal injury, it requires water-cooling, so includes long, thin flexible tubes to channel water and air to it. But neither the inventors nor dental professionals imagined that those long, thin waterlines could conceal a great number of microorganisms from water, despite the implementation of basic principles of asepsis.[1]

Dental units are supplied with drinking water, which contains small microbial load. In Brazil, Ministry of Health ordinance No. 2,914 establishes

the limit as 500 colony-forming units (CFU) per milliliter (mL) of water.[2] In 1996, the American Dental Association (ADA)[3] recommended that the water in dental units should contain no more than 200 CFU/mL.

Dental unit water can pose a risk to oral and general health due to microbial contamination and biofilm formation on waterlines.[4-10] The first signs of microbial contamination of dental unit water and biofilm formation on waterlines were described by Blake[11] and Kelstrup et al.[12], respectively. The literature includes reports of infectious diseases due to contamination in dental unit waterlines.[13,14] This is a matter of concern, since the infections caused by microorganisms resistant to antimicrobials can be fatal, mainly in immunocompromised patients.

Once biofilm is formed, it is difficult to remove. Various strategies have been reported for controlling it on dental unit waterlines, such as development of surfaces with antibiofilm activity[15], supply of sterilized water for dental units[16], and physical-chemical treatments.[17,18] However, most of the strategies used for biofilm formation control on waterlines have limitations, often related to high cost and difficulty in implementation. There is thus a need for the development and use of an efficient protocol for biofilm control on dental unit waterlines based on easy implementation, short execution times and low cost.

The aim of this study was to evaluate the microbial load of water from taps and dental units (reservoirs, air-water syringes and high-speed outputs without handpieces) before and after the implementation of a protocol for improvement and maintenance of the microbiological quality of the water in dental units.

MATERIALS AND METHODS

Samples were collected aseptically from dental unit waterlines [reservoirs (R), air-water syringes (AWS) and high-speed outputs without handpieces (HSWH)] from 27 dental units at the School of Dentistry of Ribeirão Preto (SP, Brazil). Samples of tap water (TW) used to supply the dental units were also collected. The samples were collected at baseline ("T0") and seven months after baseline ("T1"). The "T1" samples were collected after the implementation of a protocol for improvement and maintenance of the microbiological quality of water in dental units as a daily routine. The protocol consisted of supplying and draining the dental unit reservoirs at the beginning and end of the work, respectively, and recommended flushing AWS and HSWH for 30 seconds before and after each patient.[3,19] The protocol did not include any chemical agents for disinfecting reservoirs and dental unit waterlines.

TW, AWS and HSWH samples were collected after water flushing (30s). In addition, samples were collected from reservoirs after rinsing three times with TW. All samples were collected in an approximate volume of 10mL in sterile test tubes (25x150mm). The samples were placed in a cooler, and microbiological processing began no longer than 30 minutes after collection.

The experiment was conducted in a Class II Type A1 Biological Safety Cabinet (VECO, Campinas, SP, Brazil). A 50µL aliquot of 2% sodium thiosulfate was added to each sample. The samples were homogenized (Phoenix, Araraquara, SP, Brazil), diluted up to 10^4 and seeded on Petrifilm™ AC and YM (3M, St Paul, USA) plates to evaluate total aerobic bacteria and fungi (filamentous fungi and yeasts), respectively. In addition, Petri plates (60x15mm) with conventional culture media for detection of *Legionella* spp. (Legionella Agar Base® supplemented with Legionella Agar Enrichment® – BD Difco, Sparks, MN, USA) and enterobacteria (MacConkey Agar – BD Difco, Sparks, MD, USA) were employed. The plates with water samples were incubated at 37°C for 48 h (total aerobic bacteria and enterobacteria), 23°C for 5 days (filamentous fungi and yeasts) and 37°C for 48 h (*Legionella* spp.). After the incubation periods, the colonies were counted using a trinocular stereomicroscope (Tecnival) under reflected light. The number of colony forming units per milliliter (CFU/mL) of water *in natura* was determined.

The statistical tests were performed using IBM SPSS Statistics 20.0 software (IBM Corp Armonk, NY, USA). As the distribution was non-normal, non-parametric Wilcoxon test was used to compare T0 and T1. Differences between contamination of the TW, R, AWS and HSWH in T0 and T1 were analyzed by Kruskal-Wallis. Since there was no count of total aerobic bacteria in T1 for TW and R, Mann-Whitney test was used for the comparison between AWS and HSWH. Relative frequency of presence and absence of contamination for the evaluated groups in T0 and T1 was performed through Pearson Chi-square test. The significance level was set at 0.05.

RESULTS

Tables 1 and 2 show the results of this study for loads of total aerobic bacteria and fungi (filamentous fungi and yeasts).

Of 27 TW samples, 6 (22.2%) were contaminated by total aerobic bacteria at T0. No TW sample was contaminated at T1, having a reduction of 16.0 times the bacterial load (CFU/mL). Filamentous fungus and yeast counts showed that 8 (29.6%) of TW samples were contaminated at T0, and 10 (37.0%) at T1, presenting an increase of 2.2 times of CFU/mL (p=0.407).

Of 27 reservoirs (R), 1 (3.7%) was contaminated by total aerobic bacteria at T0 and none at T1, presenting a reduction of 1.9 times of CFU/mL. The filamentous fungus and yeast count showed that 13 (48.1%) R were contaminated at T0 and 8 (29.6%) at T1, a reduction of 1.8 times of CFU/mL (p=0.351). It is worth noting that only 2 R remained contaminated at T1, while the other 6 R had new

Table 1: Median and confidence interval of CFU/mL for the evaluated groups: before (T0) and after (T1) the protocol implementation for reduction of the microbial contamination of dental unit water. Ribeirão Preto, SP, Brazil, 2018.

	Total aerobic bacteria		p***	Filamentous fungi and yeasts		p***
	T0	T1		T0	T1	
TW	0.0 (0.0; 35.4)^{ab}	0.0 (-;-)	-	0.0 (0.0; 7.9)^{a,A}	0.0 (0.6; 15.7)^{a,A}	0.407
R	0.0 (0.0; 5.7)^{a}	0.0 (-;-)	-	0.0 (0.6; 2.6)^{a,A}	0.0 (0.2; 1.5)^{a,A}	0.351
AWS	0.0 (0.0; 294.4)^{a,A}	0.0 (0.0; 0.7)^{a,A}	0.225	0.0 (0.0; 60.7)^{a,A}	0.0 (0.0; 1.3)^{a,A}	0.098
HSWH	0.0 (0.0; 316.8)^{b,A}	0.0 (0; 1.5)^{a,B}	0.004	4.0 (0.0; 279.0)^{b,A}	0.0 (0.0; 34.7)^{a,A}	0.131
p	0.001*	0.096**		0.001*	0.133*	

CFU/mL: colony forming units per milliliter of water; T0: baseline; T1: after implementation of the protocol for reduction of microbial contamination in dental unit water; TW: tap water; R: reservoirs; AWS: air-water syringes; HSWH: high-speed outputs without handpieces. *Kruskal-Wallis followed by Dunn test; **Mann-Whitney; ***Wilcoxon; ab Same lowercase letters indicate statistical similarity among collection sites; AB Same uppercase letters indicate statistical similarity between T0 and T1.

Table 2: Relative frequency of presence and absence of contamination for the evaluated groups in T0 and T1. Ribeirão Preto, SP, Brazil, 2018.

	Total aerobic bacteria				Filamentous fungi and yeasts			
	T0		T1		T0		T1	
	Absence	Presence	Absence	Presence	Absence	Presence	Absence	Presence
TW	21 (77.8%)	6 (22.2%)	27 (100.0%)	0 (0.0%)	19 (70.4%)	8 (29.8%)	17 (63.0%)	10 (37.0%)
R	26 (96.3%)	1 (3.7%)	27 (100.0%)	0 (0.0%)	14 (51.9%)	13 (48.1%)	19 (70.4%)	8 (29.6%)
AWS	23 (85.2%)	4 (14.8%)	26 (96.3%)	1 (3.7%)	16 (59.3%)	11 (40.7%)	22 (81.5%)	5 (18.5%)
HSWH	14 (51.9%)	13 (48.1%)	22 (81.5%)	5 (18.5%)	6 (22.2%)	21 (77.8%)	16 (59.3%)	11 (40.7%)
Total	84 (77.8%)	24 (22.2%)	102 (94.4%)	6 (5.6%)	55 (50.9%)	53 (49.1%)	74 (68.5%)	34 (31.5%
p*	0.001		0.007		0.003		0.307	

T0: baseline; T1: after implementation of the protocol for reduction of microbial contamination in dental unit water; TW: tap water; R: reservoirs; AWS: air-water syringes; HSWH: high-speed outputs without handpieces; *Pearson Chi-square test.

contamination. Consequently, the protocol for improvement and maintenance of the microbiological quality of water in dental units as a daily routine showed a reduction in fungal contamination in 11 R.

Of 27 AWS, 4 (14.8%) were contaminated by total aerobic bacteria at T0 and 1 (3.7%) at T1, with a reduction of 539.7 times of CFU/mL (p=0.225). Moreover, this AWS contamination at T1 was considered new. The filamentous fungus and yeast count showed that 11 (40.7%) AWS were contaminated at T0 and 5 (18.5%) at T1, having a reduction of 143.7 CFU/mL (p=0.098). Thus, only 3 AWS remained contaminated at T1, and the other 2 AWS had new contamination, with a reduction in fungal contamination of 3 AWS.

Of 27 HSWH, 13 (48.15%) showed contamination by total aerobic bacteria at T0 and 5 (18.52%) at T1, presenting a reduction of 33.6 times of CFU/mL (p=0.004). Moreover, 4 HSWH remained contaminated at T1, and only one case of new contamination was reported. The filamentous fungus and yeast count showed that 21 (77.8%) of HSWH were contaminated at T0 and 11 (40.7%) at T1, with a reduction of 6.9 times of CFU/mL (p=0.131). Thus, only 9 HSWH remained contaminated at T1, and the other 2 HSWH had new contamination, with a reduction in fungal contamination of 12 HSWH.

The comparison among loads of total aerobic bacteria and filamentous fungi and yeasts from the different collection sites at T0 showed that the bacterial and fungal contamination in HSWH was greater than in AWS and R (p=0.001).

In relation to the relative frequency of cases with presence and absence of contamination at T0, HSWH contamination for total aerobic bacteria (48.1% / p=0.001) and filamentous fungi and yeasts (77.8% / p=0.003) was found to be greater than at the other evaluated sites (TW, R and AWS).

Moreover, in this study, the presence of enterobacteria and *Legionella* spp. was not detected in any of the samples (TW, R, AWS and HSWH) analyzed.

DISCUSSION

Dental units consist of reservoirs that supply water through waterlines (diameters 2 to 3 mm) to air-water syringes and high-speed handpieces.[20] Biofilm on these thin waterlines is an alarming source of microbial contamination of water[7,9,10] and can spread pathogenic microorganisms, thereby posing a threat to public health by causing respiratory infections and surgical site infections. Moreover, dentists and professional staff may become infected by aerosols generated in the dental office.[21,22] Since the microbiological quality of water for human consumption is directly related to human health, dental unit water must meet the drinking standard determined or suggested by national and international legislation or organizations.

In this study, water samples from AWS (7.4%) and HSWH (7.4%) presented a total aerobic bacterial load greater than 500CFU/mL. On the other hand, none of TW samples showed contamination above the limit permitted by Brazilian legislation.[2] According to ADA recommendations (1996), at T0, 2 AWS and 4 HSWH samples showed bacterial contamination greater than 200CFU/mL. In agreement with our results, other authors have reported contamination of water samples from dental units with counts above 200CFU/mL and 500CFU/mL.[23,6]

No national and/or international parameter has yet been established with regard to the fungal contamination of water intended for human consumption and dental units. Nevertheless, water should be monitored and controlled frequently for biosafety in dentistry, since filamentous fungi and yeasts have been isolated from dental unit water in other studies[24, 25] as well as in the current study.

Enterobacteria and *Legionella* spp. were not isolated from the water samples analyzed in the current study. Although the traditional culture technique is the main evaluation method for bacterial contamination, false-negative results or underestimated counts have been reported for *Legionella* spp. Some authors have therefore suggested the use of molecular techniques, such as polymerase chain reaction (PCR), to avoid these problems.[26,27]

Biofilm is composed of microorganisms protected by an extracellular polymeric matrix. When it develops on dental unit waterlines, it causes problems which may be resolved by applying physical/ mechanical strategies such as flushing water, as was done in the current study and in others[4, 28, 29], to partially remove microorganisms that are loosely adhered to the biofilm. Antimicrobial chemical agents are also used for this purpose, but they can compromise the structural integrity of dental unit waterlines[14], thereby increasing the

contact area for microbial adhesion and biofilm formation. Moreover, the use of chemical agents for biofilm control may present limitations related to microbial phenotypic changes[30], the difficulty of reaching the innermost microbiota in the biofilm[31], and residual toxic effects on individuals and the environment.

In this study, a protocol was implemented to improve and maintain the microbial quality of dental unit water. The protocol was inexpensive and simple to implement, created no risk to human health or the environment, and provided a partial solution to biofilm contamination on dental unit waterlines. Nevertheless, the problem remains as one of the greatest challenges in dentistry and requires further studies for better understanding, with the aim of providing a biologically safe environment in dentistry.

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Description and characterization of an alternative technique for temporary crown cementation with calcium hydroxide cement

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ABSTRACT

The purpose of this study was to describe and characterize (using the tensile test) an alternative handling technique for calcium hydroxide cement in temporary crown cementation. In the group treated with the conventional technique (n=10), the base and catalyst pastes of a calcium hydroxide cement (Dycal) were dispensed at a 1:1 ratio and mixed. The cement was then applied to the internal cervical surfaces of the provisional restoration, and the restoration was placed on the prepared tooth and kept in place with digital pressure. In the group treated with the alternative technique (n=10), the base paste of the calcium hydroxide cement (Dycal) was placed on the tooth and the catalyst paste was inserted into the temporary crown. The provisional prosthesis was placed on the tooth and kept in place with digital pressure. Tensile values were evaluated and compared between groups using Student's t test with a 5% level of significance ($\alpha = 0.05$). Use of this alternative technique resulted in significantly lower tensile strength compared to the conventional technique (0.58 ± 0.12 vs. 1.08 ± 0.13 MPa; $p < 0.001$). The technique presented here (alternative) could avoid the undesired removal of cemented cast posts or cores at the time of provisional prosthesis removal and ensures the cementation of extensive provisional prostheses with calcium hydroxide cement.

Key words: *Calcium hydroxide, dental cements, tensile strength.*

Descrição e caracterização de uma técnica alternativa para a cementação de coroa temporária com cimento de hidróxido de cálcio

RESUMO

O objetivo deste estudo foi descrever e caracterizar (utilizando o ensaio de tração) uma técnica alternativa de manuseio para o cimento de hidróxido de cálcio na cimentação de coroa temporária. No grupo tratado com a técnica convencional (n=10), as pastas base e catalisadora de um cimento de hidróxido de cálcio (Dycal) foram dispensadas na proporção de 1:1 e misturadas. O cimento foi então aplicado às superfícies cervicais internas da restauração provisória, e a restauração foi colocada sobre o dente preparado e mantido no local com pressão digital. No grupo tratado com a técnica alternativa (n=10), a pasta base do cimento de hidróxido de cálcio (Dycal) foi colocada no dente e a pasta catalítica foi inserida na coroa provisória. A prótese provisória foi colocada sobre o dente e mantida no local com pressão digital. Os valores de tração foram avaliados comparados entre os grupos pelo teste t de Student, com nível de significância de 5% ($\alpha = 0,05$). O uso desta técnica alternativa resultou em resistência à tração significativamente menor em comparação com a técnica convencional ($0,58 \pm 0,12$ vs. $1,08 \pm 0,13$ MPa; $p < 0,001$). A técnica aqui apresentada (alternativa) poderia evitar a remoção indesejada de pinos ou núcleos fundidos no momento da remoção da prótese provisória e garantir a cimentação de próteses provisórias extensas com cimento de hidróxido de cálcio.

Palavras chave: *Hidróxido de Cálcio. Cimentos Dentários. Resistência à Tração.*

INTRODUCTION

The use of provisional prostheses with temporary cements is necessary to meet the requirements of pulp protection, periodontal protection, aesthetics, and occlusion prior to cementation of the final prosthesis[1]. The operator's familiarity with the prosthesis and cement materials optimizes the results of provisional treatment. The cementing technique and the type of cement used play important roles. The retentive properties of a

temporary cement should be sufficient to prevent premature loss of the prosthesis, but should not hamper its removal when desired[2].

The choice of a cementing agent should be guided by consideration of several factors, such as the degree of tooth retention, duration of provisional prosthesis use, prosthesis fabrication technique, and tooth vitality status[3]. Temporary cementing agents should be biocompatible, have low mechanical strength, and be easy to handle; however, no single material meets all these requirements fully.

Although calcium hydroxide cements were developed for pulp capping, they are also suitable for temporary cementation. They are among the most commonly used materials for the cementation of provisional prostheses[4], but the time between the manipulation of the two pastes and insertion in the prosthesis is critical, and their mechanical resistance can be high. The aim of this study was to describe and characterize (using the tensile test) an alternative handling technique for calcium hydroxide cement in temporary crown cementation.

MATERIALS AND METHODS

An intact bovine incisor from which all debris had been removed was used in this study. The tooth was embedded in self-curing acrylic resin[5], (Jet; Artigos Odontológicos Clássico Ltd., São Paulo, SP, Brazil) in a polyethylene tube (Reforplás Indústria e Comércio Ltd., São Paulo, SP, Brazil), with the cementoenamel junction projecting 5 mm above the resin and the buccal surface oriented perpendicular to the tube. The tooth surfaces were cleaned for 10 s with a rubber cup and non-fluoridated pumice– water slurry (S.S. White, Petrópolis, RJ, Brazil), rinsed with air–water spray for 10 s, and air dried for 10 s.

A mold of the tooth was made with polydimethylsiloxane impression material (Zetaplus/catalyst; Zhermack, Badia Polesine, RO, Italy), rinsed with 150 mL distilled water, and dried. The tooth surfaces were cleaned again as described previously. A polydimethylsiloxane index (Zetaplus/catalyst; Zhermack) and a scaled periodontal probe (S.S. White) were used to control tooth reduction. A 1.2-mm-diameter diamond bur (No. 3216; KG Sorensen, Barueri, SP, Brazil) was used to achieve a 6° convergence angle and a circumferential chamfer margin of 1.2 mm at the cementoenamel junction. The incisal edge was reduced by 2 mm, and the axiogingival and axioincisal angles were rounded and finished with a multilaminated tungsten carbide bur (CF 375 R; Orthometric, Marília, SP, Brazil). After tooth reduction, self-curing acrylic resin (Dencor; Artigos Odontológicos Clássico Ltd.) was inserted into the mold. The provisional restoration was made with an L-shaped handle in the incisal for tensile testing. The provisional restoration was finished and polished. From this restoration, 20 specimens were made of self-curing acrylic resin by duplication in polydimethylsiloxane (Zetaplus/catalyst; Zhermack). The specimens were divided randomly into two groups (n=10) according to handling technique. In the group treated with the conventional technique, the base and catalyst pastes of a calcium hydroxide cement (Dycal; Dentsply, Petrópolis, RJ, Brazil) were dispensed at a 1:1 ratio (Fig. 1) and mixed (Fig. 2). The cement was then applied to the internal cervical surfaces of the provisional restoration

Fig. 1: The base and catalyst pastes of a calcium hydroxide cement were dispensed at a 1:1 ratio (conventional technique).

Fig. 2: The base and catalyst pastes of a calcium hydroxide cement were mixed (conventional technique).

(Fig. 3), and the restoration was placed on the prepared tooth and kept in place with digital pressure (Fig. 4). In the group treated with the alternative technique, the base paste of the calcium hydroxide cement (Dycal; Dentsply) was placed on the tooth (Fig. 5) and the catalyst paste was inserted into the temporary crown (Fig. 6). The provisional prosthesis was placed on the tooth and kept in place with digital pressure (Fig. 7). A single operator performed all procedures, with the order of specimens cemented by the two techniques randomized.

After curing, excess cement was removed from all surfaces of the provisional crown and the tooth surfaces were cleaned again as described previously. After 10 min temporary cementation, the tensile test was performed with a universal testing machine (EMIC DL2000; Instron Brasil Equipamentos Científicos Ltda., São José dos Pinhais, PR, Brazil). The acrylic resin handle of each provisional crown was attached to the upper arm of the testing machine, which was attached to a 1000 N load cell operated at 0.5 mm/min. Thus, the results were obtained in N and divided by the area (200 mm^2) to obtain tensile strength values in MPa.

Fig. 3: The cement was applied to the internal cervical surfaces of the provisional restoration (conventional technique).

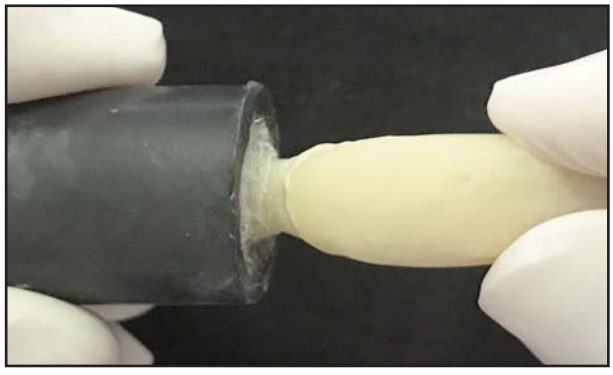

Fig. 4: The provisional restoration was placed on the prepared tooth and kept in place with digital pressure (conventional technique).

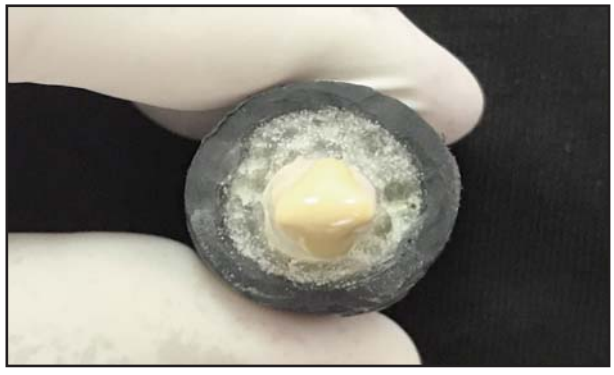

Fig. 5: The base paste of the calcium hydroxide cement was placed on the tooth (alternative technique).

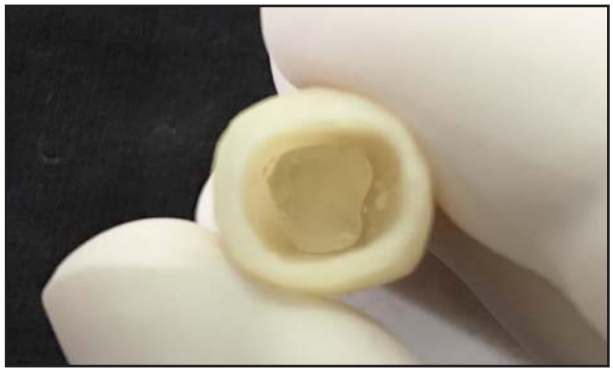

Fig. 6: The catalyst paste was inserted into the temporary crown (alternative technique).

Fig. 7: The provisional prosthesis was placed on the tooth and kept in place with digital pressure (alternative technique).

Statistical analyses were performed using Minitab 16 for Windows 8 (Minitab, State College, PA, USA). The normality of data distribution was investigated with the Shapiro–Wilk test, followed by parametric testing. Tensile values were compared between groups using Student's t test with a 5% level of significance ($\alpha = 0.05$).

Table 1: Tensile strength means (MPa) for different techniques

Technique	Tensile Strength
Conventional	1.08 (0.13)
Alternative	0.58 (0.12)

The difference between means is statistically significant ($p<0.001$). Standard deviations in parentheses.

RESULTS

None of the specimens presented cracks or fractures caused by the tensile test; therefore, none was discarded. The mean (± standard deviation) tensile values, in MPa, of the different techniques are shown in Table 1. The alternative technique showed significantly lower tensile strength compared to the conventional technique (0.58 ± 0.12 vs. 1.08 ± 0.13 MPa; $p<0.001$).

DISCUSSION

A satisfactory temporary restoration must protect the pulp from external stimuli, maintain tooth position and correct occlusion, and allow easy cleaning by the patient[6]. In addition to these basic requirements, the restoration must remain stable in the mouth during the period required for fabrication of the final restoration, with no dislodgment, which could damage the restoration and cause issues such as pulpal and periodontal alteration, modification of tooth positioning, caries development, and the patient's social constraint. Thus, an adequate temporary cement must be used for provisional restoration[7]. Calcium hydroxide cement possesses most of the required characteristics, but it has high mechanical strength and sets rapidly. Thus, an alternative handling technique for this cement was tested in the current study. Use of this technique resulted in significantly lower tensile strength compared to the conventional technique.

Good retention and strength are required for a temporary restoration to meet functional and aesthetic requirements; the cementing technique and type of cement used play major roles. The retentive properties of a temporary cement should be sufficient to avoid premature loss of the restoration, but should not complicate its removal when desired[2,7]. Some calcium hydroxide–based cements have greater mechanical retention than temporary cements[8,9], which could lead to the undesired removal of cemented cast posts or cores at the time of provisional prosthesis removal. At minimum, this issue entails the need to spend additional time re-cementing the cast posts or cores; it may also lead to damage such as cracking or fracture of the tooth involved. Thus, a technique that reduces the mechanical strength of calcium hydroxide cement is needed. The alternative technique described in the present study reduced mechanical strength by 47%. This percentage is consistent with the mechanical strength of other zinc oxide-based non-eugenol cements[8].

When used according to the manufacturer's instructions (conventional technique), the base and catalyst pastes of calcium hydroxide cement are mixed for 30 s; the setting time is 2 min and the working time is 1 min[10,11]. As this cement sets very rapidly, it is not normally used for temporary cementation of extensive provisional prostheses with several tooth elements. The alternative technique described in the present study would be of great value in such clinical situations and its use with cemented cast posts or cores and for extensive provisional prostheses is feasible. Moreover, this technique has been used clinically to cement provisional prostheses for three years at the University of North Parana, with excellent results. However, further studies are needed to evaluate properties other than the reduction in retention of provisional prostheses.

CONCLUSIONS

Based on the methodology and materials used and the results obtained in this study, the following conclusion can be drawn:

The technique presented here (alternative) could avoid the undesired removal of cemented cast posts or cores at the time of provisional prosthesis removal and it ensures the cementation of extensive provisional prostheses with calcium hydroxide cement.

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Sliding resistance of rectangular vs. beveled archwires in two self-ligating brackets: a finite element study

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ABSTRACT

The aim of this study was to use a 3-D finite element method (FEM) to compare the sliding resistance of 0.019"x0.025" stainless steel conventional archwires versus 0.019"x0.025" stainless steel beveled archwires in active (In-Ovation® Dentsply) and passive (SmartClip®, 3M) self-ligating brackets with 0.022" x 0.028" slots . A model was designed for each kind of bracket-archwire system and the following parameters were introduced in the models: friction coefficient calculated for stainless steel bracket-wire: 0.7 µm; Poisson ratio for stainless steel wire: 0.3, and elastic module: 205 GPa for bracket and 190 GPa for archwire. Static structural analysis was applied for homogeneous, linear and isotropic properties considering contacts between wire and bracket as frictional. The results indicate that the beveled archwire generates less stress than the rectangular wire in SmartClip® and In-Ovation® brackets. Comparing brackets, SmartClip® generated less stress than In-Ovation®. It is concluded that beveled rectangular arch wires provide the advantage of reduced sliding resistance, which is better in some clinical situations to improve orthodontic mechanics.

Key words: *Friction, orthodontic brackets, finite element analysis.*

Resistencia al deslizamiento entre arcos rectangulares y biselados en brackets de autoligado: estudio de elementos finitos

RESUMEN

El objetivo de este artículo fue comparar con el método tridimensional de elementos finitos (MEF) el comportamiento de la resistencia al deslizamiento expresado en esfuerzos de arcos de acero inoxidable 0.019"x0.025" convencionales y arcos de acero inoxidable con bisel de 0.019"x0.025" en brackets de autoligado slot 0.022" x 0.028" activo (In-Ovation® "R" Dentsply) y pasivo (SmartClip® 3M). Se diseñó un modelo de los brackets de autoligado In-Ovation® "R" Dentsply, SmartClip® 3M y de los arcos de acero inoxidable convencionales y arcos de acero inoxidable con bisel, el análisis se calculó con el coeficiente de fricción para el bracket de acero inoxidable con el del arco de acero inoxidable: µ 0.7. La relación de Poisson 0.3 para el arco de acero inoxidable. El módulo de elasticidad del bracket: 205 GPa y del arco: 190 GPa. La aplicación del método se realizó para un análisis estructural estático con condiciones de material homogéneas, lineales e isotrópicas y con contactos de tipo fricción entre el arco y el bracket. Se observó que el arco biselado generó menos esfuerzo que el arco rectangular en el bracket SmartClip® y en el bracket In-Ovation® "R", siendo el SmartClip® en el que se generó menor esfuerzo. Se encontró que existe una ventaja en el uso de los arcos rectangulares con bisel ya que presentan menor resistencia al deslizamiento optimizando en algunas situaciones clínicas las mecánicas ortodónticas.

Palabras clave: *Fricción, brackets, análisis de elementos finitos.*

INTRODUCTION

Resistance to sliding occurs on a continuous archwire as it slides through the bracket slot, producing frictional forces[1]. Frictional forces act parallel to the long axis of the archwire and are produced by normal forces at 90° to the archwire[1, 2]. The ligating method produces some frictional force[3-6], mostly related to the normal forces required for moving teeth[1].

Frictional forces are evident throughout all stages of orthodontic treatment and involve mesiodistal sliding between the archwire and the bracket[1].

Twelve to sixty percent of the force applied in fixed braces is lost through friction[7]. This happens not only with useful sliding mechanics such as canine retraction, but also in alignment arches, where if the arch cannot slide, buccal or lingual forces may be attenuated[1]. Friction may also be employed to open space in cases of discrepancies in arch length[1]. Although most orthodontic papers published refer to evaluation of friction, in reality they evaluate resistance to sliding[8]. Frictional resistance reduces the efficiency of orthodontic treatment due to the loss in total force applied[2, 9, 10]. Finite element analysis has shown that 60% to 80% of orthodontic force applied is lost during retraction due to the sliding mechanics of a canine along a rectangular archwire[11].

It is important to eliminate or minimize frictional forces when planning orthodontic tooth movement[12,13]. During orthodontic treatment, frictional forces should be kept to a minimum in order to enable application of lower levels of force and optimal biological response for efficient dental movement[14-16]. In orthodontics, friction occurs between the bracket, the archwire and ligature [17-20]. Resulting frictional resistance reduces the efficacy of orthodontic treatment due to the total loss of the force applied (21% to 60%) [21].

Several studies have shown a significant reduction in friction by using self-ligating brackets, with a reduction in time required to move teeth [6, 22-25]. Stefanos et al. (2010) performed a comparative study of friction with passive and active brackets, and found that passive self-ligating brackets have less static and kinetic friction than active self-ligating brackets when combined with 0.019"x 0.025" stainless steel archwires [3]. Huang et al. (2012) showed that passive self-ligating brackets are associated to lower static or kinetic friction than conventional brackets[20].

Shumacher et al. (1998) evaluated frictional forces when using conventional and beveled rectangular archwires, concluding that among the numerous parameters that affect the degree of friction exerted, archwire beveling has a positive, though secondary effect[26]. Comparison of all measurements revealed that archwires with slightly beveled edges combined with steel ligature should be preferred to an archwire without beveled edges, because a moderate bevel improves friction by approximately 10%[26].

To optimize sliding mechanics, rectangular archwire cross section has been modified by beveling the edges[26]. The 3M® company has developed beveled archwires called hybrids for use with SmartClip® 3M self-ligating appliance, but they have not yet been tested in other self-ligating brands. Therefore, we propose a study to evaluate the benefits that those archwires may provide to other types of brackets. The aim of this study is to use the 3-dimensional finite element method (FEM) to compare resistance to sliding expressed as stress of 0.019" x 0.025" conventional stainless steel wires and 0.019" x 0.025" stainless steel wires with beveled edges in active (In-Ovation® "R" Dentsply) and passive (SmartClip® 3M) self-ligating brackets with 0.022" x 0.028" slots .

MATERIALS AND METHODS

A numerical simulation was performed using the 3-dimensional finite element method (FEM). Models were designed of the active (In-Ovation® "R" Dentsply) and passive (SmartClip® 3M) self-ligating brackets with 0.022" x 0.028" slots and of the conventional 0.019" x 0.025" stainless steel archwires and 0.019" x 0.025" stainless steel archwires with beveled edges, the latter using the measurements of the 3M patented hybrid archwire. For the analysis, the friction coefficient for the stainless steel bracket with the stainless steel archwire was calculated as $\mu=0.7$[27], Poisson ratio was 0.3 for the stainless steel archwire[28, 29] and modulus of elasticity was 205 GPa for the bracket and 190 GPa for the archwire[10]. The active bracket ligating system uses a latch and the passive system uses a clip. Slot size is the same for both brackets. The method presented herein was applied for a static structural analysis with homogeneous, lineal, isotropic material conditions and friction-type contacts between archwire and bracket.

The 3D model was designed according to the technical specifications of each manufacturer. Geometry, mesh and boundary conditions were defined in each model and developed on Autodesk Inventor® software (Fig. 1).

The Autodesk Inventor® 3D geometric model was exported and imported in Ansys Workbench®. When the model was acquired by the FEM software, it was meshed and contact area refinement was employed (Table 1) in order to achieve a mesh that would enable the friction problem to be resolved.

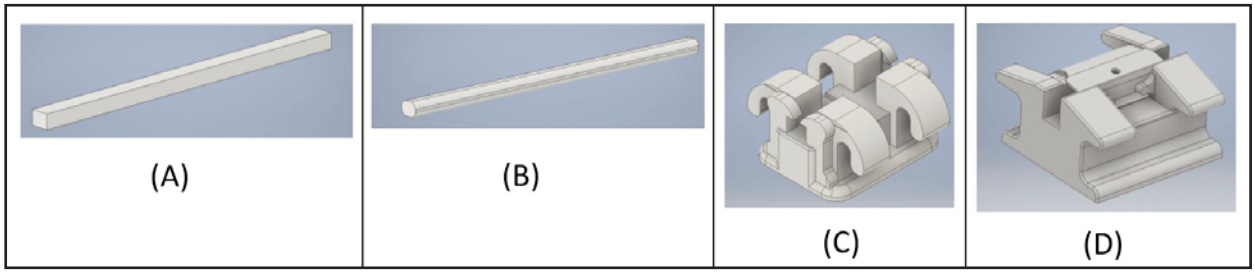

Fig. 1: Designs. (A) 0.019"x0.025" rectangular steel archwire, (B) 0.019"x0.025" beveled rectangular steel archwire, (C) Passive self-ligating bracket, (D) Active self-ligating bracket.

Table 1: Number of nodes and elements on which geometry was meshed.

Model	Nodes	Elements
Rectangular wire-SmartClip®	68849	21211
Rectangular wire-In-Ovation® "R"	92695	30087
Beveled wire-SmartClip®	76452	23094
Beveled wire-In-Ovation® "R"	104035	33215

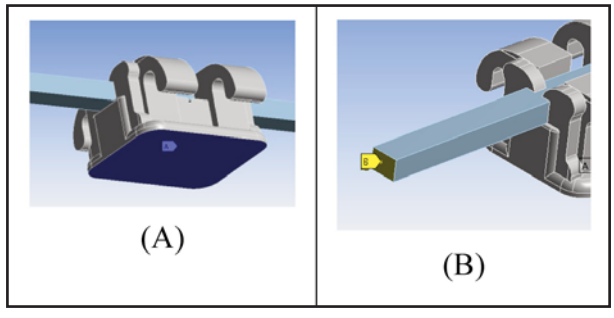

Fig. 2: Boundary conditions. (A) Restriction of movement, (B) Application of sliding.

Table 2: Working conditions.

Condition	Value
Restriction of movement	Base of bracket
Application of displacement	One end of the wire
Displacement value	1 mm

Table 3: Material properties.

Material	Modulus of elasticity(GPa) (4)	Poisson's coefficient (10)
Bracket	205	0.3
Wire	190	0.3

Static structural analysis was performed to determine the difference in behavior with regard to friction between a rectangular archwire and an archwire with beveled edges for both types of brackets under the same working conditions (Tables 2-3, Fig. 2).

RESULTS

General strain

General results are presented in kilopascal (kPa). For the passive bracket, maximum stress was 42.78 kPa with rectangular archwire and 7.062 kPa with beveled archwire. For the active bracket, stress was 42.90 kPa with rectangular and 7.062 kPa with beveled archwire (Fig. 3,Table 4).

Strain on archwires

Results for strain test on archwires are presented in kilopascal (kPa). For the rectangular archwire, maximum stress was 42.78 kPa with passive bracket and 42.90 kPa with active bracket. For the beveled archwire, maximum strain was 7.062 kPa with passive bracket and 7.062 kPa with active bracket (Fig. 4,Tabla 4).

Stress on brackets

Results are presented in kilopascals (kPa). For sliding in passive bracket, maximum stress was 30.73 kPa for rectangular archwire and 2.966 kPa for beveled archwire. For active bracket it was 42.90 kPa for rectangular archwire and 7.062 kPa for beveled archwire (Fig. 5,Table 4).

Strain in bracket contact areas

Results in contact areas are presented in kilopascals (kPa). Maximum value for passive bracket was 9.66 kPa with rectangular archwire and 0.599 kPa with beveled archwire. For active bracket, values were

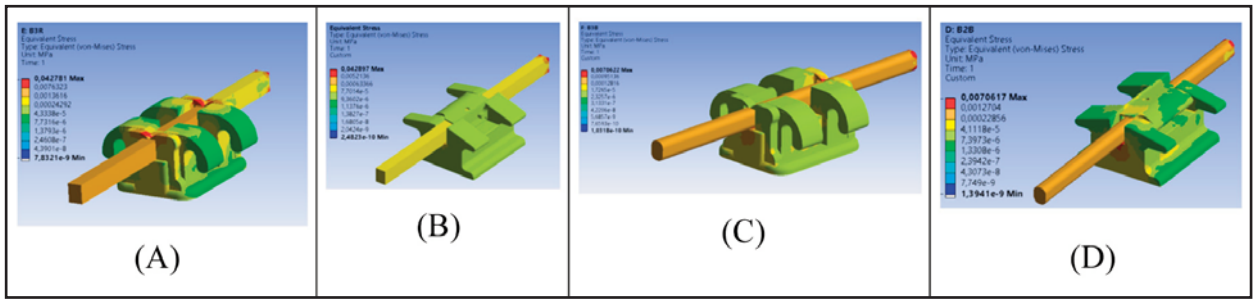

Fig. 3: Results of strains of the different models in Mpa. (A) Rectangular archwire-Passive bracket, (B) Rectangular archwire-Active bracket, (C) Beveled archwire-Passive bracket, (D) Beveled archwire-active bracket.

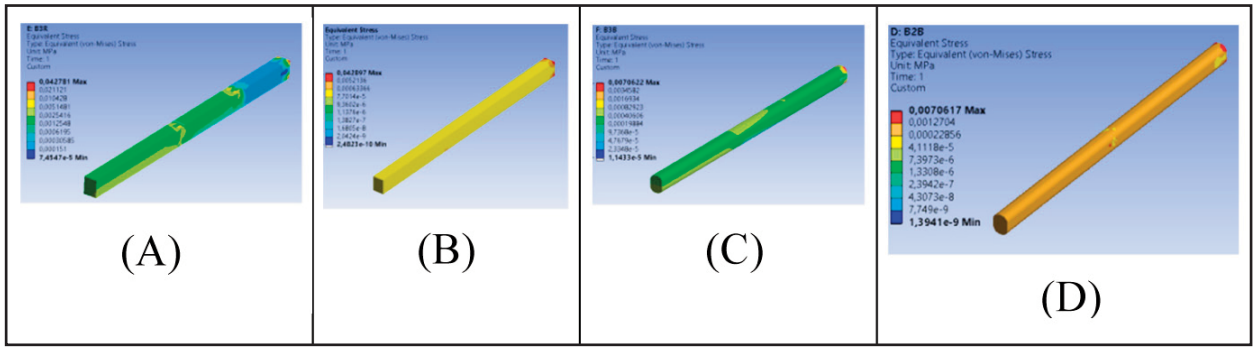

Fig. 4: Results of strains on wires in Mpa. (A) Rectangular wire, (B) Rectangular wire (C) Beveled wire (D) Beveled wire.

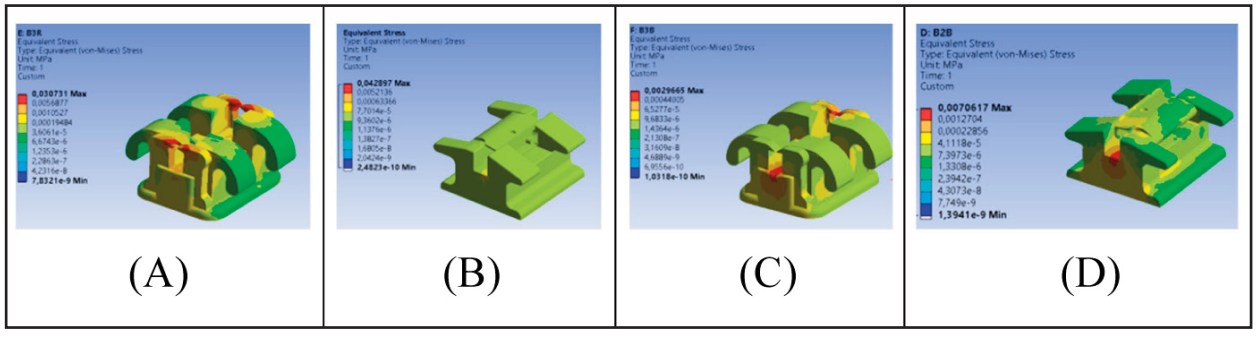

Fig. 5: Results of strains on brackets in Mpa. (A) Passive bracket, (B) Active bracket, (C) Passive bracket, (D) Active bracket.

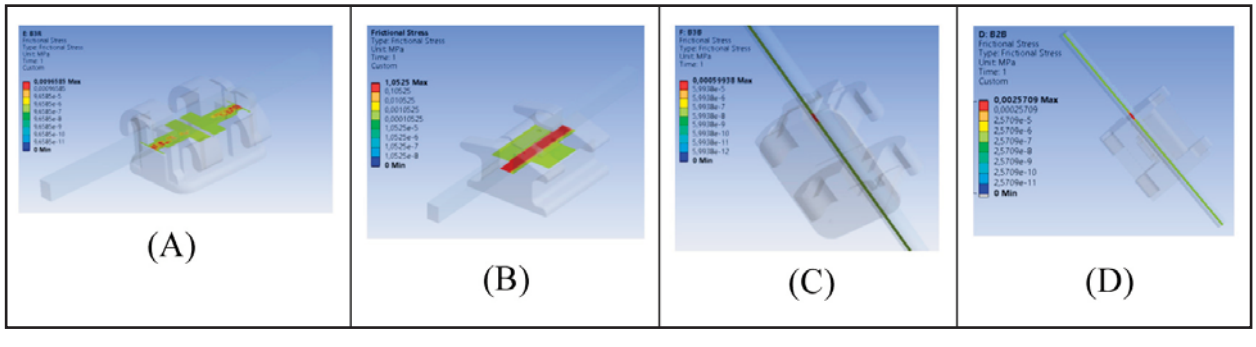

Fig. 6: Results of strains for the contact areas (areas that generate friction) for each model in MPa. (A) Rectangular wire-Passive bracket, (B) Rectangular wire-Active bracket, (C) Beveled wire-Passive bracket, (D) Beveled wire-Active bracket.

Table 4.

STRESS (kPa)	Rectangular archwire		Percentage	Rectangular archwire with beveled edge		Percentage
	Active bk	Passive bk		Active bk	Passive bk	
General	42.90	42.78	99.7	7.062	7.062	100
On the archwires	42.90	42.78	99.7	7.062	7.062	100
On the brackets	42.90	30.73	71.6	7.062	2.966	42
On the contact areas	1,052.50	9.66	0.9	2.571	0.599	23.3

Table 5.

STRESS (kPa)	Passive bracket			Active bracket		
	Rectangular archwire	Beveled archwire	Percentage	Rectangular archwire	Beveled archwire	Percentage
General	42.78	7.06	16.50	42.90	7.06	16.50
On the archwires	42.78	7.06	16.50	42.90	7.06	16.50
On the brackets	30.73	2.97	9.70	42.90	7.06	16.50
On the contact areas	9.66	0.60	6.20	1052.50	2.57	0.20

1052.50 kPa with rectangular archwire and 2.571 kPa with beveled archwire (Fig. 6,Table 4).

Stress was calculated for archwires, brackets and areas in order to determine the proportional values, assigning 100% to the highest value. Table 5 shows the results of resistance to stress for the following conditions: general, on the archwires, on the brackets and on the contact areas, for rectangular archwire and beveled archwire. The results are relevant, considering that in this model, beveled archwire with both types of brackets has the lowest stress for all conditions assessed.

The proportion of strain for variability between the passive bracket and the two kinds of archwire was calculated in the same way. For general results, beveled wire obtained 16.5% of the stress generated by the rectangular archwire. For results on archwire, beveled archwire obtained 16.5% of the stress generated by the rectangular archwire. For the results on bracket, the beveled archwire obtained 9.7% of the stress generated by the rectangular archwire. In the results on bracket contact area, beveled archwire obtained 6.2% of the stress generated by the rectangular archwire (Table 5).

Percentages of variability were calculated for rectangular archwire with the two kinds of bracket. For general results, passive bracket stress was 99.7% of the stress generated in active bracket. For archwire, passive bracket obtained 99.7% of the stress generated in the active bracket. For bracket, passive bracket obtained 71.6% of the stress generated in the active bracket. For bracket contact area, passive bracket obtained 0.9% of the stress generated in the active bracket (Table 4).

Proportions were calculated for beveled archwire with the two kinds of bracket. For general results, active bracket obtained 100% of the stress generated in the passive bracket. For archwire, active bracket obtained 100% of the stress generated in the passive bracket. For brackets, passive bracket obtained only 42% of the stress generated in active bracket. For bracket contact area, passive bracket obtained only 23.3% of the stress generated in active bracket (Table 4).

DISCUSSION

Finite element analysis has been used by different authors to evaluate resistance to sliding[10, 28-30]. The current study compares resistance to sliding with conventional rectangular archwires and beveled rectangular archwires in active and passive self-ligating brackets, in view of the need for information

to determine whether beveled archwires improve sliding mechanics.

This study found lower resistance to sliding in active and passive brackets with the 0.019" x 0.025" beveled archwire than with the rectangular non-beveled 0.019" x 0.025" archwire. This confirms the findings of Shumacher et al., 1998, evaluating frictional forces with beveled and unbeveled rectangular archwires during canine retraction using an electronic typodont and an orthodontic measurement simulation system. They concluded that among the many parameters affecting the degree of friction exerted, archwire bevel has a positive effect[26]. Comparison of all measurements shows that an archwire with slightly beveled edges combined with steel ligature is preferable to an archwire without beveled edges, since moderate bevel reduces friction by approximately 10%[26]. Current evidence suggests that further research is needed regarding these outcomes when archwires with beveled edges are used on brackets with different features from those indicated by sellers.

The current study showed more resistance to sliding in active brackets than in passive brackets when sliding rectangular archwire, in agreement with Stefanos et al. (2010), who found that passive self-ligating brackets have lower static and kinetic frictional forces than active self-ligating brackets when combined with 0.019" x 0.025" stainless steel archwire[3].

The results of the current study agree with Huang et al. (2012), who showed that passive self-ligating brackets are associated to lower static or kinetic frictional force than conventional brackets[20].

Gómez et al. (2016) compared frictional resistance between passive and active self-ligating brackets using finite element analysis and in vitro assays. They found that passive self-ligating brackets showed lowest resistance to sliding, followed by conventional brackets and active brackets. They determined that a greater contact area between the slot, the archwire and the clip increases resistance to sliding[10]. The results in the paper by Gómez are consistent with the finding in the current finite element model, where there was lower resistance to sliding in passive self-ligating brackets than in active self-ligating brackets when sliding a 0.019" x 0.025" rectangular archwire. In addition, it was established that when stress isare measured in the model, both in brackets and archwires, a larger contact area between the slot and the archwire increases resistance to sliding.

The stress generated for this finite element model in the SmartClip® bracket showed that the beveled archwire had lower resistance to sliding than the rectangular archwire.

The stress generated for this finite element model in the In-Ovation® "R" bracket showed that the beveled archwire had lower resistance to sliding than the rectangular archwire.

Analysis of the stress generated for each bracket type in this finite elements model shows that there is lower resistance to sliding in passive self-ligating brackets than in active self-ligating brackets for 0.019" x 0.025" rectangular archwire.

It is recommended to use *in vitro* and *in vivo* studies to compare the results of this model and determine its clinical application because resistance to sliding is determined by many variables such as biological parameters (saliva, plaque, tissue response, etc.), mechanical characteristics (angle, degree of malocclusion, etc.) and physical and chemical properties of the material.

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Presence and count of *S. mutans* in children with dental caries: before, during and after a process of oral health education

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ABSTRACT

Dental caries is an infectious, multifactorial, localized, transmissible process that leads to the destruction of hard dental tissue. Streptococcus mutans is considered to be the main microorganism associated with its development. The aim of this study was to determine presence and count of S. mutans in saliva samples from children with dental caries before and after an educational process including interviews, lessons, lectures, educational workshops and recreational activities on the importance of oral care and hygiene. Twenty-three 3- to 6-year-old schoolchildren provided 3 unstimulated saliva samples: one before the educational process, one at 3 months and one at 6 months into the educational process. The samples were serially diluted and plated on Mitis Salivarius agar supplemented with bacitracin and 20% sucrose, and incubated anaerobically for 2 days at 37ºC. Presumptive S. mutans isolates were identified with biochemical tests. Before the beginning of the educational process, and at 3 and 6 months into the educational process, S. mutans was found, respectively, in 22 (95.6%), 15 (65.2%) and 10 (43.5%) of the 23 children. The S. mutans count was reduced by 64.8% and 86.6% at 3 and 6 months, respectively, compared to the levels found before the educational process. These results indicate that educational intervention produced a significant reduction in S. mutans levels in the saliva of children with dental caries at 3 and 6 months into the educational process.

Key words: *Dental caries, children, Streptococcus Mutans, Health education.*

Presencia y recuento de *S. mutans* en niños con caries dental: antes, durante y después de un proceso de educación en salud oral

RESUMEN

La caries dental es un proceso infeccioso multifactorial, localizado y transmisible que se caracteriza por la destrucción del tejido dental duro. Streptococcus mutans es considerado el principal microorganismo asociado al desarrollo de esta enfermedad. El objetivo de este estudio fue determinar la presencia y recuento de S. mutans en saliva de niños con caries dental antes y después de un proceso educativo. Con este fin se tomó saliva no estimulada de 23 niños con caries dental pertenecientes a un centro educativo con edades de 3 a 6 años. En todos los niños se tomaron 3 muestras de saliva: antes del proceso educativo y a los 3 y 6 meses de iniciado el proceso educativo. El proceso educativo consistió en entrevistas, enseñanzas, conferencias, talleres educativos y actividades lúdicas sobre la importancia del cuidado e higiene oral. Después de su recolección, las muestras de saliva fueron serialmente diluidas y sembradas en Agar Mitis Salivarius con bacitracina y sacarosa al 20%. Los medios de cultivo sembrados se incubaron en anaerobiosis durante 2 días a 37ºC y los aislamientos presuntivos de S. mutans se identificaron con pruebas bioquímicas. Antes del inicio del proceso educativo, a los 3 y 6 meses de iniciado el proceso educativo se encontró S. mutans, respectivamente, en 22 de los 23 niños (95.6%), en 15 de los 23 niños (65.2%) y en 10 de los 23 niños (43.5%). En cuanto al recuento de S. mutans, se encontró una reducción de 64.8 % y 86.6% a los 3 y 6 meses, respectivamente, en comparación a los niveles encontrados antes del inicio del proceso educativo. En conclusión, los resultados indican que la intervención educativa realizada produjo una reducción significativa en los niveles de S. mutans en saliva de niños con caries dental después de 3 y 6 meses de iniciado el proceso educativo.

Palabras clave: *Caries dental, niños, Streptococcus mutans, educación en salud.*

INTRODUCTION

Dental caries is an infectious bacterial disease which is multifactorial, chronic, localized, post-eruptive and transmissible, and leads to destruction of hard dental tissue [1-3]. Its development basically requires three factors sustained over time: a susceptible host, cariogenic microflora in the dental biofilm, and an adequate substrate. *Streptococcus mutans* (primarily serotype C), and to a lesser extent, *S. sobrinus* and *S. gordonii,* as well as *Lactobacillus* and *Actinomyces* species, in that order of frequency, are the primary microorganisms associated to the development and progression of dental caries [4,5].

Different studies have found a strong correlation between number of *S. mutans* colonies in the oral cavity and prevalence and incidence of dental caries[1,4,5]. In addition, as a result of dental treatments, *S. mutans* can cause bacteremia, systemic infections and subacute endocarditis[6].

The fact that *S. mutans* has been clearly recognized as the primary bacterial species involved in dental caries has led to the implementation of preventive and control measures tending to eliminate or reduce its presence in the oral cavity[3,6-8]. Different strategies have been designed for such purpose, but because of lack of continuity, systematization, regulation and supervision, they have not been very effective[8].

Dental caries is currently one of the most frequent diseases of the oral cavity, mainly affecting 5- to 12-year old children. Because it is a chronic disease, it progresses with age unless efforts are made to control it[9,10]. The distribution and severity of dental caries varies among regions, and its onset is frequently associated to nutritional, sociocultural, economic and environmental factors[11,12].

Different comparative clinical studies on 2.5- to 7-year-old children with dental caries have shown reduction in oral levels of *S. mutans* and *Lactobacillus* species[13-15] in dental biofilm and saliva, during and after conventional and non-conventional restorative treatments[13-15]. Although the results in these studies suggest that *S. mutans* and *Lactobacillus* species counts are lower after 6 months' treatment than at baseline, they also record a tendency for these microorganisms to become reestablished over time[13-15]. With regard to oral health educational strategies, very few studies to date present successful results of restorative dental treatment in the reduction of microorganisms that are important in dental caries[16-18].

In Colombia, no study has been published evaluating the impact of oral health educational intervention on presence and count of *S. mutans* in children with dental caries. The main aim of this study was therefore to determine *S. mutans* presence and count in saliva from children with dental caries before, during and after a 6-month oral health educational process.

MATERIALS AND METHODS

Study population

This was a longitudinal prospective study which met bioethical requirements for sampling and sample management. It included 23 3- to 7-year-old children with dental caries from the school Centro Educativo Fe y Alegría – José María Velaz- in the Suba locality of Bogota city, Colombia. Exclusion criteria were: use of topical or oral antimicrobial agents within 7 days prior to sampling; undergoing orthodontic treatment with fixed or removable appliances, and/or any kind of oral infectious process different from dental caries. Each child underwent an oral clinical examination to determine dental caries experience, performed by a single calibrated examiner using artificial light and a dentist's mirror. No X-ray was taken. Dental caries clinical stage was determined according to the International Caries Detection and Assessment System (ICDAS), which distinguishes early non-cavitated lesions from cavitated or dentinal lesions[19,20]. Of the 23 children with dental caries included in the study, 12 were diagnosed as ICDAS score 3 and 11 as ICDAS score 6.

S. mutans isolation, identification and count

Three saliva samples were taken from each of the 23 children: one before starting the educational process (baseline), one 3 months into the educational process, and a final sample 6 months into the educational process. The samples were taken between 8:00 and 10:00 a.m., with prior commitment from the children not to eat anything and not to brush their teeth before sampling. Spontaneous saliva (0.2-1 ml) was sampled by gentle suction with a sterile plastic pipette[21] and samples were immediately placed on ice to be transferred to the bacteriological laboratory. At the laboratory, the saliva samples were vortexed

for 30 seconds and serially diluted (1/10, 1/100 and 1/1000) with 0.05M phosphate buffer. For *S. mutans* selective isolation and count, 35 µl of the serial dilutions were inoculated in duplicate in Mitis Salivarius Bacitracin Agar (MSB; Difco Laboratories, Detroit, MI). MSB Agar contains digested pancreatic casein, proteose peptone No.3, proteose peptone, dextrose, 20% sucrose, dipotassium phosphate, trypan blue, crystal violet, agar, Chapman's tellurite and 0.2 U/ml bacitracin. The Petri dishes with MSB agar were incubated anaerobically (H_2:CO_2:N_2, 10:10:80) for 2 days at 37°C. After bacterial growth in the MSB agar, colonies with *S. mutans* morphological characteristics[22] were counted and expressed as colony-forming units (CFU) per ml of unstimulated saliva. Then 5 to 10 colonies per sample with *S. mutans* characteristics were examined by Gram stain, catalase test and the following biochemical tests: fermentation of raffinose, mannitol, melibiose, trehalose and inulin; hydrolysis of esculin in presence and absence of bile; urease; hydrolysis of arginine; and resistance to 2 U of bacitracin. The biochemical profile for *S. mutans* is positive fermentation of raffinose, mannitol, melibiose, trehalose and inulin; negative hydrolysis of esculin in presence of bile and positive hydrolysis of esculin in absence of bile; negative urease; negative hydrolysis of arginine; and resistance to 2 U of bacitracin. The commercial system Api 20S (bioMerieux, Marcy-létoile, France) was also used in the identification of strains.

Total aerobic microbial count

Total aerobes were counted in all saliva samples (before and after the beginning of the educational process). To do so, 35 µl of all the serial dilutions of saliva with phosphate buffer (as described above) were inoculated in blood agar (BHI agar supplemented with 5% lamb´s blood). After inoculation, the dishes of blood agar were incubated aerobically at 37°C for 48 hours. Total aerobic microbe CFU/ml were counted.

Oral health educational process

The educational process began with a survey to determine parents' and/or guardians' knowledge, attitudes and practices regarding oral health, acquired previously in family and school settings. After the survey, the following activities were conducted with parent/guardian-child pairs: 1. Talks and workshops for parents/guardians and children on the importance of good oral health; 2. Educational workshops on tooth brushing, evidence of bacterial plaque or dental biofilm, and the importance of good nutrition; 3. Workshops and feedback with parents/guardians and children over the 6 month educational period, on the importance of good oral hygiene; and 4. Recreational-educational workshops for the children on dental care and the practice of good oral health. Throughout the 6-month educational process, oral hygiene kits consisting of 22 ml dentifrice and a child toothbrush were delivered individually to each child every 15 days. In addition, throughout the 6-month study, the four oral hygiene strategies described above were reinforced every 15 days.

Statistical analysis

Descriptive statistics (frequency, mean, standard deviation and maximum and minimum values) and paired Student's t-test were used to establish differences between *S. mutans* counts in the groups with caries (ICDAS 3 and 6) before (baseline) and after beginning the educational process (at 3 and 6 months). Student's t-test was performed with the program IBM SPSS Statistics version 22.0 and the level of statistical significance was set at $p<0.05$.

RESULTS

Table 1 shows caries experience and demographics for the 23 children with dental caries included in the study. The 11 children (5 female and 6 male) diagnosed with ICDAS score 3 were within an age range of 3.5-4.5 years, average age 4.2 ± 0.24 years and dmft 3.7±3.1. The 12 children (5 female and 7 male) diagnosed with ICDAS score 6 were within an age range of 5.7-6.7 years, average age 6.5 ± 0.3 years and dmft 5.3±3.9.

Table 2 shows the frequency of *S. mutans* in baseline, 3- and 6-month samples in the 23 children with caries (ICDAS groups 3 and 6). The 11 children with ICDAS score 3 had *S. mutans* frequencies of 91% (10/11), 27% (3/11) and 9% (1/11), at baseline, 3 and 6 months, respectively. The 12 children with ICDAS score 6 had *S. mutans,* frequencies of 100% (12/12), 100% (12/12) and 75% (9/12) at baseline, 3 and 6 months, respectively. Aggregate results for both groups showed *S. mutans* frequencies of 95.7% (22/23),

65.2% (15/23) and 43.5% (10/23) at baseline, 3 and 6 months, respectively (Table 2).

Fig. 1 shows average *S. mutans* values in saliva from children at the two clinical stages of dental caries for the three sampling times. For clinical stage ICDAS 3, there was a significant reduction in *S. mutans* from baseline to month 3 (p=0.0221) and from baseline to month 6 (p= 0.0182). For reduction of *S. mutans* at clinical stage ICDAS 6, baseline to month 3 had p= 0.0526 and baseline to month 6 had p=0.0607.

Table 1: Baseline: Caries experience and demographics of children included in the study.

Caries Status	Children (n=)	Age range in years	Age* (years)	Sex	dmft*
ICDAS 3	11	3.5 – 4.5	4.2 ± 0.24	Female: 21.7% (n= 5) Male: 26.1% (n= 6)	3.7 ± 3.1
ICDAS 6	12	5.7 – 6.7	6.5 ± 0.3	Female: 21.7% (n= 5) Male: 30.4% (n= 7)	5.3 ± 3.9
Total	23	3.5 – 6.7	5.4 ± 1.2	Female: 43.5% (n= 10) Male: 56.5% (n= 13)	4.2 ± 1.1

* Values expressed as means (± standard deviation).

Table 2: Frequency of *S. mutans* in saliva samples at baseline and 3 and 6 months into the educational process.

Time	Presence of S. mutans	No. of children with:		Total
		ICDAS 3	ICDAS 6	
Baseline	Positive	10	12	22
	Negative	1	0	1
	Frequency	10/11 (91%)	12/12 (100%)	22/23(95.7%)
3 months	Positive	3	12	15
	Negative	8	0	8
	Frequency	3/11 (27%)	12/12 (100%)	15/23(65.2%)
6 months	Positive	1	9	10
	Negative	10	3	13
	Frequency	1/11 (9%)	9/12 (75%)	10/23(43.5%)

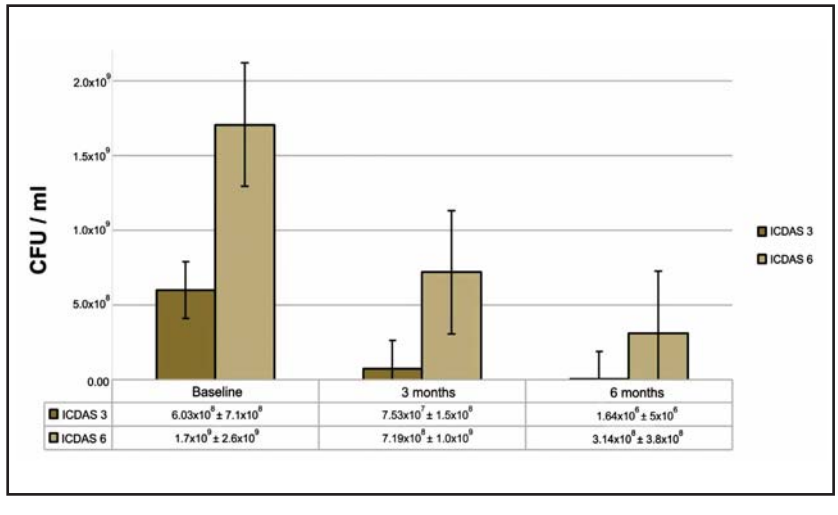

Fig. 1: S. mutans count (CFU/ml) in saliva of children classified by ICDAS into two clinical stages of dental caries. *CFU/ml (mean ± SD) are expressed on logarithmic scale. Paired Student t-test was used to analyze the differences between baseline and month 3, and between baseline and month 6. There was a significant reduction (p = 0.0221) for ICDAS 3 between baseline and month 3. There was also a significant reduction for ICDAS 3 between baseline and month 6 (p = 0.0182). No significant difference was found for ICDAS 6 between baseline and month 3 (p = 0.0526), between baseline and month 6 (p = 0.0607) or between month 3 and month 6 (p=0.0569).*

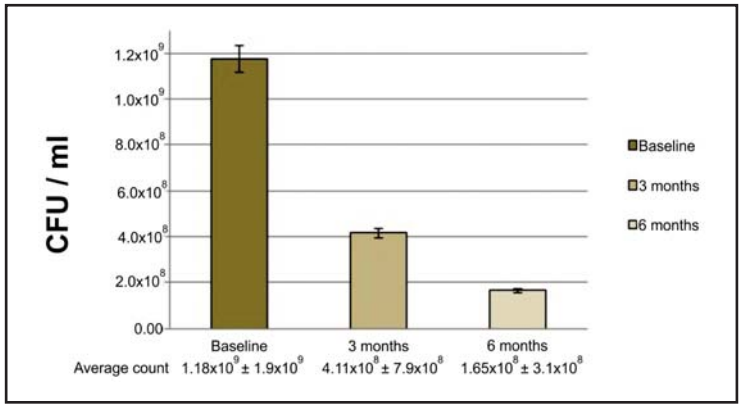

Fig. 2: S. mutans count (CFU/ml) found in saliva from children with dental caries (ICDAS 3 + ICDAS 6). * CFU/ml (mean ± SD) expressed on logarithmic scale. Paired student t-test was used to analyze the differences. S. mutans was reduced significantly between baseline and month 3 (p = 0.007) and between baseline and month 6 (p= 0.002).*

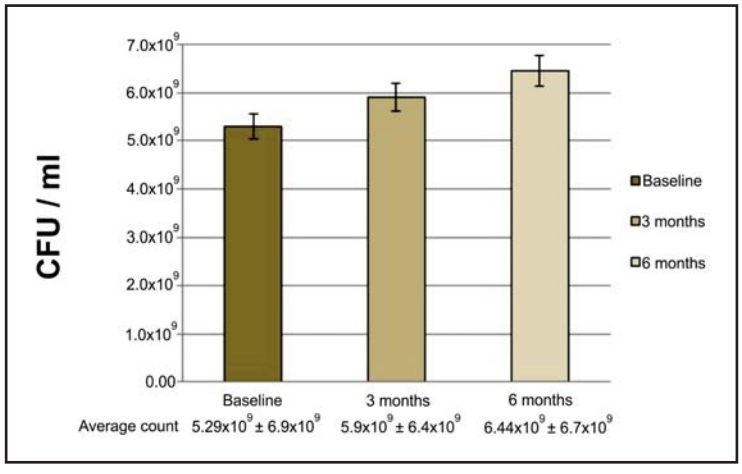

Fig. 3: Total aerobic microorganism count in saliva from children with dental caries (ICDAS 3 + ICDAS 6). * CFU/ml (mean ± SD) expressed on logarithmic scale. Paired student t-test was used to analyze the differences. No difference in aerobic microorganisms was found between baseline and month 3 (p=0.63417), between baseline and month 6 (p= 0.5711) or between month 3 and month 6 (p= 0.6124).*

Fig. 2 shows aggregate average values for *S. mutans* count in saliva from all 23 children with dental caries (ICDAS 3 + ICDAS 6) at baseline, month 3 and month 6. According to Student's t-test, average values for total count of *S. mutans* present in saliva decreased with statistically significant differences, comparing counts at baseline, month 3 and month 6 ($p<0.05$). Bacterial counts diminished significantly between baseline and month 3 (64.8%, p=0.007), and between baseline and month 6 (86%, p=0.02).

Fig. 3 shows total aerobe count, expressed as CFU/ml, for saliva samples from the 23 children with dental caries at baseline, 3 months and 6 months. Total average value at baseline was 5.29 x 10^9 CFU/ml, increasing at 3 and 6 months by 11.6% (5.9 x 10^9) and 21.8% (6.44 x 10^9) respectively, without statistically significant differences (Student t-test $p>0.05$, Fig. 3).

DISCUSSION

Dental caries is considered to be a multifactorial disease as a result of an imbalance in the oral ecosystem leading to the predominance of flora previously considered normal in the oral cavity, which then becomes pathogenic. Microbial imbalance, as well as the presence of different external factors, including nutritional, sociocultural, economic, environmental and individual behavioral, impact the development of dental caries. For a long time, *S. mutans* has been considered the primary etiological agent involved in the development of dental caries, playing a very important part in initial stages of dental enamel deterioration[23,24]. In children, *S. mutans* is associated to a specific period, known as "window of infectivity", which coincides with the eruption of teeth and occurs between 6 and 30 months of age, with higher risk from 18 to 30 months of age[24-26].

Clear recognition of *S. mutans* as the primary bacterial species involved in dental caries has led to the search for, and development and implementation of prevention and control measures aimed at eliminating or reducing *S. mutans* in the oral cavity [25]. Previous clinical studies on children with dental caries have shown the positive effect of restorative

treatment on the reduction of *S. mutans* levels[13-15]. On the other hand, with regard to educational strategies, very few studies conducted to date present similar results to those of restorative dental treatment for reduction of microorganisms which are important in dental caries[16-18].

In view of the fact that dental caries is basically a controllable disease and considering the failure of many preventive and therapeutic actions, greater efforts and resources need to be allocated to preventive and educational measures to reduce the presence and quantity of cariogenic microorganisms in the oral cavity, thereby reducing dental caries[27, 28]. Proper oral health education should be a strategy leading to a change in attitude in children and adults to achieve better individual and collective health and wellbeing indicators[27, 28]. In the current study, the 23 children complied with all the educational strategies proposed over the 6-month educational process. They received relevant oral health and hygiene education in accordance with the plans outlined: 1. Survey on knowledge of oral health; conferences and workshops for parents/guardians and children on the importance of good oral health and hygiene; 2. Personalized educational workshops on tooth brushing, identifying bacterial plaque, and the importance of healthy eating; 3. Recreational-educational workshops for the children on dental care and practicing good oral health. The oral health educational strategies were reinforced periodically, according to the proposed schedule, and also by leaving in classrooms leaflets, guides, notice boards and documents relevant to oral health and hygiene education. It should be highlighted that all the activities required constant, disciplined commitment of the dentists, preschool teachers and children's parents and/or guardians who participated in this project.

Regarding microbiological results, *S. mutans* count was found to be directly proportional to caries clinical stage, i.e., at higher levels of dental caries there were greater numbers of *S. mutans* colonies. The health and oral hygiene educational strategies enabled a reduction in *S. mutans* levels at 3 and 6 months, by 64.8% and 86% respectively, in children with dental caries (ICDAS 3 + ICDAS 6, Fig. 2). The educational strategy had greater impact on the reduction of *S. mutans* at 6 months in the group of children with ICDAS score 3, where *S. mutans* was only detected in 1 of the 11 children. The impact was weaker in the children with ICDAS score 6, since at the end of the 6 months *S. mutans* persisted in 9 of the 12 children, at concentrations higher than 500.000 CFU/ml.

The high counts at the end of the 6-month educational process in children with ICDAS score 6 may indicate the high risk for originating carious stages and that educational strategies alone were insufficient. It is highly likely that future educational activities will need to cover longer times, and/or strategies to achieve absence and/or reduction of *S. mutans* levels will need to be strengthened, as proposed in other studies[27- 29], even considering joint use of educational strategies and dentifrices with greater bactericidal power[29]. A study by Kumar et al. [29] analyzed the effect of oral health education and the use of fluoridated and non-fluoridated dentifrices on oral health status, using *S. mutans* and *Lactobacillus* counts before beginning the educational strategy, and 3, 6 and 12 months post-treatment. They found post-treatment reductions in the counts of both microorganisms, both in children who used fluoridated dentifrices and in those who used non-fluoridated dentifrices. However, the reduction was significantly greater at 3, 6 and 12 months post-treatment in children who used fluoridated dentifrices, and the authors suggest that this may have been due to the bactericidal action of fluoride. Another study on children found that the long-term use of fluoride mouthrinse led to lower levels of *S. mutans*[30]. However, long-term studies have been proposed to clarify the effect of chemotherapeutic agents on the reduction of *S. mutans* and their impact on caries incidence[31].

It is important to clarify that although the current study focuses the microbiological risk factor, other studies have shown that long-term caries prevention needs to act simultaneously on all risk factors involved in this disease[1-4, 19, 25].

Different studies have shown the direct association between dental caries incidence and *S. mutans* presence and quantity in the oral cavity[32-35]. Similarly, the current study found high frequency (95.7%, or 22 out of 23 children) and high *S. mutans* colonization rate at baseline in both groups of children with dental caries (ICDAS 3 and ICDAS 6). The current study also determined levels of aerobic microorganisms before and after the oral health educational strategies. In general, the counts remained stable, with slight, non-statistically signi-

ficant increase over time. These levels of aerobic microorganisms may also be indicative of stable oral health, in which the place left by the reduction of *S. mutans* colonies achieved through educational strategies was taken over by aerobic microorganisms, which are compatible with the healthy microflora that is part of the oral microbial ecology[8].

This study was intentionally sought children with dental caries. There was predominance of male children, with 56.5% (13/23), and overall average age was 5.4 ±1.2 years. Oral examination detected that 47.8% (11/23) of the children had ICDAS score 3 and 52.2% (12/23) had ICDAS score 6. Overall average dmft index was 4.2 ±1.1 for the 23 children. Other studies conducted in Colombia, which also used ICDAS and dmft diagnostic criteria, have reported similar results[36-38]. These results indicate a high rate of dental caries in the child population studied, which may serve as a sentinel and be an indicator of the results of the public health strategies being implemented[39]. On the other hand, microbiological analyses of saliva and monitoring children under 6 years old with dental caries may be useful for identifying individual and community risk of caries, and for improving treatment and delaying the process of *S. mutans* colonization and multiplication in the oral cavity[40].

To conclude, the results of this study indicate that the oral health educational process conducted led to a significant reduction in *S. mutans* level in the saliva of children with dental caries, 3 and 6 months into the educational process.

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Relation between periapical lesions and sinus membrane thickening assessed by Cone Beam Computed Tomography

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ABSTRACT

The aim of this study was to evaluate thickening of the Schneiderian membrane and to determine its association with periapical pathologies, using computerized cone beam tomography. An observational, analytical, cross-sectional retrospective study was conducted. A total 179 maxillary sinuses were evaluated using CBCT. The presence of sinus membrane thickening and its association with unhealthy teeth was analyzed. Results are shown as percentages with 95% confidence intervals (95%CI); Chi square test was used with a significance level of 5%.

Sinus membrane thickening was detected in 70 cases (39%; 95%CI=32% to 46%) and no sinus membrane thickening was observed in 109 (61%; 95%CI=54% to 68%) ($p<0.05$). The 70 cases showing sinus membrane thickening included 46 of odontogenic origin (66%; 95%CI=54% to 76%) and 24 (34%; 95%CI=24% to 46%) of non odontogenic origin ($p<0.05$). The frequency of odontogenic causes followed a heterogeneous distribution ($p<0.05$): penetrating caries, failing endodontic therapy, root remnants, deep restorations, implants, periodontal pathology. The main cause was caries (46%; 95%CI=32% to 60%) followed by failing endodontic therapy (26%, 95% CI=16% to 40%). The frequency distribution of involved teeth was uneven ($p<0.05$), with tooth 16 (33%; 95%CI=21% to 47%) being the most frequently involved, followed by tooth 26 (30%; 95%CI=19% to 45%).

The high incidence of sinus pathology of odontogenic origin shows the need for interdisciplinary work involving dentists and ear-nose-throat specialists. Caries, inadequate restorations, periodontal lesions, implants, and the presence of root remnants are the main causes of Schneiderian membrane thickening. The use of CBCT for diagnosis and treatment planning allows detecting maxillary sinus membrane thickening and determining its association with an odontogenic etiology.

Key words: *maxillary sinus, periapical tissues, cone beam computerized tomography, Schneiderian membrane.*

Lesiones periapicales y engrosamiento de la membrana sinusal, su relación y valoración a través de Tomografía Computarizada de Haz Cónico

RESUMEN

El objetivo del presente trabajo fue evaluar el engrosamiento de la membrana de Schneider y determinar su asociación con patologías periapicales, mediante tomografía computarizada cone beam (CBCT). Se realizó un estudio observacional, analítico, retrospectivo y transversal. Un total de 179 senos maxilares fueron evaluados utilizando CBCT. Se analizó la presencia de engrosamiento de la membrana sinusal y su asociación con piezas dentarias sin vitalidad pulpar. Los resultados se muestran como porcentajes con intervalos de confianza del 95% (IC del 95%). Se utilizó la prueba de Chi cuadrado con un nivel de significación del 5%.

Se detectó engrosamiento de la membrana sinusal en 70 casos (39%; IC del 95% = 32% a 46%) y no se observó engrosamiento de la membrana sinusal en 109 (61%; IC del 95% = 54% a 68%) ($p < 0.05$). Los 70 casos que mostraron engrosamiento de la membrana sinusal incluyeron 46 de origen odontogénico (66%; IC del 95% = 54% a 76%) y 24 (34%; IC del 95% = 24% a 46%) de origen no odontogénico ($p<0,05$). La frecuencia de las causas odontogénicas siguió una distribución heterogénea ($p<0.05$): caries penetrantes, tratamiento endodóntico deficiente, restos radiculares, restauraciones profundas, implantes, patología periodontal. La principal causa fue la caries (46%; IC 95% = 32% a 60%), seguida por endodoncia deficiente (26%, IC 95% = 16% a 40%). La frecuencia se distribuyó en forma heterogénea entre las distintas piezas ($p<0,05$). Las piezas más afectadas fueron la 16 (33%; IC95=21% a 47%) y la 26 (30%; IC95=19% a 45%).

La alta incidencia de patología sinusal de origen odontogénico implica la necesidad del trabajo interdisciplinario entre odontólogos y otorrinolaringólogos. Caries, restauraciones inadecuadas, lesiones periodontales, implantes y la presencia de restos radiculares son las principales causas del engrosamiento de la membrana de Schneider. El uso de CBCT para el diagnóstico y la planificación del tratamiento permite detectar el engrosamiento de la membrana del seno maxilar y determinar su asociación con una etiología odontogénica.

Palabras clave: *seno maxilar, tejidos periapicales, tomografía computada cone beam, membrana de Schneider.*

INTRODUCTION

The maxillary sinus is the main paranasal sinus, and is located in the body of the maxilla. At the end of dento-maxillo-facial growth, the floor of the maxilla presents its final anatomical features. At this point, the teeth and the sinus floor are in close anatomical proximity, and this close anatomical relationship only differs among individuals[1]. Accurate knowledge of the anatomy of the maxillary sinus is paramount to the general dentist, since it allows recognizing the diversity of sinus pathologies of oral origin, and preventing iatrogenic complications associated with dental and surgical procedures.

The maxillary sinuses are anatomically located in an intermediate position between the nasal and oral cavities, and are therefore susceptible to invasion by pathogenic bacteria through the nasal ostium or the oral cavity[2].

The maxillary sinuses consist of two pairs of symmetrical cavities that occupy the central part of the maxilla. The lower wall extends from the roots of the upper premolars to the roots of the molars. The canine roots may occasionally project into the sinus. The premolar and molar roots are generally immediately below the floor of the maxillary sinus; this proximity explains the causal relationship between dental pathology and pathologies of the maxillary sinus, such as sinusitis[3]. Sinus pathology is common in patients presenting dental pathologies such as periapical lesions, cysts, and tumors[4].

Alterations in the Schneiderian membrane can manifest as uniform thickening and hypertrophy, and/or the presence of polyps and solid or cystic masses, which can be observed by computed tomography. The finding of mucosal thickening in cone beam computerized tomography (CBCT) *per se* does not allow establishing differential diagnosis between acute and chronic disease, and clinical evaluation is necessary for accurate diagnosis[5].

Radiographic techniques, such as panoramic radiographs, Waters radiograph, computed tomography, magnetic resonance imaging, and CBCT, are frequently used to diagnose maxillary sinus pathology. Cone beam computed tomography is considered the "gold standard" for diagnosing maxillary sinus pathology because of its high resolution and ability to visualize bone and soft tissues[4].

Maxillary sinusitis is a common pathology worldwide, and has significant health impacts. A substantial proportion of maxillary sinus cases is of odontogenic origin, given the proximity of the roots of maxillary posterior teeth to the sinus floor[6].

The bony wall that separates the maxillary sinus from the tooth roots can vary in thickness, reaching up to 12 mm. In some cases the wall is absent, and the roots are only covered by a membrane[7].

Dental pathologies of infectious origin are very common, though they account for only 5 to 10% of all cases of maxillary sinusitis. Sinus membrane integrity can be disrupted in a number of situations, as is the case of trauma causing iatrogenic displacement of a tooth or implant, treatment of periapical lesions, periodontal pathologies, teeth with extensive caries, and teeth with apical infections. Certain materials, such as gutta-percha, are inert and therefore cause no reaction in the sinus. Hence, no treatment is necessary in the absence of symptoms. However, in the event that chronic or acute sinusitis develops, the condition will not be resolved satisfactorily until the cause is eliminated[8].

The aims of the present work were to evaluate thickening of the Schneiderian membrane and to determine its association with periapical pathologies, using computerized cone beam tomography.

MATERIALS AND METHODS

Cone beam computed tomography scans performed for diagnosis of pathology and treatment planning of patients seen at the Department of Endodontics of the School of Dentistry, University of Buenos Aires, between March 2016 and September 2016 were analyzed. The project was approved by the Ethics Committee of the School of Dentistry of the University of Buenos Aires (Res Nº 921/14).

CBCT scans of male and female patients over the age of 18 years, requested for treatment (assessment of missing teeth for implant treatment) or diagnostic purposes (evaluation of existing pathologies, endodontic evaluation), and which allowed visualization of the entire maxillary sinus in the coronal and axial sections were included in the study. CBCT scans corresponding to patients who smoked, had systemic disease, abused drugs, were taking some type of medication at the time of the CBCT, and/or failed to sign the informed consent form to participate in the study were excluded from the study.

All CBCT scans were taken with the same Scanner (Kodak 9000C 3D, FOV 5 X3.75 cm), at the Imaging diagnosis Department of the School of Dentistry, University of Buenos Aires.

The present observational, analytical, cross-sectional, retrospective study was conducted by a single operator, who evaluated the computerized images, first observing the panorex and then thoroughly examining the para-axial sections of the involved teeth. In keeping with the analyzed bibliography, sinus alteration was considered sinus mucosa thickening when membrane thickness was greater than 2mm [9-12].

Membrane thickness was determined on coronal sections, taking the maximum thickness as reference (Fig. 1). Odontogenic origin was evaluated taking into account the following 4 situations: 1) teeth with penetrating caries, root remnants with periapical lesions, and teeth with endoperiodontal lesions (with severe attachment loss); 2) teeth with restorations and recurrent caries; 3) endodontically treated teeth with unsuccessful treatment outcome (visible periapical lesions/lesions on CBCT scan), or with filling material inside the sinus; 4) no dental cause; this group comprised all cases of non-odontogenic origin[13, 14].

The recorded data were analyzed to obtain absolute frequencies and percentages. Ninety-five percent confidence intervals (95%CI) for percentages were obtained using the Wilson score method[15]. Frequencies were compared using Chi square test Statistical significance was set at 5%.

RESULTS

A total 179 CBCT scans were analyzed; significant sinus membrane thickening was observed in 70 cases (39%; 95%CI = 32% to 46%, Chi-square = 8.50; gl = 1; $p < 0.05$, Fig. 2). A significantly higher number of cases of membrane thickening were of odontogenic origin (66%; 95%CI = 54% to 76%), accounting for 46 cases as compared to 24 cases of non-odontogenic etiology 34%; 95%CI = 24% to 46%). With regard to cases of sinus pathology of odontogenic origin, an uneven frequency distribution of the different causes was observed (Chi-square = 36.96; gl = 5; $p < 0.05$; Fig. 3). Specifically, the frequency of caries and endodontic treatment (72%; 95%CI = 57% to 83%) was significantly higher than that of the remaining causes (Chi-square = 8.70; gl = 1; $p < 0.05$); however, no statistically significant differences were observed between caries and endodontic treatment (Chi-square = 2.45; gl = 1; $p = 0.12$), nor when comparing root remnants, restorations, implants, and periodontal pathology (Chi-square = 2.69; gl = 3; $p = 0.44$).

An uneven frequency distribution was also observed when analyzing the frequency of affected teeth in cases of sinus pathology of odontogenic origin (Chi-square = 42.00; gl = 7; $p < 0.05$; Fig. 4). In this group of cases, the tooth most frequently associated with sinus pathology was tooth 1.6 (33%; 95%CI = 21% to 47%), and was significantly more involved than teeth 1.4 (Chi-square = 15; gl = 1; $p < 0.05$), 15 (Chi-square = 12.25, gl = 1; p <

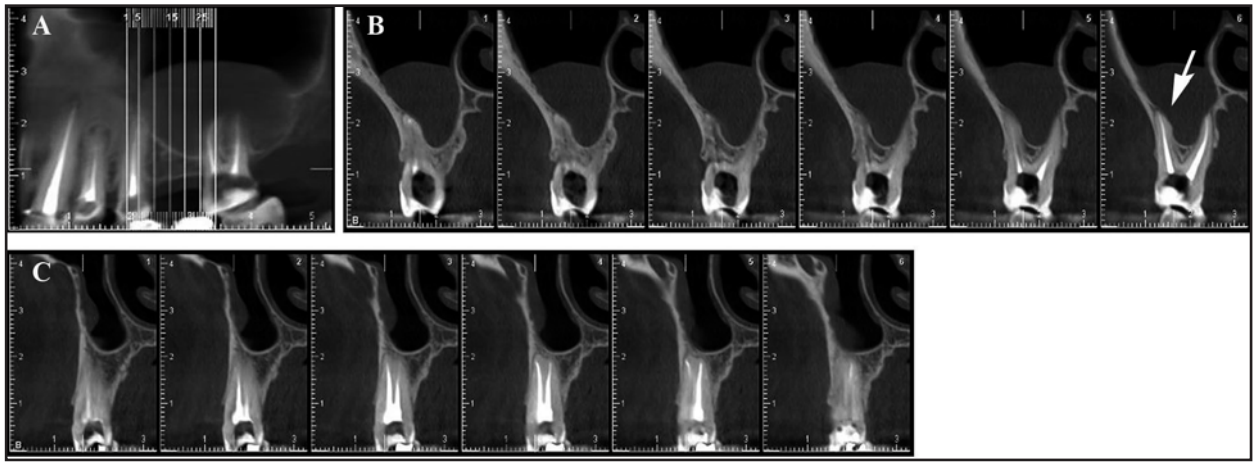

Fig. 1: A- Panorex shows membrane alteration in the right sector. B and C- The images of the corresponding sections allow evaluating the close relation between the root and the maxillary sinus floor, and the increase in the thickness of the Schneiderian membrane (↑).

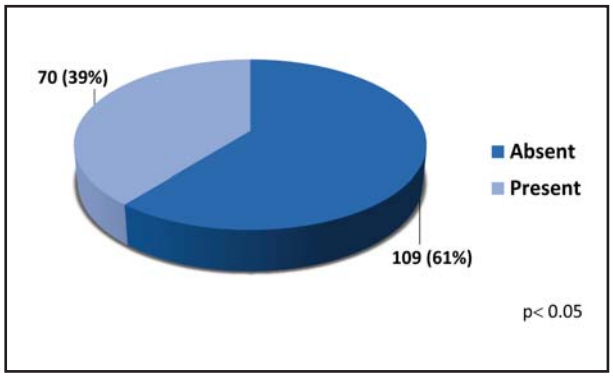

Fig. 2: Presence of sinus membrane thickening. Absolute frequencies are shown with corresponding percentage in brackets (Chi-square: p < 0.05).

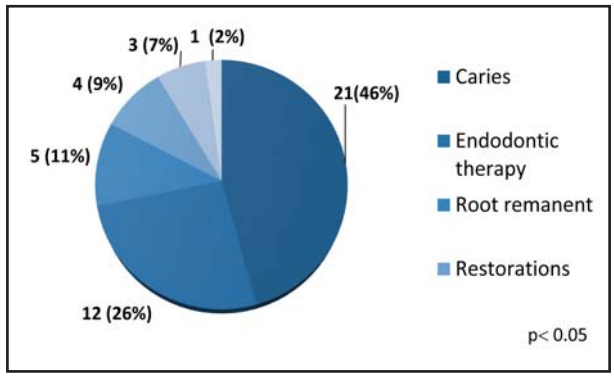

Fig. 3: Frequency distribution of odontogenic causes of sinus pathology. Absolute frequencies are shown with the corresponding percentage in brackets (Chi-square: p < 0.05).

0.05), 1.7 (Chi-square = 5.00; gl = 1; p < 0.05), 2.4 (Chi-square = 8.00; gl = 1; p < 0.05) and 2.5 (Chi-square = 12.25, gl = 1; p < 0.05); no significant differences were observed between the frequency of tooth 1.6 and teeth 2.6 (Chi-square = 0.03; gl = 1; p = 0.85) and 2.7 (Chi-square = 2.91; gl = 1; p = 0.09).

DISCUSSION

Maxillary sinusitis is a frequent pathology, and is associated with odontogenic etiologies in a number of situations. Thus, dentists must be familiar with its diagnosis and prevention. The present article sought to evaluate cases of sinus mucosal thickening greater than 2mm, considered a sign of pathology in keeping with the literature, associated with dental pathology.

Although odontogenic maxillary sinusitis has traditionally been reported to account for approximately 10 to 12% of all cases of maxillary sinusitis, a review of more recent reports suggests a higher prevalence, ranging between 30 and 40%[13]. According to a study on 770 cases, the percentage of maxillary sinusitis cases of odontogenic origin was 37-40.6%. In the present study, 66% of cases showed membrane thickening associated with odontogenic causes. Therefore, development of caries could be considered the major cause of sinus pathology. The second most frequent cause in the series of cases studied here was endodontic treatment, with no statistically significant differences in frequency as compared to caries. Usually, the roots of premolars and molars are separated from the floor of the maxillary sinus by a dense cortical bone of varying thickness. Sometimes, however,

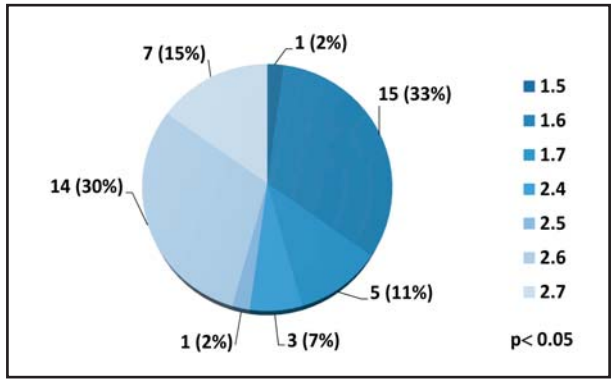

Fig. 4: Frequency distribution of teeth associated with sinus pathology of odontogenic origin Absolute frequencies are shown with the corresponding percentage in brackets (Chi-square: p < 0.05).

only the mucoperiosteum separates the sinus from the teeth[14]. Clearly, this anatomical proximity could explain the origin and development of an inflammatory process that could cause thickening of the sinus mucosa.

A number of studies found iatrogenic injury during dental procedures and chronic periodontitis to be the most common cause of spread of oral pathogens into the maxillary sinus, and considered them the main cause of sinus mucosa thickening[15]. According to a review of 35 studies, iatrogenic etiology accounted for 55.9% of cases. Extrusion of dental filling materials during endodontic treatment accounted for 22.27% of iatrogenic etiology[7]. In the present study, endodontic pathology was the second cause of sinus membrane thickening, though no significant differences were observed with regard to the frequency of caries-related cases. Examination of the CBCT scans

included in the present study allowed detecting cases of overfilling with materials, such as gutta-percha, with no sinus alteration. This could be an indication of the biocompatibility of said material, which would account for the absence of an inflammatory process at the apical level.

Proper chemical preparation, maintaining apical permeability, and use of the corono-apical technique for endodontic treatment, contribute to decreasing the likelihood of spread of microorganisms to the maxillary sinus.

Periodontal disease has long been reported as an etiological factor of sinus mucosa inflammation. For example, in their 1943 study in human cadavers, Bauer et al. demonstrated direct diffusion of oral sepsis to the maxillary sinus. More recently, Abrahams et al.[16] reported the incidence of sinusitis in patients with periodontal disease to be two-fold that of patients without periodontal disease. Recognition of the close relationship between inflammation of periapical tissues and damage to the sinus membrane led to describing the pathological entity referred to as the "endo-antral syndrome. In recent years, however, the incidence of these effects has decreased, likely due to factors such as improved oral hygiene and preventive techniques related with periodontal disease. In the present study, periodontal lesions accounted for 2% of cases of sinus membrane thickening associated with direct spread of pathogens to the maxillary sinus.

According to Panico and Adell[17,18] development of sinusitis associated with implant placement is infrequent. Reviews published in the literature found that maxillary sinus floor augmentation prior to surgical placement of a dental implant resulted in sinus alteration in 4.17% of cases, and in inadequate implant position or implant migration in 0.92% of cases[7]. In the present study on a total 179 maxillary sinuses analyzed using CBCT, sinus thickening was observed in 39% of cases, 6% of which were associated with the presence of implants in close proximity to the affected maxillary sinus.

None of the cases studied here showed signs of apicoectomy. It is of note that the latter procedure can lead to a complication that is observed less frequently than expected, likely because most professionals prefer extracting the tooth rather than performing this technique in view of the associated high risk of accidental perforation to the sinus due to its proximity[19]. Nevertheless, reports such as that by Freedman et al. involving 472 apicoectomies, none of which resulted in sinusitis, show that there is no contraindication to performing apicoectomy in antral teeth, despite their proximity to the maxillary sinus [20].

A review of 35 articles on maxillary sinusitis of odontogenic origin showed the upper molar region to be more frequently associated with maxillary sinus alterations (47.68%). The first upper molar was the molar tooth most frequently associated with maxillary sinusitis, with a 22.51% incidence, followed by the third molar tooth (17.21%) and the second molar tooth (3.97%). With regard to the upper premolar region, it was affected in 5.96% of cases only; the second premolar was the most affected premolar tooth (1.98%), and the canine was involved in only 0.66% of cases of maxillary sinusitis of odontogenic origin[7].

In keeping with the aforementioned study, the results obtained here showed that the tooth most frequently involved in sinus alteration was tooth 1.6, accounting for 33% of cases. It was significantly more affected than teeth 1.4 (0%), 1.5 (2%), 1.7 (11%), 2.4 (7%) and 2.5 (2%), but did not differ significantly compared to teeth 2.6 (30%) and 2.7 (15%).

Examination of sinus membrane thickening by cone beam computed tomography allows detecting the presence of sinus pathology. Findings must be correlated with the clinical condition of the patient. Thorough examination of the para nasal sinuses and visualization of the osteomeatal complex on axial and coronal sections obtained by computed tomography without contrast is essential to evaluate permeability of the ostium and for proper treatment planning. The retrospective nature of the present study does not allow establishing a "cause-effect" relationship between periapical pathology and changes in the maxillary sinus. Further prospective studies are necessary to correlate clinical and radiographic data and confirm the present findings. Lastly, a larger sample size and including cases classified according to disease severity could reveal more significant associations between periapical and/or periodontal pathologies and changes in the sinus mucosa.

CONCLUSION

According to the results obtained in the present study, the high incidence of sinus pathology of odontogenic origin implies the need for interdisciplinary work involving dentists and ear-throat specialists. Carious processes, poor restorations, periodontal lesions, implants, and the presence of root remnants are the main causes of sinus mucosa thickening.

The use of CBCT for diagnosis and treatment planning allows identifying the presence of maxillary sinus membrane thickening and determining whether it is associated an odontogenic origin.

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Implantes Dentales

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

IADR
International Association for Dental Research

On October 17, 18 and 19, the 51st Annual Meeting of the Argentine Society for Dental Research (SAIO) -the Argentine Division of the IADR- was held in Los Cocos, Córdoba Province. Two hundred and sixty 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 200 research works were presented.

We were honored by the presence of Francisco Tamarit, José Miguel Amenábar Céspedes and Ronell Bologna Molina. Special features of the 51st Anniversary meeting included the following lectures:
- "Current challenges in higher education, science and technology, 100 years after the University Reform" by Dr Francisco Tamarit
- "Integrating graduate and postgraduate degrees through scientific research projects" by Dr José Miguel Amenábar Céspedes
- "The low publication rate of scientific production in Argentine dental research: a pending debt" by Dr. María Elina Itoiz
- "Progress in research into odontogenic tumors" by Dr. Ronell Bologna.

A highlight of the meeting was the appointment of Dr. Ángela Argentieri as Life Member.

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

Ten propolsals for research projects were presented during the executive meeting of a Research Group related on the subject on the subject of encouraging 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 Clinical Science Professionals, Colgate-Palmolive Award for Basic Science Professionals, Colgate-Palmolive Award for Students, Federa Award, "Rodolfo Erausquin" Award, "Rodolfo Erausquin" Subsidy, Oral Health "Anibal Cobanera" Award, "Rins de David" Award, National Academy of Dentistry Award, Dental Materials Group "Prof. Dr. H. Maddalena" Award, Orthodontics Group Award, and Fundación CREO.

The Editorial Committee of the Acta Odontológica Latinoamericana met with SAIO members during the event.

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

LI REUNIÓN CIENTÍFICA ANUAL SAIO

17 al 19 de Octubre 2018

LOS COCOS, CÓRDOBA / **ARGENTINA**

Opening ceremony

Opening ceremony. From left to right: Sebastián Fontana, Susana Molgatini, Gabriel Sánchez.

Dr. Sebastián Fontana, President of the 50th Annual Scientific Meeting (left)
Dr. Susana Molgatini, President of the SAIO (centre)
Dr. Gabriel Sánchez, President-elect of the SAIO (right)

Life member:
Prof. Dr. Ángela Argentieri (left)
President of Argentine Division:
Prof. Dr. Susana Molgatini (right)

SAIO Board of Directors
From left to right: Drs. Gabriel Sánchez, Ángela Argentieri, Carlos Rozas, Daniel Olmedo, María Inés González, Esteban Funosas, Susana Molgatini, Ana Sorazabal, Aldo Squassi and Analía Garrofé.

Organizing Committee of the 51st Annual Meeting
From left to right: Drs. Centeno, Carpentieri, Rozas, Morelatto, Carleto Korber, Ponce, Gallará, González, Rocamundi, Plavnik, Fontana, Rubio, Brunengo, Sezin and Soto.

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† Resultado de un estudio de remineralización en comparación con una crema dental regular con floruro, ambas con 1450 ppm de floruro.

‡ Resultados de un estudio de 6 meses para evaluar las mejoras en caries del esmalte usando el método QLFTM (Fluorescencia Cuantitativa Inducida por Luz) contra una crema dental regular sólo con fluoruro, ambas con 1450 ppm de fluoruro.

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