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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.

Dos revisores, seleccionados por la mesa editorial dentro de especialistas en cada tema, harán el estudio crítico de los manuscritos presentados, a fin de lograr el mejor nivel posible del contenido científico de la revista.

Para facilitar la difusión internacional, se publicarán los trabajos escritos en inglés, con un resumen en castellano o portugués. La revista publicará, dentro de las limitaciones presupuestarias, toda información considerada de interés que se le haga llegar relativa a actividades conexas a la investigación odontológica del área latinoamericana.

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Although **Acta Odontológica Latinoamericana (**AOL) will accept original papers from around the world, the principal aim of this journal is to be an instrument of communication for and among Latin American investigators in the field of dental research and closely related areas.

AOL will be devoted to original articles dealing with basic, clinic and epidemiological research in biological areas or those connected with dental materials and/or special techniques.

Clinical papers will be published as long as their content is original and not restricted to the presentation of single cases or series.

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Short communications which fall within the scope of the journal may also be submitted. Submission of a paper to the journal will be taken to imply that it presents original unpublished work, not under consideration for publication elsewhere.

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The journal will publish, within budget limitations, any data of interest in fields connected with basic or clinical odontological research in the Latin America area.

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Informa que a partir del Volumen 27 (2014) la revista se editará en formato digital con el *Sistema de Gestión de Revistas Electrónicas* (Open Journal System, OJS). Se utilizará el *Portal de publicaciones científicas y técnicas* (PPCT) del Centro Argentino de Información Científica y Tecnológica (CAICYT-CONICET). A partir de este volumen la revista será de acceso abierto (Open Access). Esta nueva modalidad no implicará un aumento en los costos de publicación para los autores.

Comité Editorial

Acta Odontológica Latinoamericana
Wishes to inform that as of Volume 27 (2014) the journal will be published in digital format with the *Open Journal System* (OJS), employing the *Portal de publicaciones científicas y técnicas* (PPCT) of the Centro Argentino de Información Científica y Tecnológica (CAICYT-CONICET). From this volume on, the journal will be open access (Open Access). The publication fees for the authors will remain unchanged.

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DIFFERENTIAL REACTIVITY OF SALIVARY IgA AND IgG AGAINST *Streptococcus mutans* PROTEINS IN HUMANS WITH DIFFERENT CARIES EXPERIENCE

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ABSTRACT

Dental caries is an infectious disease which still constitutes a public health concern. It begins at an early age and is caused mainly Streptococcus mutans (S. mutans). The aim of this study was to characterize the salivary humor immune response to S. mutans proteins in patients with caries, with history of caries and without caries, in order to determine which S. mutans proteins participate in the immunological response in subjects with different caries experience. Saliva was collected by spontaneous salivation for 5 minutes from 60 subjects aged 18 to 30 years, classified according to their caries experience as: without caries (Group I), with active caries (Group II) and with history of caries (Group III). The antigens derived from S. mutans by sonication were recognized by salivary IgA and IgG by Western Blot. The results showed that all the individuals studied recognized S. mutans proteins with molecular weights in the range of 8 to 191 kDa, with similar recognition profiles for salivary IgA and IgG. Subjects without caries recognized the 29 kDa protein, also known as S. mutans Antigen A, via salivary IgA, differing from patients with caries and history of caries, who recognized it via IgG. The protective response against S. mutans is mediated by IgA. To conclude, a differential response to the 29 kDa protein between study individuals may be indicative of resistance to dental caries and may have a protective role in the induction of IgA antibodies against dental caries, as found in the group without caries, in contrast to subjects with active caries and history of caries.

Key words: *Streptococcus mutans; saliva; Immunoglobulin A; Immunoglobulin G; Dental caries; Immunoblotting.*

REACTIVIDAD DIFERENCIAL DE IgA E IgG SALIVALES CONTRA PROTEINAS DE *Streptococcus mutans* EN HUMANOS CON DIFERENTES ESTADIOS DE CARIES DENTAL

RESUMEN

La caries dental es una enfermedad infecciosa que continua siendo un problema de salud pública, inicia a temprana edad y es causada principalmente por Streptococcus mutans (S. mutans). El objetivo de este estudio fue caracterizar la respuesta inmune humoral salival, ante las proteínas de S. mutans, en pacientes con caries, historia de caries e individuos libres de caries, para así establecer que proteínas de S. mutans participan en la respuesta inmunológica en los diferentes estadios de caries. La saliva de 60 individuos entre 18 y 30 años de edad, clasificados de acuerdo al estado de caries: libres de caries (grupo I), caries activa (grupo II) e historia de caries (grupo III), se colectó por salivación espontánea durante 5 minutos. Los antígenos derivados de S. mutans por sonicación, fueron reconocidos por IgA e IgG salivales por Western Blot. Los resultados mostraron que todos los individuos estudiados reconocen las proteínas de S. mutans en el rango de 8 a 191 kDa de peso molecular con perfiles de reconocimiento similares para IgA e IgG salival. Se encontró que los sujetos libres de caries reconocen por IgA salival la proteína de 29 kDa, también llamada Antígeno A de S. mutans, de manera diferente que los pacientes con caries e historia de caries quienes reconocieron la proteína vía IgG. La respuesta protectora frente a S. mutans es mediada por IgA. En conclusión, una respuesta diferencial a la proteína de 29 kDa entre los individuos estudiados, puede ser indicativo de resistencia a la caries dental y tener un papel protector en la inducción de anticuerpos IgA frente a la caries dental, como se encontró en el grupo libre de caries, a diferencia de los sujetos con historia de caries y caries activa.

Palabras clave: *Streptococcus mutans; saliva; Inmunoglobulina A; Inmunoglobulina G; Caries Dental, Inmunodetección.*

INTRODUCTION

Dental caries is the local destruction of hard tissues in the tooth by acid products from bacterial fermentation of carbohydrates. It is usually chronic, site-specific, multifactorial, dynamic, and results in physiological imbalance between the mineral portion of the tooth and bacterial plaque, when the reduction in pH leads to loss of minerals over time.

It is an infectious disease which is produced not by a single microorganism but by many, and it can be stopped at any point in time [1-6].

Dental caries is an epidemiological problem. The latest National Oral Health Study (Estudio Nacional de Salud Bucal, ENSAB III) conducted by Colombia's Ministry of Health, reports that "caries history in permanent dentition occurs in 19.9% of seven-year olds and 71.9% of twelve-year-olds. The percentage increases during adolescence to 89.5%. At thirty-five years of age, the DFMT index (D: decayed, F: filled, M: missing, T: teeth) is 15" [7].

Given the infectious nature of caries, there is worldwide interest in developing a vaccine to prevent it. This vaccine would focus on neutralizing the virulence factors of *S. mutans*, the most widely studied causal agent, which participate in the pathophysiological mechanisms of dental caries, such as adherence to teeth and the production of insoluble glucans, by inducing a response of specific salivary antibodies of the type secretory immunoglobulin A (sIgA) and serum immunoglobulins A and G (IgG), the latter as part of the salivary component through blood extravasation through the gingival fluid [8-10].

Research groups such as Chía, Lehner and Naspitz conducted studies with the aim of characterizing the humoral immune response to different *S. mutans* proteins, in order to correlate them with the absence or presence of caries. It is deduced from the results that there is a high frequency of infection, suggesting that the immune response against *S. mutans* may be non protective. Only a small percentage of the population is unaffected by dental caries and it has not been possible to determine a factor that would explain this natural resistance. The differences in immune response to *S. mutans* between individuals who suffer from the disease and the small percentage who do not, are unclear.

Under the hypothesis that these differences that maintain or keeps an individual caries free, depends on that the humoral immune response in without caries individuals, is directed against particular antigens of *S. mutans* that neutralize its pathophysiological mechanisms and despite of being present in oral cavity, it does not cause the disease[11-26], the aim of this study was to characterize the specific salivary IgA- and IgG-mediated response against *S. mutans* proteins in patients with caries, with history of caries, and without caries.

Finding *S. mutans* antigens that induce a protective response against dental caries in naturally sensitized humans would contribute additional strategies for mass protection of the population through a vaccine designed with those antigens.

MATERIALS AND METHODS

Population and Sample

The study included 60 subjects aged 18 to 30 years, with no systemic and oral pathology other than dental caries, who visited the clinics at the School of Dentistry at Pontificia Universidad Javeriana. After a review of the clinical history and an oral examination, they were classified into 3 groups according to DFMT index (D: decayed, F: filled, M: missing, T: teeth), with 20 subjects per group, as follows: (I) without caries: DFMT equal to zero; (II) with active caries: DFMT greater than zero and D equal to 1 or more, and (III) with history of caries: DFMT greater than zero, D equal to zero and F equal to 1 or more, who received treatment for caries at least 6 months prior to being included in the study. After subjects had signed informed consent, saliva samples (5 ml) were taken by spontaneous salivation. The project was approved by the Ethics and Research Committee of the School of Dentistry at Pontificia Universidad Javeriana (CIEFOUJ).

S. mutans colony-forming units count from saliva

To determine the number of colony-forming units (CFU) per ml, we used a quantitative method for which a series of saliva dilutions were made in saline solution (1:10, 1:100, 1:1000). Aliquots (50 ml) of each dilution were plated on mitis salivarius agar (Becton Dickinson) and incubated for 48 hours in microaerophilic conditions.

For the immunological analysis, the remaining saliva in each sample was clarified by centrifugation (Eppendorf Centrifuge) at 10,000 rpm for 15 minutes and stored at –20° C (General Electric) until it was used.

Preparation of *S. mutans* extracts

To prepare *S. mutans* extracts we employed a strain from the American Type Culture Collection (ATCC, #31989) grown in Todd Hewitt broth (Difco Bacto Todd Hewitt Broth) supplemented with 1% glucose (Carlo Erba) for 18 hours at 37°C, in microaerophilic atmosphere.

The purity of the culture was tested by Gram stain (Labsar). Bacterial culture density was adjusted to

tube 4 on the McFarland scale, corresponding to a concentration of $1.2x10^9$ cells/ml in phosphate buffer solution (PBS: Sigma) – glycine (Pharmacia Biotech) (0.5g/ml). Having previously standardized the optimum conditions, the bacteria were suspended in PBS-glycine supplemented with protease inhibitor (Tris (0.1M) (Pharmacia Biotech), 10% n-propanol (Merck), 2% EDTA, phenylmethanesulfonyl fluoride (PMSF) 2 mM (Sigma) and subjected to 25 sonication cycles (Fisher Scientific) at 20W for one minute, with 1-minute rests, in ice throughout the process.
The *S. mutans* extract was analyzed by SDS-PAGE, and a Bradford test (Bradford Sigma reagent; Human spectrophotometer) was used to quantify proteins, obtaining a concentration of 2747 mg/ml.

Reactivity of salivary IgA and IgG to *S. mutans* proteins

SDS-PAGE

To separate the proteins contained in *S. mutans* extracts, we used the SDS-PAGE technique (Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis) on 10% polyacrylamide gels. The first well on each gel served as a wide range molecular weight pattern from 7.2 to 200 kDa (BIO-RAD). The other wells were filled with 110 µg/well *S. mutans* extract. Electrophoresis was done in tris-glycine buffer (Pharmacia Biotech) at 150 mV (BIO-RAD. Mini Protean Electrophoresis Cell) for approximately 1 hour. For the electrophoresis control, some gels were stained with 0.25% Coomassie Blue (BIO-RAD) for 5 minutes.
The gels were transferred to a PVDF membrane (Pall Life Sciences, BIO-RAD transfer chamber) for two hours at 100 V and 150 mA. The transfer was confirmed with 1% India ink (Pelikan) in PBS, and the gels were stained with Coomasie blue (Sigma). The membranes were rinsed for 5 minutes with TBS buffer (Invitrogen), pH 7.5: Tris (10 mM), NaCl (150 mM) and Tween 20 (Sigma) (0.1%). After rinsing, they were blocked with skimmed milk (Proleche) (5% in TBS) for two hours under constant stirring (Barnstead-Thermolyne magnetic stirrer), after which the rinsing procedure was repeated. The membranes were stored on filter paper at 4°C until they were used.

Immunoblotting

Prior to use, membranes were cut into 0.5 cm wide strips and incubated with the saliva from each subject diluted 1:5 in TBS for IgG and 1:12 for IgA for two hours under constant stirring at room temperature. After 5 rinses with TBS, they were incubated for one hour with the secondary human anti-IgA (Sigma) and anti -IgG (Sigma) antibodies conjugated with peroxidase, diluted in TBS in a proportion of 1:100,000 for IgG and 1:250,000 for IgA, having been previously standardized.

Chemiluminescence

After rinsing, the membranes were placed on the film (Light sensitive CL-Xposure Film, Pierce). Chemiluminescent substrate (Luminol Supersignal West Dura Extended Duration Substrate, Pierce) was added, which had been previously prepared following a five-minute protocol. The films were developed by autoradiography and the molecular weight for each protein in the extract, recognized by each protein, was calculated by preparing a calibration curve using the molecular weight patterns and relative mobility of each protein.

Analysis of information

The data obtained were expressed as percentages of individuals in each group that recognize each *S. mutans* protein band. Comparison between groups was done using the Chi-square test, and significance was established at values of $*p<0.05$.

RESULTS

The increase in colony forming units is related to degree of caries

Data reported in the literature [27] show that the number of *S. mutans* colony-forming units was lower in subjects without caries than in those with caries in 3- to 5-year-old children. In our study, the number of *S. mutans* CFU in saliva of adult individuals was measured to determine whether this reflect the caries experience in these groups. The group with active caries (Group II) had a significantly higher number of CFU ($p=0.001$) than the other two groups (history of caries (Group III) and caries-free (Group I)). The latter two had low counts with no difference between them (Fig. 1).

Salivary IgA response to *S. mutans* proteins in different caries experience

Analysis of the results from the 60 subjects included in this study, shows that 29 different *S. mutans* proteins are recognized by IgA, with molecular weights ranging from 8 to 191 kDa (Table 1, Fig. 2).

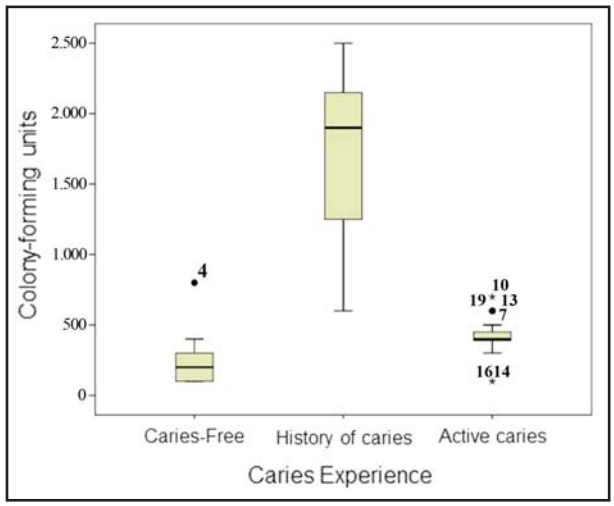

Fig. 1: Microbiological analysis. The graph shows interquartile distribution (median and range) of the colony-forming units (CFU) obtained from plating saliva on mitis salivarius agar, for subjects from each study group. The dots represent extreme values with respect to the median.

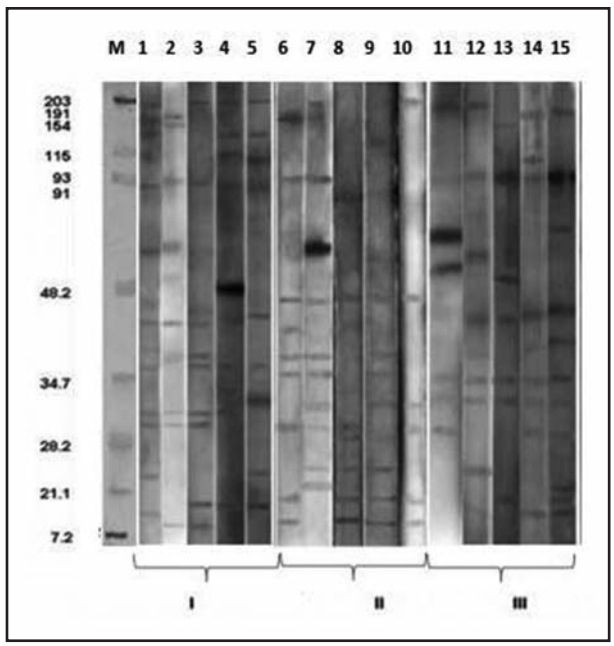

Fig. 2: Response of salivary IgA to S. mutans proteins. Image showing chemiluminescence of 5 samples per group, revealing recognition by salivary IgA of different S. mutans proteins of individuals in the three study groups. Lines 1-5 Group I (active caries); lines 6-10 Group II (history of caries) and lines 11-15 Group III (without caries). Line M is the pattern of molecular weight used. The S. mutans extracts obtained by sonication were subjected to electrophoresis to separate proteins, transferred to PVDF membranes and incubated with saliva from the study subjects. Human anti-IgA conjugated with peroxidase was used for immunoblotting. It was developed by chemiluminescence.

Table 1: Percentage of individuals with different caries experience that recognize S. mutans proteins via salivary IgA and IgG. Proteins separated by molecular weight.

	Salivary IgA			Salivary IgG		
MW (kDa)	*AC*	*HC*	*CF*	*AC*	*HC*	*CF*
191	35	70[a]	75[a]	20	15	15
163	0	15	0	0	0	0
154	20	30	40	35	40	30
139	0	5	5	10	0	0
125	45	10	40	0	15	5
119	5	5	0	0	0	0
113	0	5	5	5	5	5
107	0	10	0	0	0	0
91	55[a]	85[a]	100[a]	100[a]	90[a]	100[a]
78	0	5	0	0	0	0
66	0	5	0	0	0	0
54	0	5	0	0	0	5
49	5	10	10	0	10	10
45	95[a]	40	65[a]	55[a]	50[a]	50[a]
43	15	15	35	90[a]	50[a]	40
39	65[a]	60[a]	85[a]	75[a]	85[a]	55[a]
35	30	60[a]	45	90[a]	75[a]	50[a]
33	5	15	5	15	0	0
32	95[a]	95[a]	100[a]	75[a]	60[a]	85[a]
29	60[a]	35	80[a]	60[a]	60[a]	30
26	50[a]	35	20	40	30	30
23	35	15	30	15	30	20
21	15	15	5	0	0	0
20	5	10	5	10	5	5
17	20	30	30	20	0	0
15	50[a]	15	50[a]	35	30	40
12	5	5	5	0	0	0
10	55[a]	20	55[a]	35	0	25
8	5	0	0	0	0	0

MW: molecular weight in kilodaltons (kDa). AC group of individuals with active caries. HC: group of individuals with history of caries. CF: group of individuals caries-free. [a]: recognized by 50% or more of the study individuals, corresponding to the most immunogenic proteins.

On average, each individual recognized 8 proteins, in a range of 5 to 12. The response with highest diversity was observed in an individual with active caries and in an individual without caries. Table 1 shows the different percentages of salivary IgA response to the different *S. mutans* proteins.

Patients in Group I have the highest diversity in recognition (average 9 proteins of different molecular weights), followed by Group II (8 different proteins), and Group III (7 proteins). Comparative analysis between groups shows no significant difference in diversity in recognition among the three groups ($p>0.05$). The individual with the highest recognition (12 different proteins) belonged to the caries-free group. Table 1 describes the most immunogenic antigens for each study group.

Chi-square analysis of the number of individuals who recognize *S. mutans* proteins of different molecular weights via IgA showed significant differences for some proteins between groups. Only the recognition of the 29 kDa protein showed differences among all three groups ($p=0.015$). Highest recognition occurred in individuals without caries, followed by those with active caries, and lastly those with caries history (Fig. 3).

Recognition of 191 kDa and 91 kDa proteins is significantly lower in the active caries group than in the group without caries, but similar between the group with caries history and the group without caries. The 125 and 10 kDa antigens are recognized by fewer individuals in the group with caries history than in the other two groups ($p= 0.036$) (Fig. 4).

Salivary IgG response to *S. mutans* proteins in different caries experience

The IgG from the 60 individuals included in this study recognized 21 different *S. mutans* proteins ranging in molecular weight from 10 to 191 kDa (Table 1, Fig. 3). On average, each individual recognized 6 proteins, in a range of 2 to 12 proteins. The response with highest diversity was observed in an individual with active caries. Table 1 shows the percentages of specific salivary IgG-mediated response to *S. mutans* proteins.

On average, the patients from the group with active caries recognized the greatest diversity of proteins of different molecular weights (8 proteins), followed by the group with caries history (6) and group without caries (6). Similarly to the observations for IgA, no difference was found among groups regarding the diversity recognized via IgG. However, patients who recognized the highest number of different proteins belong to the group with active caries (18) and the group with history of caries (12). Table 1 shows the most immunogenic antigens for each group.

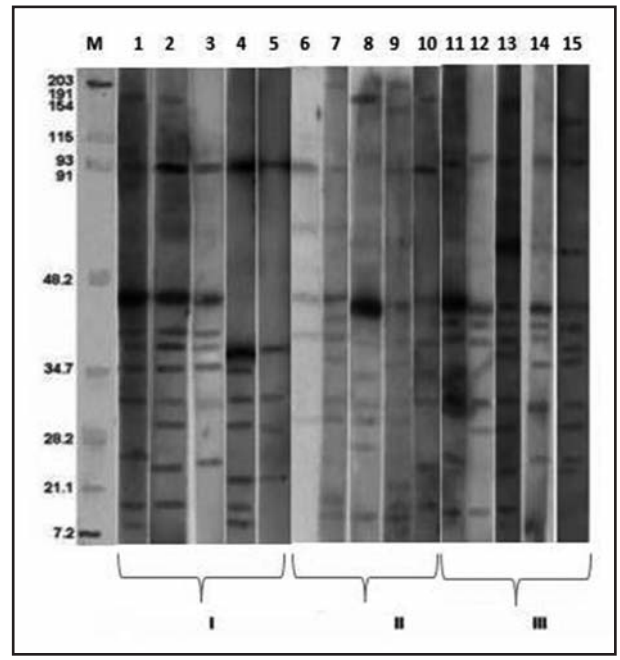

Fig. 3: Response of salivary IgG to S. mutans proteins. Image showing chemiluminescence of 5 samples per group, revealing recognition by salivary IgG of different S. mutans proteins of individuals in the three study groups. Lines 1-5 Group I (active caries); lines 6-10 Group II (history of caries) and lines 11-15 Group III (without caries). Line M is the pattern of molecular weight used. The S. mutans extracts obtained by sonication were subjected to electrophoresis to separate proteins, transferred to PVDF membranes and incubated with saliva from the study subjects. Human anti-IgG conjugated with peroxidase was used for immunoblotting. It was developed by chemiluminescence.

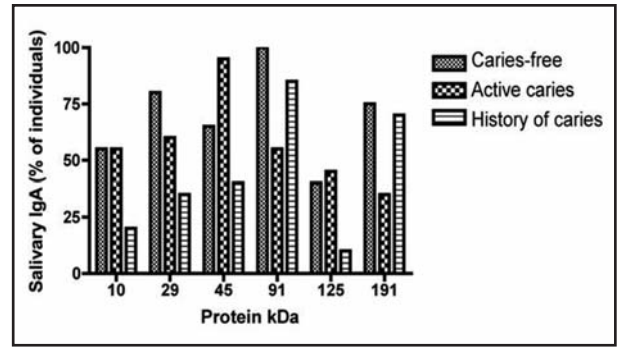

*Fig. 4: IgA recognition of S. mutans proteins in study groups. Behavior of recognition frequencies by IgA of Streptococcus mutans proteins of different molecular weights, which showed some significant differences among the three study groups. The signals obtained by chemiluminescence were taken as proteins recognized by salivary IgA, and the molecular weights of each peptide in the extract recognized was calculated by preparing a calibration curve using molecular weight patterns as a reference and the relative mobility of each protein. Frequencies between groups was compared by Chi-square and a value *p< 0-05 was considered significant.*

Statistical analysis by Chi-square of the number of patients whose IgG recognizes *S. mutans* proteins of different molecular weights showed significant differences between groups for proteins of molecular weights 17kDa (p= 0.01376), 36 KDa (p= 0.0179) and 43 KDa (p= 0.0029), which were recognized in greater numbers by patients from the group with active caries than by individuals without caries. The 29 kDa protein was recognized less by subjects without caries (p=0.09), and the group with history of caries did not recognize the 10 KDa antigen (p= 0.01720) (Fig. 5).

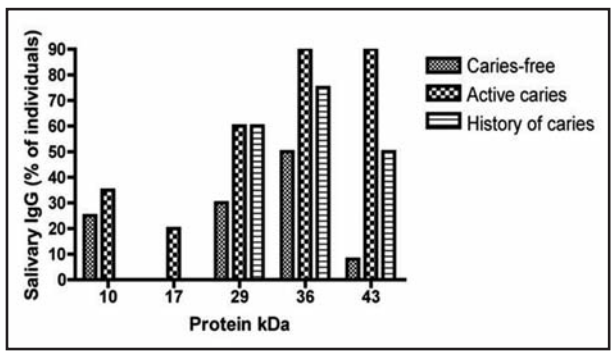

*Fig. 5:IgG recognition of S. mutans proteins in study groups. Behavior of recognition frequencies by IgG of Streptococcus mutans proteins of different molecular weights, which showed some significant differences among the three study groups. The signals obtained by chemiluminescence were taken as proteins recognized by salivary IgG, and the molecular weights of each peptide in the extract recognized was calculated by preparing a calibration curve using molecular weight patterns as a reference and the relative mobility of each protein. Frequencies were compared between groups by Chi-square and a value *p< 0-05 was considered significant.*

Analysis of simultaneous IgA and IgG recognition of *S. mutans* 29 kDa protein

An analysis of the recognition behavior of proteins with significant differences between groups showed a pattern in the recognition of the 29 kDa protein by the two types of IgA and IgG antibodies simultaneously, which differed significantly among the three groups (p=0.00001).

Of the 14 caries-free individuals that recognized the 29 kDa protein via IgA, only 3 recognized it simultaneously via IgG, in contrast to the active caries group, where most of the individuals that recognized the protein via IgA (12) also did so via IgG (8) (p=0.0001716) (Fig. 6).

Of the 6 individuals with caries history who recognized the 29 kDa protein via IgA, 4 also recognized it via IgG. The simultaneous expression in this group differs significantly from the group without caries (p=0.0002994) (Fig. 6).

There was no significant difference in the simultaneous expression of IgA and IgG against the 29 kDa *S. mutans* protein between the group that has the disease and the group that has had the disease (p=0.7709) (Fig. 6).

DISCUSSION

This study was designed to determine whether individuals with and without caries recognize *S. mutans* antigens differently, which might explain natural resistance. It looks at one group of persons free from the disease and another group of diseased patients, in turn divided into two groups, considering that persons with active carious lesions have higher

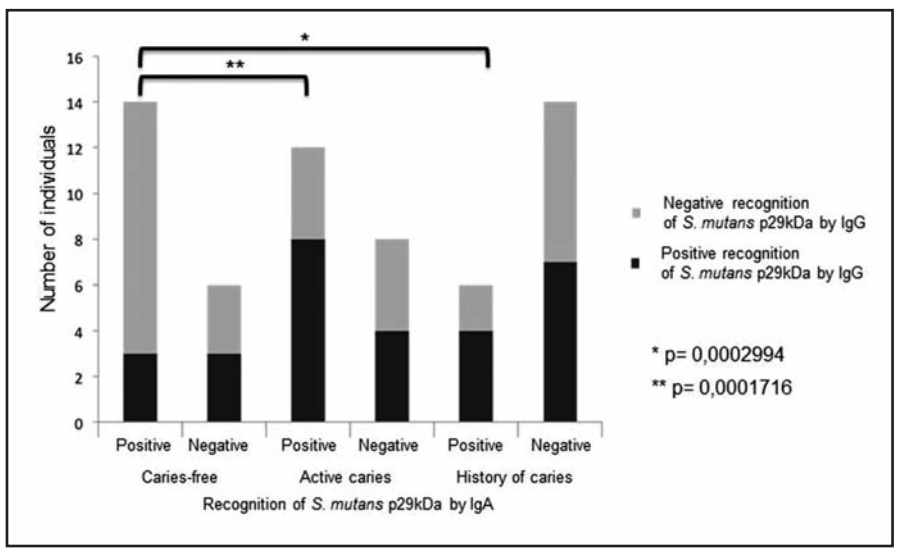

Fig. 6: Simultaneous expression of IgA and IgG against the 29 kDa S. mutans protein. Shows the number of individuals in each study group that recognized 29 kDa S. mutans antigen by the IgA type antibody (whole bar); the black section on each bar represents simultaneous recognition in these individuals via IgG, and gray section shows individuals that did not recognize it via IgG. There are significant differences among the three groups (p= 0.00001), when the number of individuals recognizing the S. mutans 29 kDa protein via IgA and IgG is compared by Chi-square.

levels of *S. mutans* than those who have had the disease but no longer have lesions (group with caries history), even though they might have the same susceptibility, as reported in the consensus in the literature about the association between caries and *S. mutans* [28].

The profile of *S. mutans* proteins recognized by salivary IgA and IgG was established for each patient. Salivary IgA represents local immunity, while IgG represents systemic immunity but has an influence on the response in the oral cavity because it is present in whole saliva through the gingival fluid [29].

There is controversy in the literature about the salivary IgA and IgG response regarding the number and type of *S. mutans* proteins recognized. For example, Chia et al. report that the proteins most often recognized by salivary IgA and serum IgG are those having molecular weight 60 to 63 kDa [30], whereas in our study, this molecular weight range was not recognized by salivary antibodies in most of the study subjects. The most immunogenic proteins for both classes of antibodies reported here were those with molecular weights 91, 45, 32 and 29 kDa. The 191 kDa protein induced an IgA response, while the 43, 38, 36 and 32 kDa proteins primarily activated an IgG response.

Chia et al.[30] report that a protein similar in size to the 191 kDa protein was recognized by IgG in most patients, but not by IgA, as it was in our study. This antigen is particularly relevant due to its proximity to the molecular weight reported for the protein PAc, also known as antigen I/II (Ag I/II),[31] which is one of the primary *S. mutans* virulence factors.

Other *S. mutans* proteins are immunogenic for different population groups; for IgA, the proteins of approximately 92 kDa,[30] 170 and 190 KDa [32-34] (which may correspond to glycosyltransferases (GTFs) and Ag I/II, respectively) and for IgG, the 39 and 97 kDa proteins [27].

In addition to different studies reporting diversity in recognition, the molecular weights of some of the most immunogenic proteins are different according to the populations studied, possibly due to genetic influence of the Major Histocompatibility Complex (MHC)[35-40] and other factors such as diet, oral hygiene and exposure to fluoride [32-34].

The comparative analysis of results between groups showed that all three groups recognize a similar number of proteins; however, the response differs according to the experience of disease. For IgA, recognition is similar in caries-free individuals and patients with caries history, while it differs in patients with active caries. In contrast, IgG recognition is similar in patients with caries history and active caries, and different in caries-free individuals, whose response is lower in most cases.

Within this comparison it was found that only the 29kDa protein is significantly recognized by IgA in individuals without caries compared to subjects with caries history. In contrast, subjects without caries tend to respond less via IgG to the same protein, compared to the other two groups, which respond similarly. The 29 kDa protein has been described as *S. mutans* antigen A since 1979 [41] and has been a candidate for a vaccine antigen after being tested in monkeys, because it induced significantly high levels of serum IgG [42], but the quantity of IgA induced has not been assessed.

This study assessed simultaneous recognition by IgA and IgG of *S. mutans* 29 kDa protein, and found that there are significantly more individuals who recognize it via IgG in the groups which had or have the disease, although most individuals who recognize it via IgA belong to the group without caries. Taking into account that a lower quantity of *S. mutans* was found in caries-free individuals, it suggests that the quantity of specific IgA against the 29 kDa protein and low amount of IgG protected them at the local level.

In contrast, among individuals who had or have the disease, although there are fewer individuals with specific IgA for the 29 kDa protein, most individuals simultaneously recognized it via IgG. This may be because higher numbers of CFUs were found in their mouths than in caries-free individuals. The IgA present in groups with caries or with history of caries did not control the disease. This may be explained from two points of view: (1) the response observed in individuals with history of caries may be due to a mechanism of tolerance because the microorganism present in the oral cavity may be ingested in small amounts during food intake, [43] which could in turn explain why having had the disease is considered one of the primary risk factors for having it again [44]; and (2) subjects with active caries who also had the highest number of CFUs probably trigger a humoral immune response due to the bacterial challenge, but it is not as high and not enough to be effective.

A high percentage of individuals with active caries recognized the 45 kDa protein primarily, as well as

the 125 kDa protein via IgA, and the 36, 43, 17 and 10 kDa proteins via IgG. These proteins have not been described in the literature as dominant natural antigens in terms of antigenicity and immunogenicity, and may be related to the activity of the disease [30] and correlated to the fact that caries-free individuals and individuals with caries history reacted poorly to them. This behavior reflects differences between the two types of response: the local IgA-mediated response and the systemic IgG-mediated response. In the presence of large quantities of *S. mutans*, an initial systemic IgG-mediated response is activated, which subsequently changes to IgA in terms of predominance. The process that takes place to generate the antibodies found in saliva is different for each type of immunoglobulin [29]; this is one of the few studies reported in the literature describing the IgG response to *S. mutans* in the oral cavity.

Even though deficient memory response to *S. mutans* antigens has been shown,[45] information gathered from the literature and data from our study suggest that the protective response may be mediated more by IgA than by IgG. Our study found a greater number of caries-free individuals who responded to several *S. mutans* proteins which have been classified in the literature as potential vaccine antigens, including the 191 kDa, 29 kDa and 38 kDa proteins; [30] in contrast to the results reported by Smith et al., who did not find significant differences in the pattern of response between patients with and without *S. mutans* infection [15].

With regard to the antigen corresponding to the PAc protein, Takahashi et al. claim that IgA type antibodies against it play a part in protection against colonization by *S. mutans* [31]. A higher IgA response has been shown in caries-free individuals than in patients with active caries [39]. In our study, the 191 kDa protein was similarly recognized by IgA in caries-free individuals and in those with caries history, but differently between individuals with caries history and those with active caries. The deviation of the response towards antigens which are not relevant in the pathophysiology of the disease, when there is a large number of microorganisms in the environment, may be an S. *mutans* evasion mechanism, in addition to the fact that it has been shown that this protein is recognized by adults but not by children, showing how the immune response matures as the individual grows [27].

It is important to design studies that help clarify the role of antibodies against the protein PAc in the disease, considering that artificial induction of maturation of the response to this antigen early in life could provide protection against dental caries [46].

To date, it has not been possible to find antigens that explain natural protection from the disease. Differences might be observed by conducting studies of specific antibody avidity and affinity. Until these aspects are studied, the possibility of finding an *S. mutans* antigen which will activate a protective response should not be left aside.

Other topics that should be considered are *S. mutans* evasion mechanisms that enable it to avoid or regulate the specific immune response, and knowledge of tolerance mechanisms that may be generated by the microorganism and its constant ingestion. Future strategies for controlling dental caries should aim to control these mechanisms.

CONCLUSIONS

All the individuals studied have antibodies that recognize *S. mutans* proteins via salivary IgA and IgG, but the protective response against *S. mutans* seems to be more mediated by local IgA than by IgG. The 29 kDa protein, formerly known as Antigen A, was recognized mainly via IgA in individuals without caries, and simultaneously via IgG in individuals with caries history and active caries, showing an association between this protein in the protection of caries-free subjects, and tolerance and low response in subjects with caries history or active caries, respectively.

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DENTAL STATUS AND DENTAL TREATMENT DEMANDS IN PRESCHOOLERS FROM URBAN AND UNDERPRIVILEGED URBAN AREAS IN MENDOZA CITY, ARGENTINA

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ABSTRACT

The aim of this study was to establish the association between dental status and demand for dental care in preschoolers at urban and underprivileged urban schools in the city of Mendoza.

Dental status was diagnosed in a purposive sample of preschoolers at urban schools (Group U: n = 148) and underprivileged urban schools (Group UnU: n = 155) in Greater Mendoza city, by determining the following indicators: (a) caries-free children (%), and (b) dmft/DMFT and its discriminated components, including active non-cavitated enamel caries. The characteristics of demand for care were determined using an ad hoc structured questionnaire. The following were determined: frequency distributions and confidence intervals for categorical variables, measures of central tendency and dispersion, tests for differences in means (Student's t test), association (chi squared) and correlation among variables (Pearson's r), at a significance level p< 0.05.

Comparison of dental status variables between groups showed significantly higher values in group UnU for: d+D tooth (= 5.4± 3.8; t = 2.887; p = 0.004); dmft+DMFT (= 5.7±4.1; t = 0.466; p = 0.020); d+D surface (= 7.62± 6.2; t = 0.956; p = 0.014); f+F surface (= 0.12±4.5; t = 2.71; p = 0.007) and percentage of caries-free children (x^2= 25.377; p= 0.018). The following trends were found in this group: higher demand on the government subsystem, fewer visits to the dentist (x^2= 7.02, p = 0.008) and greater difficulty in getting appointments (x^2= 19.91, p 0.001). Frequency of visits was associated to the severity of dental status (x^2 = 19.412; p 0.001), but no correlation was found between frequency of visits during the past year and dmft+DMFT (Pearson's r coefficient = 0.091; p = 0.0426)

Group U showed preferential demand for the private or "obra social" (trade union managed health insurance) systems (x^2 = 78.85 p = 0.00) and there was no statistically significant association between visits to the dentist and dmft+DMFT categories (x^2 = 2.781; p = 0.427), although there was direct correlation between frequency of visits during the past year and dmft+DMFT (Pearson's r coefficient = 0.486, p = 0.000).

Preschoolers at UnU schools had higher caries indicators than preschoolers at U schools. For UnU the demand for care was related to the severity of dental status and situations of urgency, while U preschoolers demanded dental care in both health and disease, with a tendency to greater adherence to treatment.

Actions to promote oral health in preschoolers should take into account both internal and external barriers to access to and use of oral health services.

Key words: *Dental caries; dental health services; school age populations; access to health care.*

ESTADO DENTARIO Y DEMANDA DE ATENCIÓN ODONTOLÓGICA EN PREESCOLARES DE AREAS URBANAS Y URBANO-MARGINALES EN MENDOZA, REP. ARGENTINA

RESUMEN

El objetivo de este estudio fue establecer la asociación existente entre el estado dentario y la demanda de atención en alumnos de nivel inicial asistentes a escuelas del ámbito urbano y urbano-marginal de la ciudad de Mendoza.

Material y métodos: Sobre una muestra intencionada de alumnos/as asistentes a cuatro escuelas de nivel inicial de ámbito urbano (Grupo AU: n=148) y de ámbito urbano-marginal (Grupo AUM: n=155) del Gran Mendoza, se realizó el diagnóstico del estado dentario determinando los siguientes indicadores: (a) niños libres de caries (%), (b) ceod/CPOD y sus componentes discriminados, incluyendo caries adamantinas activas no cavitadas. Las características de la demanda de atención fueron establecidas mediante una encuesta estructurada ad hoc. Se establecieron distribuciones de frecuencias e intervalos de confianza para varaibles categóricas, medidas de tendencia central y dispersión, pruebas de diferencias de medias (t de Student), de asociación (chi cuadrado) y de correlación entre las variables (r de Pearson), con un nivel de significación de p< 0.05.

Resultados: Al comparar las variables del estado dentario entre ambos grupos se encontraron valores significativamente mayores en el grupo AUM para: c+C diente (=5,4± 3,8; t= 2,887; p=0,004); ceod+CEOD (=5,7± 4,1; t=0,466; p=0,020); c+C superficie (=7,62± 6,2; t=0,956; p= 0,014); o+O superficie (=0,12± 4,5; t=2,71; p=0,007) y porcentajes

de niños libres de caries (x^2= 25,377; p= 0,018). En este grupo, se registraron las siguientes tendencias: mayor demanda al subsistema estatal, menor asistencia a la consulta dental (x^2= 7.02, p= 0,008) y mayor dificultad para obtener turno (x^2=19,91, p 0,001). La frecuencia de las consultas estuvo asociada con la gravedad del estado dentario (x^2=19,412; p 0,001), pero no se registró correlación entre la frecuencia de las consultas durante el último año y ceod+CPOD (coeficiente r de Pearson =0,091; p=0,0426)

El grupo AU demandó preferentemente en los subsistemas privado o de seguridad social (x^2=78,85 p=0,00) y no existió asociación estadísticamente significativa entre la concurrencia del niño a la consulta odontológica y las categorías de ceod+CPOD (x2=2,781; p=0,427), pero sí correlación directa entre la frecuencia de consulta durante el último año y el ceod+CPOD (coeficiente r de Pearson =0,486, p=0,000).

Los preescolares asistentes a escuelas de AUM presentaron indicadores de caries más elevados respecto a los encontrados en el grupo AU. La demanda de atención para AUM se relacionó con la gravedad del estado dentario y con situaciones de urgencia. Los preescolares de AU demandaron atención dental tanto en salud como en enfermedad, con tendencia a mayor adherencia al tratamiento. Las acciones tendientes a promover la salud bucal en los preescolares deberán tomar en cuenta tanto las barreras internas como las externas en el acceso y utilización de los servicios de salud bucal

Palabras clave: *Caries; servicios de salud bucal; poblaciones escolares; acceso al cuidado dental.*

INTRODUCTION

Early childhood caries (ECC) was defined by the *American Academy of Pediatric Dentistry* (AAPD) as the presence of one or more decayed, missing (due to caries) or filled tooth surfaces in any primary tooth in a child 71 months of age or younger. Severe early childhood caries is the presence of any sign of softening of tooth surfaces in children under 3 years of age or the presence of one or more decayed, missing (due to caries) or filled primary anterior teeth from ages 3 through 5, dmfs = 4 at 3 years, 5 affected surfaces at 4 years or 6 surfaces affected at 5 years of age [1].

ECC is currently recognized as a public health problem with defined biological, social and behavioral etiological components [2]. Its impact is higher when recurrence of caries in children who have had them previously is analyzed. Approximately 40% of children who have been treated for ECC develop new lesions within a year of completing the treatment [3,4]. Family and community socio-economic variables act as potentiating factors for the severity and progression rate of the caries process in early infancy. Milnes[5] reports that in developed countries, prevalence was 1% to 12%, but in developing countries and within disadvantaged populations in developed countries, it was as high as 70%. These findings have been confirmed by different studies [6].

One model explaining the etiology of early childhood caries attributes it to family stress caused by joint socio-economic variables, particularly in mothers, leading to dysfunctional parenting behaviors and thus greater risk of caries in children [7]. In developing countries, studies on the prevalence of early childhood caries and caries in preschoolers match the association found between high caries experience and disadvantaged socio-economic status [8-12].

Ly et al.[13] in Xiamen, China, studied a population of 1570 children under 5 years of age and found that 56.8% to 78.31% were affected, and evidence of increasing tendency with age. These findings were confirmed by Karla et al.[14] in a study in Gurgaon, Haryana (India) on 600 preschoolers of middle socio-economic class, where caries prevalence was 68%, with mean dmft 2.85, and increasing values with age. Pridaryarshini et al.[15] found caries prevalence of 37.3% among low income preschoolers in the city of Bangalore, India, of whom 94.3% had high levels of untreated disease. Ramírez et al.[16] analyzed a sample of 659 individuals and found that 67% were affected by dental caries according to ICDAS II criteria.

An increase in prevalence of ECC has been found even in developed countries. The US 2007 national health survey showed that caries prevalence is on the rise and that 28% of 2- to 5-year-olds have caries experience [17].

Previous studies [18] showed that preschoolers in Greater Mendoza city have high levels of caries, which are significantly higher in children who attend underprivileged urban schools, with a high level of decayed component (d) indicators and indication of extraction (ie). This background justifies the importance of this study, the aim of which was to analyze the characteristics of demand for care and the dental care itself, discriminating the differences between socio-economically distinct groups, and to advocate the adoption of evidence-based policies.

MATERIALS AND METHODS

This was a cross-sectional correlational descriptive epidemiological study on a cluster sample of preschoolers from 2 schools in an underprivileged urban area (School No. 17 Silvia Puebla and School No. 18 Xumec) and 2 urban schools (Hipólito Yrigoyen and Manuel Belgrano Schools) located in Greater Mendoza (n=155 and n=148, respectively). Demographics at the schools were established according to the criteria of the General Directorship of Schools of the Government of Mendoza, based on percentages of families with unmet basic needs. This was used to include children either in group U (urban environment, with basic needs satisfied) or group UnU (underprivileged urban environment, with unmet basic needs).

The parents or legal guardians of the children at all four schools were informed about the study and they provided written consent for children's participation. All children in the sample were provided with a preventive program at which oral hygiene techniques were taught and 1.2% acidulated sodium fluoride, pH 3.5 was applied professionally.

An oral clinical examination was performed by two calibrated examiners (inter-judge Kappa index = 77%). The caries category included the white spot lesion category (ICDAS II criterion 2) [19]. The indices dmft, dmfs, DMFT and DMFS were calculated [20]. "Caries free" was used to describe children with dmft plus DMFT equal to 0.

Data were grouped into four ordinal categories according to the severity of the variable "sum of dmft+DMFT":

a) dmft+DMFT = 0.
b) dmft+DMFT = 1 to 3.
c) dmft+DMFT = 4 to 6.
d) dmft+DMFT = 7 or higher

An *ad hoc* structured survey (Fig. 1) was used to collect data about demand for dental care. It was answered by the parents of the preschoolers included in the study, administered by the teachers from each class.

The survey asked about whether the child had ever visited a dentist (Question 1). If the answer was 'yes', the following information was requested:

a) which subsector care was requested at,
b) number of visits to the dentist in the past year,
c) whether they were satisfied with the care received, and
d) whether it was difficult to get an appointment.

The category "Demand according type of school" was established by associating school setting to the yes/no variable (has/has not visited a dentist).

To establish the association between dental status and demand for care in each group, we used:

- the ordinal categories according to severity (dmft + DMFT), and
- the answers to survey questions 2, 3 and 4.

The data were processed with SPSS 18.0 software. The following were determined at a significance level smaller than 0.05:

- Frequency distribution and confidence intervals for each variable,
- Measures of central tendency and its dispersal, and
- Comparison between groups (Student's t-test, chi square and Pearson's r correlation coefficient).

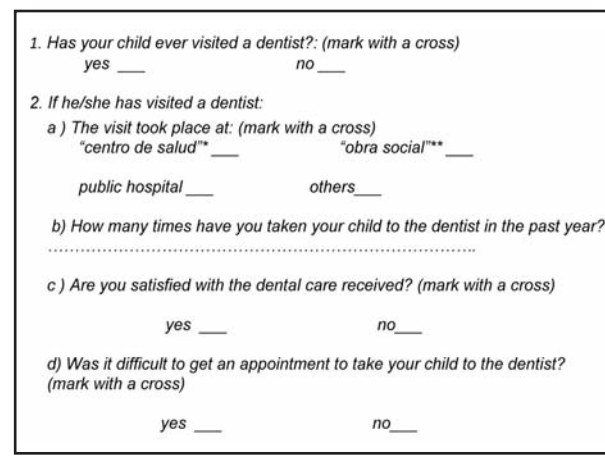
RESULTS

Dental status

Caries experience was 85.8% for children from underprivileged urban schools (UnU) and 75% for children from urban schools (U). The percentage of caries-free children was significantly lower in group UnU (14.2%) than in group U (25%) (x^2= 25.377; p= 0.018).

A comparison of the groups revealed significantly higher values in children from UnU schools for the following indicators (Table 1):

- d+D tooth (= 5.4± 3.8; t = 2.887; p = 0.004);
- dmft+DMFT (= 5.7± 4.1; t = 0.466; p = 0.020);


1. Has your child ever visited a dentist?: (mark with a cross)
yes ___ no ___

2. If he/she has visited a dentist:
a) The visit took place at: (mark with a cross)
"centro de salud"* ___ "obra social"** ___
public hospital ___ others___

b) How many times have you taken your child to the dentist in the past year?
.....

c) Are you satisfied with the dental care received? (mark with a cross)
yes ___ no___

d) Was it difficult to get an appointment to take your child to the dentist? (mark with a cross)
yes ___ no___

Fig. 1: Model of the survey used for classifying demand for dental care. "centro de salud" refers to a government-run community health center. ** "obra social" refers to a trade union-managed health insurance plan.*

- d+D surface (= 7.62± 6.2; t = 0.956; p = 0.014), and
- fewer filled surfaces than children from U (f + F surface: = 0.12± 4.5; t = -2.71; p = 0.007).

When mean dmft+DMFT was discriminated according to grouped categories, distribution was more homogeneous among preschoolers from in the U group, with 52.7% corresponding to the sum of classes 0 and 1/2/3. In children from the UnU group, the problem was more serious: the sum of children with class 0 and 1/2/3 was 34.2% while 41.9% had dmft+DMFT equal to or higher than 7 (Table 2).

Table 1: Association tests for dental status variables and percentage of caries-free children from each school environment (Student's t-test for independent samples and chi-squared).

Indicator	Type of school	n	%	mean	SD	t	x2	p
D+Dt	Underprivileged Urban	155		5.40	3.78	2.887		0.004
	Urban	148		4.08	4.12			
ie+Mt	Underprivileged Urban	155		0.25	0.72	0.766		0.444
	Urban	148		0.18	0.70			
f+F	Underprivileged Urban	155		0.14	0.79	-1.496		0.136
	Urban	148		0.28	0.85			
dmft+DMFT	Underprivileged Urban	155		5.70	4.07	2.342		0.020
	Urban	148		4.54	4.55			
d+Ds	Underprivileged Urban	155		7.63	6.2	2.475		0.014
	Urban	148		5.76	6.89			
ie+Ms	Underprivileged Urban	155		1.23	3.61	0.766		0.444
	Urban	148		0.91	3.50			
f+Fs	Underprivileged Urban	155		0.12	0.45	-2.717		0.007
	Urban	148		0.45	1.44			
dmfs+DMFS	Underprivileged Urban	155		8.89	8.39	1.902		0.058
	Urban	148		6.99	8.96			
Caries-free percentage	Underprivileged Urban	155	14.2					
	Urban	148	25				25.377	0.018

Table 2: Contingency table for dmft+DMFT categories for each Type of school.

			Type of school		Total
			Underprivileged Urban	Urban	
dmft+DMFT categories	dmft+DMFT = 0	Count	22	37	59
		% for Type of school	14.2%	25.0%	19.5%
	dmft+DMFT = 1,2,3	Count	31	41	72
		% for Type of school	20.0%	27.7%	23.8%
	dmft+DMFT = 4,5,6	Count	37	30	67
		% for Type of school	23.9%	20.3%	22.1%
	dmft+DMFT = 7 y +	Count	65	40	105
		% of Type of school	41.9%	27.0%	34.7%
Total		Count	155	148	303
		% for Type of school	100.0%	100.0%	100.0%

Demand for dental care

The answers to 'yes/no' question No. 1: *"Has your child ever visited a dentist?"* differed significantly according to school setting (x^2 with Yates' correction = 7.022; p = 0.008), with 55% of the "yes" answers corresponding to U and 63% of the "no" answers to UnU (Figure 2, Table 3).

For the cases that answered "yes" (n = 180) there was association between type of school and place where the visit to the dentist took place (x^2 = 78.851; p = 0.000):

- For U, 85.6% of the answers were "*obra social*" (health insurance managed by trade unions) and "others", whereas
- for UnU 80.3% of the answers were "*centro de salud*" (government-run community health centers) and "public hospital" (Figure 3).

No significant difference was found between groups for:

- type of school and average number of visits to the dentist during the past year (UnU = 1.82±1.5; U = 2.07±2.1; t = -0.865; p = 0.394);
- school setting and satisfaction with dental care received (x^2 with Yates' correction =1.368; p=0.242).

Among participants from the underprivileged urban group (UnU), 64% said they had encountered difficulty in getting an appointment for the visit to the dentist, while among participants from the urban group (U), 71% said they had not had difficulty (x^2 with Yates' correction = 19.910; p = 0.000).

Severity of dental status and demand for dental care

To establish possible associations between severity of dental status and demand for dental care, we analyzed frequency distribution between the dmft+ DMFT ordinal categories and the 'yes/no' question about visiting the dentist (Question 1) for each type of school. To establish whether there was a pattern in demand for dental care related to caries severity indicators, the correlation between number of visits in the past year (Question 2b) and dmft+DMFT categories was analyzed.

Table 3: Frequency distribution and association test for answers to the 'yes/no' question "Has your child ever visited a dentist?" according to type of school (Chi squared).

	Has your child ever visited a dentist?		chi square (withYates' correction)	p
Type of school	YES	NO		
Underprivileged urban	81	73		
	45.0%	61.3%		
Urban	99	46	7.022	0.008
	55.0%	38.7%		
total	180	119		
	100.0%	100.0%		

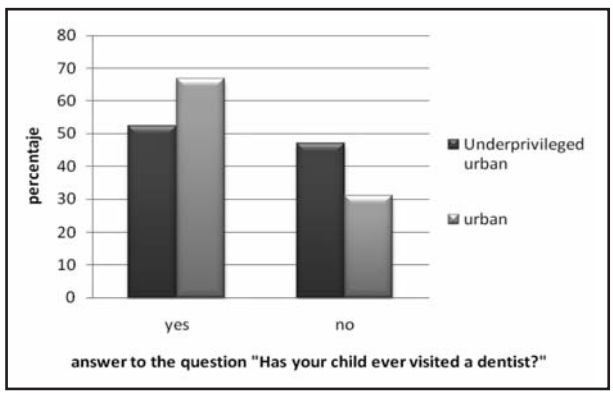


Fig. 2: Percentages of answers to the 'yes/no' question "Has your child ever visited a dentist?" according to type of school.

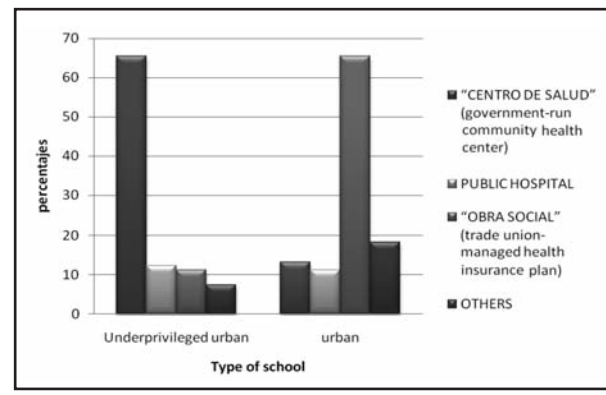


Fig. 3: Percentages of use of oral health subsystems according to type of school.

For children from schools there was association between visits to the dentist and dmft+DMFT categories ($x^2 = 19.412$; p = .000). A high percentage of preschoolers whose parents answered "yes" to survey Question 1 about going to the dentist corresponded to the category dmft+DMFT = 7 or higher (59.3%), whereas among children without caries experience, only 9.9% answered 'yes' (Table 4). No correlation was found between frequency of visits to the dentist during the past year and dmft+DMFT (Pearson's r coefficient = 0.091; p = 0.0426), as shown in Table 5. Children from the UnU group with more severe dental status had visited a dentist, but not as frequently or as regularly as required by their diagnosed dental status. Most children without caries experience had never visited a dentist, i.e. they had not been to preventive visits.

In children from urban (U) schools, no evidence was found of association between the child visiting the dentist and dmft+DMFT categories ($x^2 = 2.781$; p = 0.427). However, direct correlation was found between frequency of visits during the past year and

Table 4: Contingency table for the 'yes/no' question "Has your child ever visited a dentist?" according to dmft+DMFT categories and type of school.

			Dmft+DMFT categories								Total	
			dmft+DMFT= 0		dmft+DMFT= 1,2,3		dmft+DMFT= 4,5,6		dmft+DMFT= 7 or more		dmft+DMFT = 0	
			UU	U	UU	U	UU	U	UU	U	UU	U
Has your child ever visited a dentist?	Yes	Count	8	26	13	24	12	19	48	30	81	99
		% of "Has your child ever visited a dentist?"	9.9%	26.3%	16.0%	24.2%	14.8%	19.2%	59.3%	30.3%	100%	100%
		% of groups dmft+DMFT	36.4%	70.3%	41.9%	60.0%	33.3%	65.5%	73.8%	76.9%	52.6%	68.3%
	No	Count	14	11	18	16	24	10	17	9	73	46
		% of "Has your child ever visited a dentist?"	19.2%	23.9%	24.7%	34.8%	32.9%	21.7%	23.3%	19.6%	100%	100%
		% of groups dmft+DMFT	63.6%	29.7%	58.1%	40.0%	66.7%	34.5%	26.2%	23.1%	47.4%	31.7%
Total		Count	22	37	31	40	36	29	65	39	154	145
		% of "Has your child ever visited a dentist?"	14.3%	25.5%	20.1%	27.6%	23.4%	20.0%	42.2%	26.9%	100%	100%
		% of groups dmft+DMFT	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%

Table 5: Association and correlation tests for questions 1 and 2 regarding demand for dental care according to severity of dental status at schools in urban and underprivileged urban settings (Chi squared and Pearson's correlation coefficient).

Survey question on demand for dental care	Type of school	Type of statistical test and n		Asymptotic signficance (bilateral)
Has your child ever visited a dentist?/dmft+DMFT categories	Urban	Pearson's Chi-squared N of valid cases	2.781 145	0.427
	Underprivileged urban	Pearson's Chi-squared N of valid cases	19.412 154	0.000
How many times have you taken your child to the dentist in the past year?/dmft+DMFT categories	Urban	Pearson's correlation coefficient N of valid cases	0.486 98	0.000
	Underprivileged urban	Pearson's correlation coefficient N of valid cases	0.091 81	0.426

dmft+DMFT (Pearson's r coefficient = 0.486; p = 0.000), (Table 5). Children from urban schools visited the dentist in both health and disease, but there was a tendency to increasing the frequency of visits to the dentist when the severity of the dental status was higher.

DISCUSSION

Caries experience in both the populations of children that were studied in Mendoza is high, in agreement with studies on caries prevalence in preschoolers in other developing countries.[13-17]

Early childhood caries in preschoolers in the study sample seems to be influenced by multiple factors related to socio-economic status. Dental status in children from underprivileged urban schools corresponded to high degrees of ECC severity and was worse than in children from urban schools. However, despite the prevalence of caries, only 47% of those children had ever demanded dental care, and the frequency and consistency of the visits were not sufficient to ensure the resolution of these dental problems. Kopycka-Kedzierawski and Billings [21] reported similar results in Rochester (USA), finding that in a population of 246 children aged 1 to 4 years at child care centers, 28% had caries, but only 39% of them had demanded dental care.

Another important finding is that the demand for dental care due to caries problems in preschoolers from the underprivileged urban group was not related to the number of visits completed, from which it may be inferred that there was little adhesion to treatment and that the demand was related to urgencies. This agrees with a study conducted in New York (USA) by Uargarkar et al.,[22] who analyzed the demand and gradual increase in costs of care for children under 6 years old, which they claim is due to an increase in caries in low income sectors, the existence of physical barriers, lack of health insurance, lack of knowledge in parents and care givers, and the limited number of dentists willing to attend to preschoolers or who have contracts with the health insurance system in force (Medicaid in this case).

The health sub-system primarily used by the sample from UnU schools was the government system, which involved difficulty in getting appointments. This constitutes a real barrier to access to oral health care. To make matters worse, oral health is undervalued compared to other health needs, particularly in low income populations. This points to the need to deal with oral health problems by means of policies involving both clients and health care providers [23].

An outstanding study with regard to this issue was conducted by Grembosky et al.[24] on the oral health status of children from low income families, showing that children whose mothers had a regular source of dental care were healthier than children whose mothers did not have that benefit.

In our study, preschoolers from the urban setting enjoyed a better socio-economic status, which was related to access to trade union-managed health insurance (*obra social)* or private subsystems. They tended to demand dental health care for both healthy and diseased status. Correlation tests showed that the more often they had visited the dentist, the higher was the dental status indicator (dmft+DMFT), and vice versa. This may be interpreted as higher adherence to treatment and it may be assumed that treatment was completed.

The association between school setting (defined by unmet basic needs in the school population) and dental status may be considered upon establishing subpopulations at risk of the disease. This concept is supported by studies such as Da Rosa et al., reporting a direct association between school deprivation indices and dental status in preschoolers in Quebec (Canadá) [25]. Muirhed et al. also propose school socioeconomic context as an adequate indicator for mean dmft values in preschoolers [26].

It would be helpful to explore whether the results of this study are attributable to:

- Different perceptions in the families regarding the impact of oral status on the child's quality of life
- Structure and dynamics of the health care system fostering or hindering the entry and engagement of socially deprived population groups
- The current health care system, which does not focus on risk in individual or group programs.

Awareness needs to be created regarding the problem of early childhood caries and severe early childhood caries. It should be prioritized by political decision makers as a real and urgent public health problem in order to establish strategies for preven-

tive and curative intervention, with the participation of human resource training institutions, so that they will be prepared to deal with the situation [27.28,22].

CONCLUSIONS

- Preschoolers from underprivileged urban settings had higher caries indicators than preschoolers from urban settings, although this was not reflected by higher demand for dental care.
- Preschoolers from underprivileged urban settings tended to demand dental care only for more severe dental problems, and the severity of their status was not related to the number of visits demanded. It may be inferred that demand was for urgencies.
- Children from urban schools demanded dental care for both healthy status and diseased status, and were more likely to adhere to the treatment.
- Action to promote oral health in preschoolers should take into account internal and external barriers against access to and use of oral health care services.

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THE INFLUENCE OF MAXILLARY AND MANDIBULAR OSTEOPOROSIS ON MAXIMAL BITE FORCE AND THICKNESS OF MASTICATORY MUSCLES

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ABSTRACT

The aim of this study was to examine the bite force and masseter and temporal muscle thickness in individuals with maxillary and mandibular osteoporosis. 72 individuals were distributed into two equal groups: (1) facial osteoporosis and (2) healthy controls. Bite force on the right and left molar regions was recorded with a dynamometer and the highest value out of three measurements was recorded as the maximal bite force. Muscle thickness was measured with a SonoSite Titan ultrasound scanner. Ultrasound images were obtained of the bilateral masseter and temporal muscles at rest and at maximal voluntary contraction. The means of the measurements in each clinical condition were analyzed with multivariate statistical analysis (SPSS 19.0). Student's t test indicated no significant difference for muscle thickness, but indicated significantly lower bite force values in the osteoporosis group (p> 0.05). Lower bite force in individuals with facial bone loss demonstrates functional impact of osteoporosis on the complex physiological stomatognathic system.

Key words: *Osteoporosis; ultrasound; bite force; masticatory muscles.*

A INFLUÊNCIA DA OSTEOPOROSE MAXILAR E MANDIBULAR NA FORÇA DE MORDIDA E ESPESSURA DOS MÚSCULOS MASTIGATÓRIOS

RESUMO

Este estudo teve como objetivo analisar a força de mordida e a espessura dos músculos masseter e temporal em indivíduos com osteoporose maxilar e mandibular. 72 indivíduos distribuídos em dois grupos equivalentes: (1) osteoporose facial e (2) controles saudáveis. Força de mordida nas regiões de molar direita e esquerda foi gravada com o dinamômetro e o valor mais alto das três medidas foi registrado como a força de mordida máxima. A espessura muscular foi mensurada com ultrassom SonoSite Titan. As imagens de ultrassom foram obtidas dos músculos masseter e temporais bilateral em repouso e em contração voluntária máxima. As médias das medidas em cada condição clínica foram analisadas com a análise estatística multivariada (SPSS 19.0). Teste t de Student não revelou diferenças significativas para a espessura músculos, mas indicou valores significativamente mais baixos de força de mordida no grupo com osteoporose (p> 0,05). Força de mordida menor em indivíduos com perda óssea facial demonstra um impacto funcional da osteoporose na fisiologia complexa do sistema estomatognático.

Palavras-chave: *Osteoporose; ultrassom; força de mordida; músculos mastigatórios.*

INTRODUCTION

Increasing longevity of the world population has led to osteoporosis being considered the "epidemic of the twenty-first century" [1,2]. Osteoporosis is a serious public health problem for middle-aged and elderly women and increases after menopause [3]. By 2050, the worldwide incidence of hip fracture is projected to increase by 240% for women and 310% for men. The estimated number of osteoporosis hip fractures worldwide is expected to rise from 1.66 million in 1990 to 6.26 million in 2050, even if age-adjusted incidence rates remain stable [4]. The International Osteoporosis Foundation in 2013 reports that it is one of the most important diseases associated with aging.

Systemic osteoporosis affects femoral, radial, and spinal bones, in addition to affecting craniofacial bones and oral structures, directly influencing vari-

ous oral conditions and dental procedures [5,6]. Clinical and scientific dental interest in the effects of osteoporosis on facial structures has been growing. In a preliminary study, Siéssere et al. [7] evaluated the electromyographic activity of the masseter and temporal muscles of patients with maxillary and mandibular osteoporosis compared to a control group. They found that the decrease in the amount of maxillary and mandibular bone tissue that supports the muscle structure in individuals with osteoporosis does not cause a change in the level of electromyographic pattern activation.

Dental radiographs might be useful for screening for osteoporosis. Some studies indicate the use of the relationship between mandibular bone mineral density (BMD) and other skeletal sites commonly used for bone densitometry in the detection of osteoporosis [8]. The evaluation of dental radiographs may have a role in the detection of individuals with osteoporosis [9]. Other oral signs of osteoporosis could be alveolar ridge resorption, tooth loss and chronic destructive periodontal disease [10].

Mastication is one of the functions of the stomatognathic system, which comprises a functional and physiological entity integrating a set of organs and tissues whose biology and physiopathology are absolutely interdependent and therefore require complex evaluation. In addition to the electrical activity previously evaluated [7], structural evaluation of masticatory muscles and their ability is essential for complete understanding of the possible influences of osteoporosis on the masticatory process.

In this context, the aim of this study was to investigate the thickness of the masseter and temporal muscles and the bite force of patients with mandibular and maxillary osteoporosis. The data from these osteoporotic patients was compared to data obtained from healthy individuals.

MATERIALS AND METHODS

Volunteers

Seventy-two individuals of both genders, with an average age of 53.0±5 years, with no distinction of ethnicity or social class, took part in this study. They were divided into two groups of 36. Group 1 consisted of thirty-six individuals selected at random from the pool of users of the Radiology Clinic at the Ribeirão Preto Dental School, University of São Paulo, Brazil, with mandible and maxillary osteoporosis, who had been diagnosed by means of panoramic radiographs, obtained through the acquisition of digital image indirectly, with the chassis plans 15x30 or 18x24cm, using the panoramic X-ray machine and cephalometric brand - Siemens, model - Orthophos CD with kVp: 90 mA and: 16 and turnover time 14.1s. Lorente – Ramos et al. in 2011 reported that panoramic radiographs showed low bone mineral density (BMD), confirmed by BMD values of the lumbar spine (L_1–L_4) as measured by the exam Dual Energy X-ray Absorptiometry or DEXA, which has high diagnosis accuracy and a low dose of radiation compared to other methods. The DEXA exam was used to diagnose skeleton osteoporosis in each individual. The scanner takes a picture of the bones in the spine, hip, total body and wrist, and calculates their density. To take a DEXA bone density scan, the patient lies on a bed underneath the scanner, a curving plastic arm that emits X-rays. These low-dose X-rays form a fan beam that rotates around the patient. During the test, the scanner moves to capture images of the patient's spine, hip or entire body. The test takes about 20 minutes to perform and is painless. Group 2 (control) included thirty-six individuals, who were employees, and relatives of patients and students, paired subject-to-subject by gender and age (Table 1) with the subjects with osteoporosis.

The sample and inclusion/exclusion criteria were selected by means of anamneses and clinical examinations. The anamneses provided information on the participants' personal data, medical and dental history, any existing parafunctional habits, and possible temporomandibular dysfunction symptoms. All subjects were completely dentate or orally rehabilitated by means of partial fixed dentures or dental implants and had no periodontal problems. The following exclusion criteria were applied during the anamnesis: any systemic or local disorders other than osteoporosis, which could compromise craniofacial growth or the masticatory system, such as neurologi-

Table 1: Demographics of the two groups evaluated. Age, gender and standard deviation (±) in osteoporosis and control group.

Groups	N	Age	Gender
Osteoporosis	36	53.0 ± 5 years	33 female and 3 male
Control	36	51.0 ± 6 years	33 female and 3 male

cal disorders, cerebral palsy, and others; taking any medication that could interfere with muscle activity, such as antihistamines, sedatives, homeopathy, or central nervous system depressors; being under any kind of treatment that could, directly or indirectly, interfere in muscle activity during the period in which the study was performed, such as speech therapy and otorhinolaryngology treatment. Subjects were informed about the purposes and stages of the study and they all provided written consent, signing the form previously approved by the National Health Council (process number 2006.1.242.58.3). Thirty-six control patients were matched individual to individual with the osteoporosis sample. Each subject was assigned to one of two groups, named 1 and 2, and only one examiner knew which group the numbers referred to (control or osteoporosis). All examinations were performed without the researchers knowing which group the subjects belonged to, which made it a double-blind study.

Ultrasound analysis

Muscle thickness was analyzed with a SonoSite Titan ultrasound tool using a high-resolution real-time 56mm/ 10 MHz linear-array transducer placed transversally to the muscle fibers. The middle of masseter muscle was considered to be located between 1.5 and 2.0 cm above the jaw angle towards the upper eyelid, and the anterior portion of the temporal muscle between 1.0 and 1.5 cm to the back and above the external palpebral commissure. The muscle location was confirmed by palpation and transducer movement at the time of image acquisition. The ultrasound program enables measurements with a precision of 0.1 mm. Three acquisitions were made in each muscle condition (rest and dental clenching at maximal habitual optimized imaging). Ultrasound images were obtained from bilateral temporal and masseter muscles at rest and maximal voluntary contraction. During the examination, the participants remained seated, leaning on the backrest with the head unrestrained. Measurements were taken at intercuspidation, with an interval of 2 min between each acquisition for the participants to rest their muscles after dental clenching.

Bite Force analysis

Bite force measurements were collected with the volunteers sitting on a comfortable chair (office-like), with arms extended along the body and hands resting on their thighs. The records were taken with a digital dynamometer, model IDDK (Kratos, Cotia, São Paulo, Brazil), with a capacity of 1000 N, adapted to the mouth. The apparatus has a "set-zero" key, which allows the exact control of the values obtained and also "peak" registers that facilitate the record of the maximal force during measurements. It has two arms with plastic disks on each end, on which the force to be measured is applied. Its high precision charge cell and electronic circuit to indicate force supply precise measurements easily viewed on a digital display. The dynamometer was cleaned with alcohol, and disposable latex finger cots (Wariper, São Paulo, Brazil) were positioned on the biting arms as a biosafety measure. The participants were given detailed instructions and bite tests were performed before the actual recordings were made in order to ensure the reliability of the procedure. The volunteers were then asked to bite the dynamometer three times with maximal force, with a 2-min rest interval between records. Evaluations were performed at the first molars (left and right). Maximal bite force was measured in N through the "peak" force record indicated on the screen, for subsequent analysis. The highest value out of three records was considered as the individual's maximal bite force.

Method Error

The method error of muscle thickness measurements was performed on 18 individuals. Recordings were obtained at two different sessions with a 7-day interval. At each session, an average of three measurements was considered for each side and used later to assess the results. The method error (Se) was calculated using Dahlbergs's formula: $Se = \sqrt{\sum d^2 / 2n}$, where "d" is the difference between the two recordings of the individual and "n" the number of double recordings. Percentage errors were calculated using the formula % = (Se/mean) 100%, where "Se" is the result from Dahlberg's formula and mean corresponds to the mean value of the total of the initial and second measurements. A small difference was found between the first and second (1 week later) series (2.57 – 6.37 %).

The method error of bite force measurements was performed on five subjects. Recordings were obtained at two different sessions with a 7-day interval. At each session, the mean of three bites was considered for each side and used later to assess the results. Paired measurements were analyzed to identify systematic errors. No difference was found between the first and second (one week later) series.

Data analysis and statistics

The maximal molar bite force and muscle thickness measurements on both sides were analyzed using Student's T - test (SPSS 19.0 for Windows; Chicago, USA). A 5% ($p \leq 0.05$) level of significance was adopted.

RESULTS

There was no significant difference between the osteoporosis and control groups regarding masseter and anterior temporalis muscle thickness during rest or dental clenching (Table 2).

The bite force of the osteoporosis group was statistically significantly lower ($p<0.01$) than the bite force of the control group (Table 3).

DISCUSSION

Ultrasound scanning imaging (US) allows real-time evaluation of human masticatory muscle morphology. It is a considerable improvement over computed tomography and magnetic resonance imaging because it does not produce cumulative biological effects, and it has greater clinical availability and lower cost, making it suitable for large-scale studies [11,12].

The thickness of the masseter and temporal muscles, as measured by US, has been related to occlusion, temporomandibular dysfunction, and gender [13]. Thus, this measurement deserves special attention when studying mastication [14,15]. The generalized bone loss in the skeleton found in osteoporotic patients can cause disturbances in the masticatory system, such as modification of muscular position and masticatory muscle hyperactivity and thus, increases the chances of temporomandibular or muscular disorders [7].

It is therefore essential to examine the thickness and bite force of osteoporotic patients in order to analyze possible functional changes associated with this disease.

In the present study, both the osteoporosis and control groups presented higher masseter and temporalis thickness during contraction than at rest, which is in accordance with other studies [11,15-18].

Table 2: Mean, standard deviation (±) and statistical significance of US thickness (mm) of the right and left masseters (RM and LM) and anterior temporalis (RT and LT) muscles during rest and dental clenching, in osteoporosis and control group.

Clinical conditions and muscles	N	Osteoporosis	Control	Significance
Rest				
RM	36	0.89 ± 0.02	0.93± 0.03	ns
LM	36	0.91 ± 0.02	0.96 ± 0.03	ns
RT	36	0.59 ± 0.01	0.58 ± 0.02	ns
LT	36	0.60 ± 0.02	0.56 ± 0.01	ns
Dental clenching				
RM	36	1.38 ± 0.15	1.36 ± 0.08	ns
LM	36	1.31 ± 0.12	1.48 ± 0.14	ns
RT	36	1.07 ± 0.07	1.23 ± 0.09	ns
LT	36	1.04 ± 0.07	1.07 ± 0.05	ns

ns no significance

Table 3: Mean, standard deviation (±) and statistical significance of maximal bite force (N) in osteoporosis and control group.

Clinical conditions and muscles	N	Osteoporosis	Control	Significance
Right molar	36	14.01 ± 1.81	23.51 ± 2.60	**
Left molar	36	14.87 ± 1.71	28.87 ± 3.50	**

** statistical significance p<0.01

The absence of differences in muscular thickness between groups also indicates that facial osteoporosis does not interfere in masseter and temporalis morphology. According to Siéssere et al.[7], the masticatory efficiency of osteoporotic patients is similar to that of healthy individuals when evaluated by electromyography. The normal activity of masticatory muscles may explain the normal thickness of these muscles.

On the other hand, osteoporosis has a strong association with the progressive reduction in muscle mass, strength and function (sarcopenia) [19-22] that affects older people [23]. In age-related muscle atrophy, a decrease in both muscle fiber size and number has been reported [24].

The osteoporosis group had significantly lower bite force than the control group. Because of the reduction of bone mass, it is suspected that the patients with osteoporosis tend to have less masticatory muscle strength than healthy patients. If the musculature is not trained over several years, there is a reduction in bite force [25]. In one study, a Brazilian urbanized population was found to have lower bite force when compared to a Brazilian indigenous population, because the soft food consumed by the white population fostered non-trained masticatory musculature [26]. Thus, if osteoporotic patients do not exert masticatory muscles for a long period, this reduced function is expected to affect muscular thickness.

Osteoporosis is a disease that occurs principally in elderly people. Good nutrition is crucial to the reduced morbidity of osteoporotic patients. There is an effective participation of bite force in mastication. Thus, if bite force increases, masticatory efficiency increases as well [12,17]. Bone tissue is continuously remodeling in response to mechanical stress. The alveolar bone mass and the cross-sectional dimension of the alveolar bone increase with increasing functional loading [26,27].

A thicker masseter muscle is associated with a higher local bone density [26]. Thus, the maintenance of a higher muscular loading may contribute to bone loss control in osteoporotic patients. However, further studies are required to evaluate the possible positive effects of muscular stimulation therapy on the jaw muscles of osteoporotic patients.

This study verified lower bite force in patients with osteoporosis than in healthy controls. In addition, both the osteoporosis and control groups presented higher masseter and temporalis thickness during contraction than at rest. If bite force is positively correlated to masticatory efficiency, then it very important to plan for the treatment of patients with osteoporosis via the training of masticatory muscle force as a way to improve masticatory efficiency.

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DO RESIN CEMENTS INFLUENCE THE CUSPAL DEFLECTION OF TEETH RESTORED WITH COMPOSITE RESIN INLAYS?

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ABSTRACT

The aim of this study was to evaluate the influence of different resin cements on the cuspal deflection of endodontically treated teeth restored with composite resin inlays. Sixty upper premolars were randomly divided into five groups (n=12): 1 – sound teeth; 2 – cavity; 3 - Rely X ARC; 4 – RelyX Unicem; 5 – SeT. The teeth from groups 2, 3, 4 and 5 received a MOD preparation and endodontic treatment. Impressions were made with vinyl polysiloxane and poured using type IV die stone in groups 3, 4 and 5. Inlays with composite resin were built over each cast and luted with the resin cements. A 200 N load was applied on the occlusal surface, and cuspal deflection was measured using a micrometer. After 24 h, cuspal deflection was measured again using a 300 N load. The Student t-test showed that there was no statistically significant difference between the 200 N and 300 N occlusal loads only for the sound teeth group (p = 0.389) and the RelyX ARC group (p = 0.188). ANOVA and Tukey'test showed that the sound teeth had the lowest mean cuspal deflection, differing statistically from the other groups (p< 0.05). The highest cuspal deflections were obtained in the SeT group and the cavity group, with no statistical difference between them. Intermediate values were obtained in RelyX ARC group and RelyX Unicem group, which differed statistically. The self-adhesive resin cements RelyX Unicem and SeT showed less capacity to maintain the stiffness of the tooth/restoration complex than the conventional resin cement RelyX ARC.

Key words: *endodontics; resin cements.*

OS CIMENTOS RESINOSOS INFLUENCIAM A DEFLEXÃO DE CÚSPIDES DE DENTES RESTAURADOS COM INLAYS EM RESINA COMPOSTA?

RESUMO

O objetivo deste estudo foi avaliar a influência de diferentes cimentos resinosos na deflexão de cúspides de dentes tratados endodonticamente e restaurados com inlays em resina composta. Sessenta pré-molares foram divididos aleatoriamente em cinco grupos (n=12): grupo 1 – dentes hígidos; 2 – cavidade; 3 - Rely X ARC; 4 – RelyX Unicem; 5 – SeT. Os dentes dos grupos 2, 3, 4 e 5 receberam preparos cavitários MOD e tratamento endodôntico. Foram realizadas moldagens com silicone por adição nos grupos 3, 4 e 5, seguido de vazamento de gesso tipo IV. Inlays em resina composta foram construídas sobre os modelos de gesso, sendo as inlays cimentadas com os cimentos resinosos. Uma carga de 200 N foi aplicada na face oclusal, e a deflexão de cúspide foi medida usando um micrômetro. Após 24 h, a deflexão de cúspide foi medida novamente sob carga de 300 N. De acordo com o teste t-Student, não houve diferença estatística na deflexão de cúspides apenas para o grupo dos dentes hígidos (p = 0.389) e o grupo do RelyX ARC (p = 0.188) quando comparada as duas cargas. De acordo com ANOVA e o tese de Tukey, os dentes hígidos tiveram a menor media de deflexão de cúspides, diferindo estatisticamente dos outros grupos (p< 0.05). A maior deflexão de cúspides foi obtida com o grupo SeT e o grupo cavidades, não diferindo estatisticamente entre si. Valores intermediários foram obtidos para os grupos RelyX ARC e RelyX Unicem, diferindo estatisticamente entre si. Os cimentos resinosos autoadesivos RelyX Unicem e SeT mostraram menor capacidade de manter a rigidez do complexo dente/restauração em comparação com o cimento resinoso RelyX ARC.

Palavras-chave: *tratamento endodôntico; cimento resinoso.*

INTRODUCTION

As a result of their structural design, posterior teeth naturally suffer cuspal deflection under load. When endodontic treatment and mesio–occlusal–distal (MOD) preparations are performed, this trend towards cuspal deflection under masticatory loads is increased [1,2] due to the decrease in the stiffness of the tooth [3]. It is a consequence of the removal of dental tissues, which leads to greater fragility of the dental structure [4,5].

Numerous techniques and restorative materials have been indicated to recover the stiffness of endodontically treated teeth [6,7]. The most frequently used materials are composite resins and ceramics, due to esthetic demands. In the case of composite resin restorations, the indirect technique is considered the best treatment option to restore teeth with large cavities and to overcome polymerization shrinkage [8]. These indirect restorations are luted with adhesive materials, such as adhesive systems associated with resin cements, which favor reinforcement of the weakened tooth [9-11].

The conventional adhesive luting procedure consists of applying an adhesive system followed by a resin cement. However, self-adhesive resin cements have gained popularity with clinicians because they are easy to use, and the luting procedure takes less time than resin cements, which require the application of an adhesive system. Without the adhesive system, part of the sensitivity of the technique is eliminated [12,13].

Self-adhesive resin cements interact superficially with tooth hard tissues, and the formation of a hybrid layer or resin tags was not observed [14-16]. Self-adhesive resin cements have lower bond strength with enamel than do resin cements requiring an adhesive system [12, 17]. In relation to dentin, studies have shown that self-adhesive resin cements perform comparably to multistep systems on coronal dentin [12, 14, 17- 19]. In contrast, other studies have shown significantly lower bond strengths of these materials to dentin [20- 22].

In an inlay restoration, the luting material occupies the space between the restoration and the tooth, and is responsible for connecting these different substrates. Regardless of the luting technique, the tooth/restoration complex must be capable of restoring the stiffness of the original tooth to a certain degree, in order to decrease mechanical fatigue of the cusps [23], which increases with higher occlusal loads [1]. However, there is no study showing the influence of resin cements on cuspal deflection under different occlusal loads.

The aim of this study was to evaluate the influence of three resin cements on cuspal deflection, under two different occlusal loads, of endodontically treated maxillary premolars restored with composite resin inlays. This study was conducted under the hypotheses that there are differences in the amount of cuspal deflection between inlays luted with the different resin cements.

MATERIALS AND METHODS

MOD preparation

Sixty sound maxillary first premolars, extracted for therapeutic reasons, were obtained from a tooth bank after the approval of the Ethics Committee of the Pontifical Catholic University of Rio Grande do Sul. The teeth were cleaned and disinfected in 10% thymol for 24 h and stored in 4° C distilled water. The water was changed every week, and the teeth were used within 6 months. The buccal–palatal and mesio–distal dimensions of each tooth were measured with a digital caliper (Mitutoyo, Suzano, SP, Brazil). A variation of 0.5 mm was allowed for each measurement to standardize the dimensions of the teeth.

Each tooth was mounted vertically in a plastic cylinder with self-cured acrylic resin (Jet Classico, São Paulo, SP, Brazil), up to 2 mm below the cement–enamel junction (CEJ). The teeth were randomly divided into 5 groups (n=12): Group 1, sound teeth (control); Groups 2, 3, 4, and 5, teeth with MOD preparation and endodontic treatment. Group 2 was not restored, and the other groups were restored with composite resin inlays, which were luted with RelyX ARC (Group 3), RelyX Unicem (Group 4), and SeT (Group 5). Table 1 shows the composition and manufacturer details of the materials used.

Table 1: Resin cements used in the study.

Material	Composition	Manufacturer
RelyX ARC (conventional)	Bis-GMA, tri-ethyleneglycol dimethacrylate, zircon/silica filler, photoinitiators, amine, benzoic peroxide, pigments	3M/ESPE, St. Paul, MN, USA
RelyX Unicem Clicker (self-adhesive)	Methacrylate monomers containing phosphoric acid groups; methacrylate monomers, silanated fillers, initiator components, stabilizers	3M/ESPE, St. Paul, MN, USA
SeT (self-adhesive)	Acidic monomer, camphorquinone, fluoroaluminosilicate glass, urethane dimethacrylate	SDI, Bayswater, VIC, AS

Bis-GMA: bisphenol-A-glycidyl methacrylate; HEMA: 2-hydroxyethylmethacrylate; GPDM: glycerol phosphate dimethacrylate.

A single operator performed the MOD preparations using a standardized preparation machine. This device consisted of a high-speed handpiece (Kavo, Joinville, SC, Brazil) attached to a mobile base. The mobile base moves vertically and horizontally, in 3 mm increments, with the aid of a micrometer (Mitutoyo, Tokyo, Japan). The long axis of the tooth was positioned vertically on the preparation machine, and the tooth was cut using a #4159 diamond bur (KG Sorensen, Barueri, SP, Brazil) attached to the high-speed handpiece under constant water and air cooling. The preparations presented rounded internal angles, divergent walls, and an occlusal box width of two-thirds of the intercuspal distance. The depth of the proximal boxes was located 1 mm above the CEJ. The preparations had only buccal and palatal walls, and a common floor from mesial to distal, so that the pulp floor of the occlusal box and the gingival floor of the proximal boxes were unified on the same level. The diamond bur was replaced after every five preparations.

After the preparations were completed, endodontic access was prepared with a #8 spherical carbide burr (SS White, Lakewood, NJ, USA). The preparation of the chamber was round and expulsive. Flexo-File files (Kerr, Orange, CA, USA) from number 15 to 40 were manually placed in the root canals to standardize the preparation. A 2.5% sodium hypochlorite solution was used to irrigate and clean the root canal. After the root canal preparation, all teeth were filled with gutta-percha cones (Dentsply Maillefer, Ballaigues, Switzerland) and N-Rickert endodontic sealer (Inodon, Porto Alegre, RS, Brazil) using the lateral condensation technique. Excess sealer was removed from the cavity using a cotton pellet soaked in 70% ethanol. The access to root canals was covered with gutta-percha.

Restorative procedures

Impressions of the preparations were taken with Express XT polyvinyl siloxane (3M Espe, Saint Paul, MN, USA) using individual trays made from self-cured acrylic resin (Jet Classico, São Paulo, SP, Brazil) with the putty/wash one-step technique. The impression material was allowed to set for 10 min before removal from the preparation. After 1 h, the impressions were poured using Durone Type IV stone (Dentsply, York, PA, USA). After 1 h, the casts were removed from the impression, numbered according to their group, and placed in dry storage. Cavity surfaces were lined with two coats of a die spacer, maintaining a distance of 1.0 mm to the marginal areas. Four horizontal layers of Filtek Z250XT composite resin (3M Espe, Saint Paul, MN, USA) were inserted in the casts, with Thompson spatulas no. 2 and 12, which resulted in a 90° inclination between the internal slopes and cusps. Each resin layer was light cured for 40 s. Restorations were then light cured for 60 s on each free surface, followed by finishing with flexible disks (TDV, Pomerode, SC, Brazil) and 8093F and 8093 FF silicone tips (KG Sorensen, Cotia, SP, Brazil). Throughout the experiment, a quartz-tungsten-halogen curing unit (Optilux Plus, Ribeirão Preto, SP, Brazil) was used for photopolymerization. The light intensity was controlled by a radiometer (model 100, Demetron/Kerr, Danbury, CT, USA) in the interval between 450 and 500 mW/cm^2.

Luting procedures

The internal surfaces of the inlays were sandblasted with 50 µm aluminum oxide for 5 s, followed by silane application (Ceramic Primer, 3M Espe, Saint Paul, MN, USA). In Group 3, Scotchbond Multi-Purpose adhesive system (3M Espe, Saint Paul, MN, USA) was applied. The tooth preparations were etched with 37% phosphoric acid for 15 s, followed by rinsing with air and water spray for 15 s. Excess water was removed with absorbent paper. A layer of primer was applied, followed by gentle air drying for 5 s. Then the bond was applied with a microbrush and light cured for 10 s. Equal lengths of base and catalyst pastes of RelyX ARC resin cement were mixed for 15 s and put on the inlay and preparation. In Group 4, equal quantities of base and catalyst pastes of RelyX Unicem were mixed and applied on the inlay and preparation. In Group 5, the internal content of a capsule of SeT was activated for 10 s and applied on the inlay and preparation. In Groups 3, 4, and 5, the inlay was placed on the preparation and a 10 N load was applied by means of a metallic tool. After 2 min, excess cement was removed with a microbrush, followed by light curing for 60 s on each free surface. The specimens were stored in distilled water at 37° C for 72 h and then submitted to the cuspal deflection test.

Cuspal deflection testing

Resin spheres (approximately 1.5 mm in diameter) were fixed with adhesive on both cusps. Following the methodology described by Gonzáles-López et al.[2] the spheres were positioned on the cuspal apexes and

served as reference points for measuring the intercuspal distance, using a precision micrometer (Mitutoyo, Suzano, SP, Brazil) to the nearest 1 µm. A device was used to fix the micrometer in the same position for every measurement. Each specimen was attached to the lower platen of a universal testing machine (Emic DL-2000, EMIC, São José dos Campos, PR, Brazil), and a steel sphere with an 8 mm diameter was used to apply a 200 N occlusal load at a cross-head speed of 0.5 mm/min. The load was applied parallel to the long axis of the tooth, simultaneously contacting the buccal and palatal cuspal inclines (Fig. 1). When the 200 N load was achieved, the machine was locked and three consecutive measurements of the cuspal deflection were made. The mean distance of the composite resin spheres prior to application of the load was subtracted from the mean distance of the spheres after application of the load. Thus, the cuspal deflection value was obtained. After 24 h, the cuspal deflection was measured again using a 300 N load.

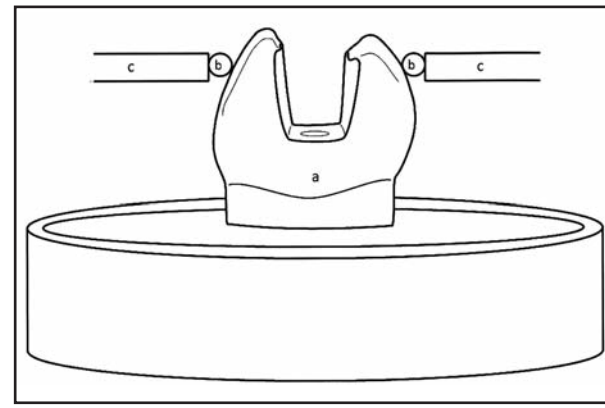

Fig. 1: Schematic figure of the cuspal deflection test: a) tooth; b) resin spheres; c) micrometer.

Statistical analysis

All statistical analyses were performed using SPSS version 10.0 (SPSS Inc., Chicago, IL, USA). After data collection, cuspal deflection data were submitted to the Kolmogorov–Smirnov normality test. To compare the study groups under the same load, the ANOVA and Tukey parametric statistical tests were applied. To compare each group under the different loads, student t-test for dependent samples was applied. The significance level was 5%.

RESULTS

The ANOVA showed that there were statistically significant differences among the groups ($p<0.05$). For both occlusal loads, the sound teeth had the lowest mean cuspal deflection, differing statistically from the other groups. The highest cuspal deflections were obtained in SeT group and cavity group, with no statistical difference between them. Intermediate values were obtained in RelyX ARC group and RelyX Unicem group, which differed statistically. The Student t-test showed that there were statistically significant differences between the 200 N and 300 N occlusal loads for the cavity group ($p = 0.002$), the RelyX Unicem group ($p = 0.000$), and the SeT group ($p = 0.004$). There was no statistical difference for the sound teeth group ($p = 0.389$) or the RelyX ARC group ($p = 0.188$) (Table 2).

DISCUSSION

The hypothesis was accepted, as there were differences in cuspal deflection among the experimental groups.

Premolars were used in this study because they have an unfavorable anatomic shape, crown volume and crown/root proportion, making them more suscep-

Table 2: Mean cuspal deflection and standard deviation of the groups under 200 N and 300 N loads.

Group	n	Mean (µm) and standard deviation 200 N	Mean (µm) and standard deviation 300 N
Group 1 – Sound teeth	12	3.42 ^{aA} (1.44)	3.92 ^{aA} (1.68)
Group 3 – RelyX ARC	12	6.83 ^{bA} (3.16)	8.83 ^{bA} (3.41)
Group 4 – RelyX U100	12	13.42 ^{cA} (8.75)	16.92 ^{cB} (8.68)
Group 5 – SeT	12	42.83 ^{dA} (12.71)	57.00 ^{dB} (14.02)
Group 2 – Cavity	12	65.50 ^{dA} (18.58)	79.00 ^{dB} (20.85)

Means in the columns followed by the same lowercase letter did not differ statistically according to Tukey's test at a 5% significance level.
Means in the rows followed by the same capital letter did not differ statistically according to Student's t-test for dependent samples at a 5% significant level.

tible to cusp fractures than other posterior teeth when submitted to occlusal load [24]. The dimensions of the cavity preparation were standardized in all groups, representing a clinical situation of advanced caries, for which preparation becomes extensive.

Cuspal deflection is a non-destructive methodology that verifies the deformation of the cusps when a load is applied in the occlusal region. In this study, a 200 N occlusal load was applied to perform the cuspal deflection test. The 200 N load is the intermediate value between 100 and 300 N, which corresponds to the range of normal biting force for maxillary premolars [25,26]. Cuspal deflection under a 300 N load was also verified. According to Jantarat et al.[1], a load of up to 300 N can be applied without the risk of tooth fracture. The results showed that the application of greater occlusal load produced an increase in intercuspal distance, in agreement with the study by González-López et al. [2]. This finding proves that the deformation depends on the intensity of the force applied [1].

The highest cuspal deflection was obtained for the cavity group, and the lowest values for the sound teeth. This small cuspal deflection in sound teeth is due to the very stiff behaviour of sound teeth under load [1]. Intact teeth with a complete enamel covering are very stiff, and an occlusal load causes only a small deformation. Sound teeth distribute load-generated stress more homogeneously, because the enamel is not appreciably deformed and the deformation is transferred to the more resilient dentin [27]. When the continuity of the enamel is lost as a result of preparation, the properties of the dentin play a major role in cusp behaviour [28]. Loss of dental structure causes a decrease in tooth stiffness, and consequently there is an increase in cuspal deflection under occlusal loads [1,2]. It is important to recover the stiffness of the teeth after restoration. Nevertheless, when the teeth were restored with composite resin inlays and luted with RelyX ARC, RelyX Unicem or SeT, none of the groups replicated the stiffness of natural teeth.

Among the restored groups, RelyX ARC had the lowest cuspal deflection. RelyX ARC is a conventional resin cement that is applied with an adhesive system. In this study, the preparation was etched with 37% phosphoric acid, followed by primer and bond applications, which allow hybrid layer formation on enamel [29] and dentin [30]. The micro-mechanical retention obtained with the hybrid layer may explain the smaller cuspal deflection in the RelyX ARC group than in the RelyX Unicem and SeT groups.

RelyX Unicem had the third lowest value of cuspal deflection. It is a self-adhesive resin cement that consists of alkaline fillers and specific multifunctional phosphoric-acid methacrylates, which are ionized at the time of mixing and which react with the hydroxyapatite of the mineral tissues of the tooth [31]. On dentin, this material was unable to demineralize or dissolve the smear layer completely, no decalcification and infiltration of dentin occurred and no hybrid layer or resin tag was observed [14,17,32]. The bond mechanism of RelyX Unicem to the tooth appears to be more chemical rather than micromechanical in nature. As RelyX Unicem have lower bond strength to enamel [12,19] and dentin [20-22] than do multistep systems, the bond to the dental tissues may not have been as strong as the bond encountered at the DEJ or when a hybrid layer is formed. This may explain the higher mean cuspal deflection of the RelyX Unicem than of the sound teeth and RelyX ARC groups.

An alternative for increasing the bond strength of self-adhesive resin cements was to apply phosphoric acid etching on the enamel [12,19,33], and polyacrylic acid on the dentin [32,34] before the application of self-adhesive resin cements. The presence of the smear layer has been recognized as the weak link in bonding self-adhesive resin cements [18,32]. These pretreatments represent an additional step in the application of self-adhesive resin cements, which were developed with the aim of simplifying the application procedure. However, it would be interesting to conduct the same study of cuspal deflection when specific pretreatments are applied on enamel and dentin before luting with RelyX Unicem, once all the inlay margins of the prepared teeth were within enamel, and a significant quantity of dentine was exposed by the inlay preparation.

The self-adhesive resin cement SeT contains an acid monomer that is responsible for etching the tooth surface. However, it is not known specifically what type of acid monomer is used, or whether it has a chemical interaction with the tooth. In the study conducted by Stona et al.[32], the adhesion of SeT to dentin did not withstand the cutting methodology used to obtain the test specimens (beams) for evaluating microtensile bond strength. It probably presented less interaction with dental substrates than RelyX Unicem, explaining the highest cuspal deflection.

Comparing 200-N and 300-N occlusal loads, RelyX ARC was the only resin cement that allowed an increase in cuspal deflection without statistical difference. The micro-mechanical retention obtained with the hybrid layer formed on enamel and dentin by the 37% phosphoric acid etching and subsequent adhesive polymerization is probably important for bond stability when the load is increased.

In the case of inlay restoration, the stiffness of the tooth tends to be restored when the material used for luting bonds strongly with the tooth tissues and restorative material, with the formation of a monobloc restoration, in which two bond interfaces are formed, corresponding to the tooth/luting material and restoration/luting material. Among the experimental groups in the present study, variation occurred only at the tooth interface. The same treatment was used at the restoration interface, which consisted of sandblasting with 50 µm aluminum oxide, followed by silane application. According to Zang and Degrange [35], the adhesion of self-adhesive resin cements to the restorative material depends on the nature of the multifunctional monomer contained in the formulation, and these luting agents have potential for specific adhesion to selective restorative substrates. In addition, the different viscosities and the different penetration capabilities of the RelyX ARC, RelyX Unicem and SeT into surface irregularities may have influenced the adhesion of the resin cements to the inlay restoration.

The clinical importance of cuspal deflection is that the greater the magnitude of this deflection, the greater the deformation, and consequently, the greater the possibility of fatigue failure. This type of failure, characterized by fracture in the presence of stress far below the maximum strength of the restored tooth, occurs in most dental fractures [36].

The result obtained for cuspal deflection showed that the self-adhesive resin cements RelyX Unicem and SeT showed less capacity to maintain the stiffness of the tooth/restoration complex than did the conventional resin cement RelyX ARC, which may foster greater longevity of the restored tooth. However, clinical studies are necessary to confirm this supposition.

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SOCIAL REPRESENTATIONS OF DENTAL TREATMENT IN A GROUP OF ENVIRONMENTAL HEALTH GRADUATE STUDENTS IN LIMA (PERU)

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ABSTRACT
Social representations are a type of common sense knowledge shared by different groups based on their experience. This study identified the social representations of dental practice in a group of environmental health graduate students in Lima, Peru. Method: We interviewed 25 graduate students using a "focus group" technique and a semi-structured guide. Three groups were formed with purposive sampling. The data were collected during the years 2010-2011, and analyzed using open, axial, selective coding with Atlas-Ti software. Results: Three substantive categories were identified: dental practice, characteristics of the dental care provider and dental practice setting. The social representations that the students identified with dental practice were fear and pain. Conclusions: The negative social representations of dental practice may affect viability and adherence to treatment, so it is important to identify them in time in order to intervene effectively.

Key words: *dentist-patient relationships; social perception; dentistry.*

LA PRÁCTICA ODONTOLÓGICA Y SU REPRESENTACIÓN SOCIAL EN UN GRUPO DE ESTUDIANTES DE POSTGRADO EN SALUD AMBIENTAL DE LIMA (PERU)

RESUMEN
Las representaciones sociales son un tipo de conocimiento de sentido común que comparten diferentes grupos, basados en su experiencia. En este trabajo se identificaron las representaciones sociales de la práctica odontológica en un grupo de estudiantes de postgrado en salud ambiental de Lima Perú. Se entrevistaron a 25 estudiantes de posgrado con la técnica "focus group" y con una guía semi-estructurada. Se conformaron tres grupos con muestreo intencionado. Los datos se recolectaron durante los años 2010-2011. La información se analizó con codificación abierta, axial y selectiva mediante el software Atlas-ti. Se identificaron tres categorías sustantivas: práctica dental, características del profesional de odontología y entorno de la práctica dental. Las representaciones sociales que identificaron los estudiantes con la práctica odontológica fueron miedo y dolor.
Las representaciones sociales negativas de la práctica odontológica pueden afectar la viabilidad y apego al tratamiento, por lo que es importante identificarlas oportunamente para intervenir con eficacia.

Palabras Clave: *relaciones dentista-paciente; percepción social; odontología.*

INTRODUCTION

Oral diseases are a major public health concern due to their high prevalence and incidence around the world[1]. Even today, dental caries is the disease that causes the greatest oral health problems in the world. In the year 2000, the Ministry of Health of Peru announced that "dental pathology" had the third most frequent morbidity, was associated with economic, sociocultural, environmental and behavioral factors, and should be dealt with urgently[2].

Dentistry works on the mouth, as a biological component, and on the connections between somatic individuality and environmental and social surroundings. Evidence of early impact on oral morbidity calls for a theoretical-practical discussion of the traditional, highly prevalent approaches of current dentistry[3,4]. Dental treatment as a social process may be approached from the social representation theory based on an epistemology of common sense that brings meaning to everyday knowledge. This knowledge is the fruit of social interactions based on a mental perception of reality, which transforms social objects in their context into symbolic categories. Social representations thus work as a sys-

tem for interpreting reality that governs people's relations with their physical and social surroundings and determines their behavior or practices[5].

From this standpoint, social representations (SR) constitute cognitive systems in which we can recognize stereotypes, opinions, beliefs, values and norms that often lead to positive or negative feelings[6]. SRs are constructed as systems of codes with values and interpretations defining how men and women act in the world, and are therefore a valuable tool for explaining people's behavior in this study, which is not limited to the particular circumstances of the interaction but transcends to the cultural aspect and the most widespread social structures. For example, when people refer to social objects, they classify, explain and assess them because they have constructed a social representation of those objects.

Some studies on the SR of dental treatment have established the importance of identifying beliefs, myths, habits and behavior related to oral health[7]. Some studies look at social representations of the oral health-disease process in an underprivileged urban population where different aesthetic, biological and sometimes emotional aspects are involved[8]. Other studies focus on the patient-dentist relationship and the importance of communication[9,10]. Marin et al. (2007) found that SR of dentists from the professional practice perspective differed among participants: the dentist considers anesthesia as the core, while among patients, it is teeth for women and fear for men[11]. Fear of pain is deemed one of the main causes for refusing to seek dental health care[8,12], which according to the World Health Organization, leads to high morbidity rates[13,14].

This study contributes initial information, which will surely be expanded on in future studies on different groups of dental service users. No paper was found in the literature review referring to the social representations of healthcare graduate students and dentistry, although there are studies on other kinds of users and on the general population, most of which focus on oral health, with very few focusing on dental treatment. Thus, the aim of this study is to identify the SRs held by a group of environmental health graduate students from Lima, Peru.

MATERIALS AND METHODS

Population

The main inclusion criteria for participants in the study were: to be a member of the professions, a graduate student in environmental health, of legal age and of either sex. Purposeful sampling was performed based on two criteria: environmental health graduate students who had had contact with dental care and who studied at that time at the General Environmental Health Directorate (DIGESA).

Twenty-five students were divided into three groups (two groups of eight and one group of nine). Environmental health students were chosen for the SR study because due to the nature of their education they had a more holistic view of the nature of health. In addition to scientific knowledge and technical skills, their training includes personal development of a positive attitude that could be related to the meaning of social representations in matters of health.

Construct preparation

Information was gathered by using the "focus group" technique, based on a collective semi-structured interview of a heterogeneous group of students, during which a participative, calm, friendly, relaxed atmosphere prevailed. This enabled the participants to express their opinions about, attitudes towards and experiences with dental treatment openly.

A thematic interview guide was prepared to direct the participants' conversation and personal disclosures, including the subject of dental treatment. Two assistants took notes at the sessions in order to record behavioral information that would have been impossible to obtain by means of audio recording only.

This study was made from a qualitative perspective with the support of ethnographic techniques, in the city of Lima, Peru in 2011.

Information was gathered from the interviews and transcribed in its entirety into a text processor. Kernels of meaning that came up during the communication and terms of presence or frequency that were meaningful for the analysis of the objective were identified. In qualitative terms, the presence of certain themes, and the behavioral models present in the discourse were reflected by frequency values. This enabled us to isolate patterns and processes of common and different factors and take them to the field in the following data-gathering stage, in a new interview of another focus group. The interviews were considered complete once the subject was exhausted. The interview was conducted at the DIGESA auditorium and lasted approximately two hours per group.

Data analysis

The analysis strategy involved formulating and sifting inferences and distinctions from the data as well as identifying the major meanings in order to codify the information into thematic categories.

The analysis process took place in two stages. The first was the descriptive distinction (descriptive aspects) where abstract codes were created based on particular meanings that allowed us to learn the dominant conceptualization of the social representations of dental treatment and that are included in the results.

The second stage used relational discrimination (explanatory aspects) where relationships or connections found in the descriptive results were established. They will be presented in the discussion by means of an open codification with a "line-by-line" examination of the gathered data as well as data that produced questions and reflections, the category grouping and lastly selective axial[15] coding until a polished category structure was obtained and saturation and integration were achieved. Coding and information analysis were done with ATLAS.ti version 2.4 software.

Participants were sent personalized invitations which included an explanation of the aims of the study, and any questions they had were answered. Each person was asked to specify an interview session schedule and confirmation, which enabled us to form groups within a minimal period of a week.

Ethical considerations

The interviews were conducted with the participants' informed consent; the protocol was reviewed and approved under number IISO/CI/18/08 as provided by the 2008 Declaration of Helsinki on Ethical Principles for Research Involving Human Subjects.

RESULTS

Three substantive areas were identified under different categories during the distinction stage when we looked for kernels of meaning making up the communication and whose presence was meaningful for the description of the social representation about how dental treatment is understood: 1) dental care; 2) dentists' professional demeanor; and 3) dental practice setting. The categories were fear of pain, economics/cost, poor attitude in the dentist, poor dentist-patient communication, and hygiene and annoying instruments.

This paper includes the most important results, obtained by means of an open coding procedure, which underscore some of the identity, performance and understanding traits of dental treatment based on the comments of graduate students in environmental health.

The representations of dental treatment that they hold and which are determinant for their day-to-day actions with regard to dentistry are reflected by the following statements:

1. Substantive area of dental care

"It is very important to take care of your teeth and receive continuous dental care."

"I feel mistrustful and uncertain about dental care when I hear other people's bad opinions about deficient interventions and poor handling of dental instruments."

- Connection between fear and pain as causes associated with lack of dental care

"Dental care scares me because I think dental treatment will hurt. I only see a dentist when I have to, especially when I have a toothache. I consider it a necessary evil."

"I don't go to the dentist because I'm afraid of pain. I've always believed it's going to hurt a lot, and that scares me and makes me feel anxiety."

- Economics/Cost as an important factor for not seeing a dentist.

"I think dental care is quite expensive and treatments are unaffordable. I think it is a highly lucrative profession."

"I don't go for dental care because I don't earn much and I can't afford dental treatment,"

Fear of pain and the high cost of dental treatment are recurrent themes in the participants' SR of dental treatment in the substantive area of dental care. These feelings lead the interviewees to refuse to seek dental and oral healthcare, preferring to avoid it and escape from what they consider a threat, even though they believe that maintaining good dental health is important.

2. Substantive area of dentists' professional demeanor

- Aspects related to a poor patient-dentist relationship due to poor communication.

"The dentist doesn't have a good attitude because he has no patience and doesn't take enough time to attend to his patients."

"I don't think there is good communication between dentists and their patients because I feel they don't explain the dental procedures they're performing and their effects."

This substantive area of dentists' professional demeanor reveals the main SR surrounding the patient-dentist relationship. It highlights the need to implement individual and social strategies, to learn about bio-psycho-social alterations, the characteristics of dental patients and their management, and endeavor to improve interpersonal relations in the patient-dentist experience. Interpersonal relationships are needed to cope with the demands of a reality subject to permanent changes. A person's attitude towards a dentist may be influenced and conditioned by this reality and the dental treatments he or she has undergone. During dental treatment, patients come into contact with the dentist, assess his/her behavior and at the same time form an opinion about him/her; feelings emerge that influence the kind of relationship that will be established.

3. Substantive area of dentistry setting

- Postures regarding dental hygiene

"The dentist's office should be clean and tidy, and disposable material should be discarded between one patient and the next."

"There shouldn't be any kind of animals in a dentist office for hygiene reasons."

- Important equipment and instrument aspects that annoy patients during dental treatment

"I don't like the noise made by the equipment, especially the handpiece."

"I dislike the lighting very much, especially when it's in your face to light up your mouth directly, and then to feel the water splashing from your mouth when instruments are being used inside it."

The above factors occasionally produce negative attitudes of mistrust and anxiety in patients, leading to fear, scant motivation, dissatisfaction and poor dentist-patient interaction. These situations should be analyzed from different perspectives in order to understand patients' demands, limitations and wishes and thus take action to adapt as much as possible to the situation and its possibilities, fostering changes both in what annoys patients and in patients' attitudes and behavior.

The results of this research show that the most dominant SR is fear of pain, followed by the expensive treatment, poor dentist attitude, poor communication, hygiene and annoying dental equipment. This suggests that dentistry has developed a disturbing scientific-technical reference about the purpose of its work and about itself, without producing social and epidemiological impact on oral health and disease. Individual SRs are distanced from or contrary to the theoretical-practical principles underlying dentistry because patients perceive the atmosphere of the dental practice as the least appropriate place for the work done by dentists.

DISCUSSION

The main SR in this group of environmental health graduate students regarding dental treatment was fear of pain. Fear of dental procedures is common because it has an impact on them and their quality of life. The appearance of extreme dental fear (dental phobia) leads to high levels of anxiety and progressive avoidance behavior in those suffering from it, with situations such as putting off making a dental appointment, avoiding periodic checkups, displaying behavior during the visit such as closing their mouth, leaning their head away, standing up, slapping the dentist's hand, screaming, complaining or crying, all of which create a problem for dentists to work. It also induces the patient to abandon preventive habits. The patient will only visit a dentist when he has extreme pain or dental problems[16,17].

Newton[4] claims that the most frequent triggers for fear of dental work are seeing the syringe, the anesthesia injection, and hearing the sound of the handpiece. The most feared interventions are tooth extractions and root canal. These emotions of fear may have been acquired in the social environment, particularly at home. They lead people to believe that they should only go to the dentist when there is pain or a serious problem. This representation is shared by people who believe that regular visits are not important for maintaining oral health, but rather that they are "a waste of time and money". Most of the people who hold these beliefs also complain about the excessive cost of dental treatment[10,11,13,18,19].

Other substantive areas present in the study population's SR were expense, poor dentist-patient relationship, and poor setting for practicing dentistry. These images held by the participants condition their behavior, which in this case may be refusal to seek dental care.

We need to recognize and differentiate contradictory feelings that produce negative attitudes, determine whether they are human creations due to mistaken ideas, thoughts or beliefs, possibly acquired either during their upbringing at home or due to negative experiences they have undergone or heard about related to dental procedures.

One of the most highly valued practical aspects in a dentist's office is hygiene, which involves the personnel, procedures and work systems. Any negligence in this area could cause cross-infection defined as the transmission of contagious agents between patients and dental care providers or vice versa[20-22] because the everyday work of dental care providers involves physical contact with blood and saliva in a septic environment..

A negative setting may be the outcome of an accumulation of several factors such as mistrust, deficient communication, and inadequate resources and work environment, as found in the SR of these participants.

The idea that dental work is expensive and annoying is based on real perception. Constant technological developments mean that dental equipment and instruments have to be renewed frequently, requiring constant investments to prevent them from falling behind and becoming obsolete. This makes dental treatment more expensive. Some people, especially the underprivileged, have limited access to dental care, and the cost of dental care is a topic that lends itself to great controversy and confusion.

The underlying causes of expensive dental care should be studied. Some of these causes are expensive equipment and materials, and the lack of prevention of oral and dental problems. The avoidance of at least a yearly dental checkup makes treatment more complicated and increases costs. When patients visit a dentist for an emergency or in the presence of pain, the situations are more complex and thus more expensive to resolve[23,24]. Scientific and technical breakthroughs have not made dental care more affordable and less bothersome, nor have they created a pleasant, comfortable environment for patients during treatment[25,26].

Dentistry is a profession that produces sensations of anxiety, fear, pain and discomfort in patients[27]. Cortes[28] claims that dentists seem to care more about the organism than the body, the sign than the symptom, the individual than the subject. There is no possibility of establishing an intersubjective encounter that would enable the body to speak based on its symptoms, where the subject is represented during dental treatment. During clinical treatment, the dentist asks the patient to open his mouth but not to talk. He only wants to hear information related to the disease, not the history of the person suffering from it. His attention is not focused on the body that suffers but on the painful organism and he becomes engaged in work resulting in biological-mechanical reductionism.

This leads to the perception of dentists as unfeeling and cold towards their patients during dental procedures. Cançado[29] claims that the success of a dental practice depends on the dentist's skill in winning over his patients. Another study suggests implementing communication strategies: listen more and talk less. Begin with topics brought up by the patients because they are important to them, and implement strategies to improve dentist-patient communication[30].

One cause of poor dentist-patient relationships is that dentists are exposed to physical and psychological fatigue in addition to work-related, social and personal circumstances, which affect them more than they do other healthcare providers. This may result in personality changes and mood swings and in extreme situations the "hateful dentist syndrome", causing negative feelings in patients, who react defensively, and inevitably contaminating the dentist-patient relationship and leading to rupture[31].

Previous studies on social representations of oral health[8,32] have discovered that oral health and disease are influenced by culture, employment, poverty, aesthetics and emotions. Alzate[33] and Romero[34] claim that social representations of the mouth and hygiene are deeply rooted in tradition and that very little is done to promote nourishment although it is recognized as a part of general health. Similarities among these studies lie in the social representations related to emotions (fear of pain) and poverty (economics). The differences between them and our study could be explained by the fact that they were undertaken with different focuses and populations and deal with different situations such as satisfaction, aesthetics, the relationship with discourse and institutional practices[8,34-36].

CONCLUSION

This study concluded that fear of pain, costly treatment, dentists' poor attitude, poor communication, hygiene and annoying dental equipment make up

the social representations of the cognitive system in environmental health graduate students. They were discerned within an explanatory framework of opinions and beliefs that marked their behavior.

We should note that although the formal education, social status and economic resources of the participants in our study differed from those in the abovementioned studies, the same SR of fear of pain prevails in both kinds of populations. This shows how difficult it is to modify this SR and indicates that professional education, even in the field of health, does not alter the SR of dental treatment, so that the perception is the same in professionals andh other populations.

New strategies should therefore be devised to identify the origin of this SR, in order to intervene effectively and change it. This would encourage patients to seek dental care, thereby improving the oral health of the general population.

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TITANIUM ALLOY ORTHODONTIC MINI-IMPLANTS: SCANNING ELECTRON MICROSCOPIC AND METALLOGRAPHIC ANALYSES

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ABSTRACT

Anchorage control is one of the determining factors of successful orthodontic mechanics. In mini-implants, fractures due to placement and removal have been related to implant design and titanium alloy quality. This study assessed the topography and microstructure of five brands of mini-implants (Neodent, SIN, Morelli, Conexão, Foresta Dent). Scanning electron microscopic analyses of the head, transmucosal neck, threaded body, and tip were performed to assess implant design and manufacturing defects (n=3/group). Metallographic analysis of longitudinal sections (n=15) and cross-sections (n=15) was performed under conventional light microscopy according to international standards of "American Society for Testing and Materials". The results showed significant differences in mini-implant design. Surface irregularities in the threaded body and tip were observed. Microstructural analyses revealed an alpha/beta-phase grain structure, in compliance with the ETTC-2 ("Technical Committee of European Titanium Producers" –2nd edition). No structural defects were detected. We conclude that differences in mini-implant design and the presence of surface irregularities may influence the effectiveness of orthodontic anchorage.

Key words: *orthodontics; dental implantation; titanium.*

MINI-IMPLANTES ORTODÔNTICOS DE LIGA DE TITÂNIO: ANÁLISES AO MICROSCÓPIO ELETRÔNICO DE VARREDURA E METALOGRÁFICA

RESUMO

O controle da ancoragem é um dos fatores decisivos no sucesso da mecânica ortodôntica. Fraturas devido ao estresse de inserção e remoção de mini-implantes são associadas ao design das peças e à qualidade da liga de titânio. O presente estudo analisou a topografia e a microestrutura de cinco marcas de mini-implantes (Neodent, SIN, Morelli, Conexão, Foresta Dent). Análise ao microscópio eletrônico de varredura da cabeça e perfil transmucoso, porção rosqueável e ponta ativa foi realizada com o propósito de avaliar o design e defeitos de fabricação (n=3/grupo). A análise metalográfica baseou-se nas normas internacionais da "American Society for Testing and Materials" e revelou a microestrutura em cortes longitudinais (n=15) e transversais (n=15) por meio do microscópio óptico. Os resultados demostraram que os mini-implantes apresentam diferenças significativas no design. Irregularidades superficiais na porção rosqueável e na ponta ativa foram também observadas. A análise da microestrutura revelou uma estrutura de grãos fases alfa e beta distribuídas de acordo com os padrões definidos pelas normas ETTC-2 ("Technical Committee of European Titanium Producers" – 2ª edição). Além disso, não foram detectados defeitos na estrutura interna das ligas. Conclui-se que diferenças no design dos mini-implantes e a presença de irregularidades superficiais podem influenciar na efetividade da ancoragem durante o tratamento ortodôntico.

Palavras-chave: *ortodontia; implante dentário; titânio.*

INTRODUCTION

Orthodontics is based on the exertion and control of forces acting on the teeth and supporting structures. Therefore, control of anchorage is essential for the success of orthodontic treatment[1].

Traditional orthodontic anchorage depends on patient compliance. Furthermore, the number or quality of teeth is often insufficient for effective anchorage[2]. Therefore, several anchorage devices have been used in recent decades. Prosthetic implants, plates, and

onplants have been replaced with mini-implants because they eliminate the need for invasive surgical procedures, high cost, placement site limitations, and considerable time for osseointegration[3].

Mini-implants are popular because of their ease of insertion and removal, less discomfort for patients, possibility of immediate loading, high versatility, and low cost[4-6]. Clinical and laboratory outcomes, however, have shown failure rates of 10 to 30%, mostly related to inflammation of peri-implant tissues, characteristics of soft tissues, and mini-implant placement site[7-9]. Screw diameter, length, thread form, presence of flutes, and screw material have also been implicated in poor primary stability of these devices[10-13].

The optimal material of mini-implants would exhibit excellent corrosion resistance, biocompatibility, and sufficient mechanical strength to resist placement and removal[14]. Titanium alloys have been used in these devices. The use of vanadium and aluminum have significantly enhanced their performance and mechanical properties[15]. Nevertheless, studies on the internal microstructure of mini-implants rare in the literature[14-16].

Because of the diameter and length restrictions of mini-implants, optimal shape design is important for primary stability. The strength resistance of a titanium alloy depends on its microstructure, which is influenced by the composition, heat treatment, and machining processes of the mini-implant[17]. Thus, studies analyzing the topography and microstructure of mini-implants are essentially important. The objective of the present study was to analyze the topographical and microstructural features of mini-implants used for orthodontic anchorage.

MATERIALS AND METHODS

Topography and microstructure were analyzed on Ti-6Al-4V (Grade 5 titanium alloy) self-drilling orthodontic mini-implants from five different dental implant manufacturing companies (four Brazilian and one imported). They were allocated into five groups: Group 1 – Neodent® (Curitiba, Paraná, Brazil); Group 2 – SIN® (São Paulo, São Paulo, Brazil); Group 3 – Morelli® (Sorocaba, São Paulo, Brazil); Group 4 – Conexão® (Arujá, São Paulo, Brazil); and Group 5 – Foresta Dent® (Pforzhein, Baden-Württemberg, Germany).

Scanning electron microscopy (SEM) analysis was conducted to obtain a descriptive analysis of implant design and detect potential manufacturing defects. Three mini-implants of each of the five brands were analyzed. The implants were bonded to aluminum stubs (Sigma Chemical Co., St. Louis, Missouri, USA) with cyanoacrylate adhesive (Super Bonder Gel, Loctite, Diadema, São Paulo, Brazil) and immediately analyzed under high-vacuum SEM (Philips XL 20, FEI, Eindhoven, The Netherlands) at 20kV accelerating voltage. Images of the screw head, transmucosal neck, threaded body, and tip were analyzed at 50x, 100x, and 200x magnification.

Metallographic analysis was conducted to detect discontinuities and to assess the presence of alpha- and beta-phase titanium. The methodology was based on the "American Society for Testing and Materials" (ASTM International). The standards applied were ASTM E3-01 (Standard Guide for Preparation of Metallographic Specimens)[18], ASTM E407-99 (Standard Practice for Microetching Metals and Alloys)[19], and ASTM E7-03 (Standard Terminology Relating to Metallography)[20]. Six mini-implants (three in longitudinal section, three in cross section) from each of the five brands were analyzed.

For longitudinal sections, the samples were cold-embedded in methyl methacrylate polymer before being sectioned lengthwise. The mini-implants were ground and polished with a series of silicon carbide abrasive sheets - 220, 320, 400, 600, and 1200 grit (3M, Sumaré, São Paulo, Brazil) under water. The samples were polished in a DP-10 sander (Panambra, São Paulo, São Paulo, Brazil), using diamond abrasive compound (3M, Brazil) with 1- and 2-mm grains. The longitudinally sectioned and polished mini-implants were etched in a solution of 10mL HF, 5mL HNO_3, and 85mL H_2O (Kroll's reagent) for 20 seconds, dried with hot air, and analyzed under light microscopy (Union MC 85800, OptiTec Ltd., Japan) at x50 and x400 magnification.

Cross sections were obtained at TORK (Controle Tecnológico de Materiais Ltda, São Paulo, São Paulo, Brazil). Samples were cold-embedded in acrylic resin. Metallographic analysis was based on the ISO 5832-3 standard (Implants for surgery - Metallic materials - Part 3), and the alpha/beta phases were compared with the European Technical standards (ETTC-2) published by the Technical Committee of European Titanium Producers. The mini-implants were sectioned with a circular table saw (Arotec, São Paulo, São Paulo, Brazil) and a cutting disc (Norton,

Worcester, Massachusetts, USA). The specimens were sanded in a round device with silicon carbide abrasive sheets - 150, 220, 320, 400, and 600 grit (3M, Brazil), on a tabletop grinding machine, using water as a lubricant to obtain a flat, homogeneous surface. The specimens were then polished in a sander with 6-µm and 3-µm diamond abrasive compound and buffed with 1-µm diamond compound. The cross-sectioned specimens were etched with a solution composed of 6 g NaOH, 60 mL H_2O, and 10 mL H_2O_2 for 20 seconds and dried with hot air. This process revealed the microstructure of the mini-implants, in which an effective contrast between alpha and beta phases was observed. The cross sections were examined under light microscopy (OPTON / TNM-07 PL, Cotia, São Paulo, Brazil) at x200 magnification.

RESULTS

The mini-implants exhibited significant differences between brands in screw head and transmucosal neck; pitch and shape of threads; and active tip design (Fig. 1).

Surface irregularities can result from machining process, polishing defects, crystal growth deposits, and areas of detritus. The greatest amount of surface irregularities and detritus was found on the tips of Groups 1, 2, and 3. The best surface finish along the threaded body was found in Groups 1 and 5. Mini-implants in all groups had adequate surface finish and no evidence of irregularities on the screw head or transmucosal neck (Fig. 1).

Despite the differences in the size of the orthodontic accessory on the screw head, they all had uniform structure and good surface finish. In Groups 1, 2, 3 and 4, the accessory was in the shape of an orthodontic button, whereas Group 5 mini-implants had bracket-shaped screw heads (Fig. 1).

Mini-implants in Group 4 had a greater number of threads and flutes at the tip and a screw head diameter equal to that of the transmucosal neck. In Groups 1, 2, 3, and 5, the screw head and transmucosal neck had different diameters. Although all mini-implants were of the self-drilling variety, those in Groups 1, 3 and 5 had sharper tips (Fig. 1).

Longitudinal sections were assessed to detect defects in the internal microstructure of each mini-implant, whereas cross-sections were compared against the ETTC-2 regarding the distribution of alpha and beta phases in the alloy.

There were no visible imperfections in the inner structure of any mini-implants, and no internal defects were detected on the longitudinal sections.

The mini-implants had a fine microstructure composed of an alpha matrix into which spheroidal beta-phase particles were dispersed. On cross sections, the internal microstructure of the alloys was consistent with ETTC-2 standard class A1. Alpha phase titanium appears light, whereas beta-phase granules appear darker. The small granule size of both phases and balanced alpha/beta ratio are indicative of high internal structure quality (Fig. 2).

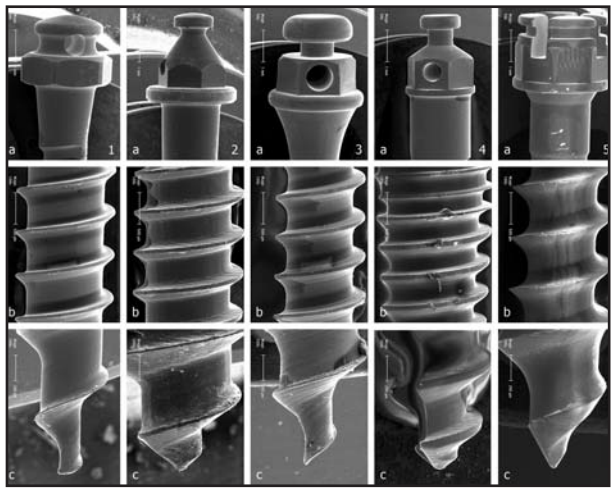

Fig. 1: Head and transmucosal neck (a) threaded body, (b) and active tip, (c) of mini-implants in the Neodent® (1), SIN® (2), Morelli® (3), Conexão® (4), and Foresta Dent® (5) groups.

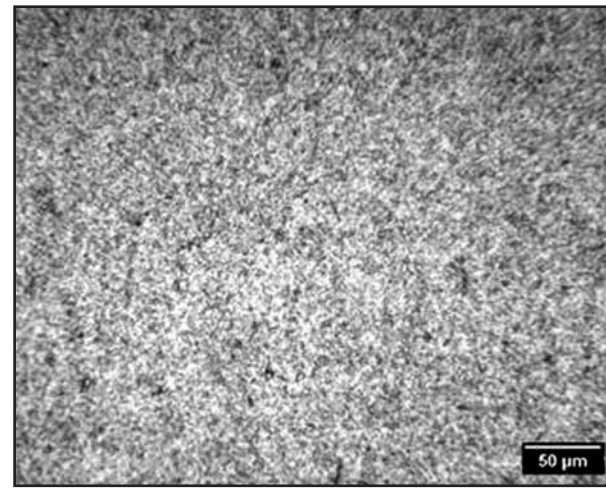

Fig. 2: Photomicrograph showing the microstructure of the mini-implants (cross section)

DISCUSSION

We found significant differences in screw head, threaded body, and tip design between the five brands of mini-implants. Furthermore, SEM analysis showed surface irregularities and detritus, particularly at the implant tip. Metallographic analysis did not show any defects in the microstructure. All mini-implants tested met the European standard for titanium alloy production.

Mini-implants are an effective and very well tolerated tool for skeletal anchorage, and have become the gold standard for orthodontic biomechanics in adults[2]. They are available in a variety of shapes, diameters, lengths, and titanium alloy compositions. However, it bears stressing that failure has been reported during mini-implant placement and removal. Mini-implant fractures are usually due to torsional strain caused by their small diameter[7,15,21]. Reicheneder et al.[22] reported that different mini-implant systems showed comparable elementary composition. They stressed that differences in mechanical properties can be attributed to mini-implant design, and that implant morphology plays an essential role in ensuring primary stability.

In our study, the surface defects found in most samples, particularly at the active tip, may be caused by the machining process. These defects may be a starting point for electrochemical degradation processes that can alter the surface finish of the implant and its resistance and other material properties[23]. According to Sebbar et al.[24] improvements in the surface treatment of mini-implants could improve their corrosion resistance. Mini-implants in Group 5 had fewer surface irregularities at the tip and better polish along the threaded body.

The machining process determines the surface finish of the piece. Machining leads to a rough surface. Therefore, the biocompatibility of the surface texture has major influence on the type and progression of reactions in the tissues adjacent to the implant surface. Furthermore, changes in surface morphology that may occur during the sterilization process and mechanical damage that may be sustained during mini-implant placement and removal may induce changes in osteoblast growth and differentiation[25,26].

Studies have also analyzed changes in mini-implant design that might lead to improvements in the mechanical properties[11]. A greater number of threads and a finer pitch in the implant are associated with greater mechanical locking ability, enhanced resistance during mini-implant placement, improved resistance to displacement, and improved primary implant stability[8]. Mini-implants in Group 4 may improve the distribution of applied forces because they have a greater number of threads. Furthermore, the presence of flutes may be linked to greater fracture resistance, as it prevents concentration of excessive strain in the adjacent tissues[8,26]. Conversely, thread design may also interfere with the distribution of strain under load[11]. Hence, further studies are required to ascertain the influence of design on the mechanical properties of mini-implants.

Lee et al.[27] found that many undesirable outcomes are attributable to the design of mini-implants. They claim that a coarsely finished or poorly designed mini-implant active tip may compromise final implant placement and primary stability. All mini-implants we tested were of the self-drilling variety, and those in Groups 1, 3 and 5 had the finest and sharpest tips, which suggests greater ease of placement without pilot hole drilling. The diameter of the mini-implant head is an important design factor. It should be wider than the transmucosal neck to prevent overgrowth of soft tissues. All implants had this design feature, except for those in Group 4. Casaglia et al.[12] showed that small transmucosal neck diameter is a site of increased fragility. The authors detected microfissures and grooves on the surface and concluded that these irregularities may predispose to mini-implant fracture.

Most mini-implants are made of Ti-6Al-4V (ASTM Grade 5 titanium alloy). This alloy has greater mechanical resistance than pure titanium and is more appropriate for small-diameter devices. Furthermore, its lower bioactivity facilitates implant removal because of less osseointegration[18].

Titanium alloys must be free of external irregularities and internal imperfections to avoid interference with fracture resistance, mechanical retention, displacement resistance, and primary stability[16]. Our metallographic analysis did not reveal any internal defects, corroborating the findings of Cotrim-Ferreira et al.[15] and Eliades et al.[16].

The alpha phase of titanium alloys is a soft alloy showing high resistance and tensile strength, but low ductility. Alpha-stabilizing elements increase the temperature range at which the alpha phase remains stable. The beta phase, in turn, has superior forming and fatigue resistance, but is highly vulnerable to

atmospheric contamination. Beta stabilizers make the beta phase stable at low temperatures[18].

The matrix of all mini-implants assessed contained beta and alpha titanium, which indicates that the small amount of vanadium in the alloy was sufficient to retain significant amounts of beta-phase titanium, thus enhancing the properties of the alloy, as demonstrated by Iijima et al.[14].

All mini-implants complied with the ETTC-2 standards, namely in class A1. Cotrim-Ferreira et al.[15] found that the microstructure of Conexão® brand implants was class A9, showing differences in hardness, resistance, and elastic modulus because of different alpha-beta phase ratio. However, this may not interfere with mechanical resistance, as alloys of any class from A1 through A10 can be used for mini-implant manufacturing according to the ETTC-2 standards.

Despite the advantages of titanium alloy mini-implants, practitioners must be aware of the topographic and microstructural features of mini-implants, since they influence the effectiveness of orthodontic anchorage. Orthodontic treatment depends on reliable and effective anchorage. Primary stability, mechanical resistance, and clinical performance of mini-implants, in turn, depend on their topographical and microstructural characteristics.

The present study concluded that the orthodontic mini-implants assessed exhibited significant differences in the design of the screw head, transmucosal neck, threaded body, and active tip. Furthermore, surface irregularity and debris were found in all groups, particularly at the active tip. Conversely, no internal defects were detected, and all groups complied with the international standards for mini-implant manufacturing. Further studies of orthodontic mini-implants should prioritize topographic and microstructural analysis combined with mechanical testing.

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ORAL HEALTH IN DRUG ADDICT ADOLESCENTS AND NON PSYCHOACTIVE SUBSTANCE USERS

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ABSTRACT

The purpose of this study was to compare oral health between adolescents who are recovering drug addicts and adolescents who report not having used psychoactive substances. A retrospective observational Case-Control study was conducted on 60 subjects per group, aged 15 to 25 years, paired according to sex, age and educational vulnerability. Dental and sialochemical examinations were used to determine oral health/disease/care indicators. Psychoactive substance use habits were obtained from clinical records. DMFT index for Case adolescents was 8.58±4.34, doubling the mean value for the Control group, which was 4.33±4.30. CPI was compatible with gingival-periodontal health in 45% of the Control subjects, but only 20% in the Case group. CPI categories 2 and 3 had different distributions according to the study group, with CPI2=33%, CPI3=0% for the case group and CPI2=57%; CPI3=5% for the control group. Mean values for stimulated saliva for Case and Control groups, respectively, were: salivary flow (ml/min) 1.42±1.08; 0.98±0.41, salivary pH 6.96±0.33¸ 6.86±0.27, and buffer capacity expressed as final pH, 6.73±0.29, 6.61±0.28. Wilcoxon's test for independent samples showed significant differences (p<0.05) between Case and Control for the variables White Spot, Non-Cavitated Carious Lesions, Cavitated Carious Lesions, DMFT, Components D and M, Salivary Flow and Buffer Capacity. There was significant association between the D component in DMFT and use of psychoactive substances, both in single drug and polydrug users. Oral component status was worse in recovering drug addicts than in non-users of psychoactive substances.

Key words: *Dental Caries Susceptibility; Oral Health; Drug users.*

SALUD BUCAL EN ADOLESCENTES DROGODEPENDIENTES Y EN NO CONSUMIDORES DE SUSTANCIAS PSICOACTIVAS

RESUMEN

El propósito de ésta investigación fue comparar el estado de salud bucodental en adolescentes drogodependientes en recuperación y los que no refieren consumo de sustancias psicoactivas. Se realizó un estudio observacional tipo Caso y Control, retrospectivo de 60 adolescentes de 15 a 25 años para cada grupo, apareados según: sexo, edad y vulnerabilidad educativa. A través de examen clínico odontológico y sialoquímico se relevaron indicadores de salud-enfermedad-atención del componente bucal. Los hábitos de consumo de sustancias psicoactivas se obtuvieron de historias clínicas. El índice CPOD en los adolescentes Casos resultó 8,58±4,34 valor que dobla la media que presenta el grupo Control 4,33±4,30. El Índice IPC presentó una situación compatible con salud gíngivo-periodontal en el 45% de los sujetos Control, mientras que sólo alcanzó al 20% en el grupo Caso. Las categorías 2 y 3 del IPC mostraron distribución diferente según el grupo de estudio siendo IPC2=33%; IPC3=0% y IPC2=57%; IPC3=5%, respectivamente para casos y controles. Los valores medios de los registros de saliva estimulada fueron para flujo salival (ml/min) 1,42±1,08; 0,98±0,41, pH de saliva 6,96±0,33¸ 6,86±0,27, y la capacidad amortiguadora o buffer expresada como pH final 6,73±0,29, 6,61±0,28 para el grupo Caso y Control respectivamente. La prueba de Wilcoxon para muestras independientes puso en evidencia diferencias significativas (p<0,05) entre Caso y Control para las variables Mancha Blanca, Caries no Cavitada, Caries Penetrante, CPOD, Componentes C y P, Flujo salival y Capacidad buffer. Se observó asociación significativa entre el componente C del CPOD y el consumo de sustancias psicoactivas tanto para la modalidad de monoconsumo como para la de policonsumo. La situación del componente bucal de los sujetos drogodependientes en recuperación, presenta mayor deterioro, respecto a los no consumidores de sustancias psicoactivas.

Palabras claves: *caries; salud bucal; drogadependencia.*

INTRODUCTION

The increasing complexity of the modern world has led to a sharp increase in misuse of psychoactive substances (PAS), which produce severe physical and mental effects, leading to individual, family and social conflict, with serious impact on society. Reliable research is needed to support intervention strategies for regional programs for promotion, prevention and control.

Changes in the interrelationship between human beings and their world include the use of psychoactive substances (PAS)[1]. The analysis and treatment

of PAS addiction disorders are currently tackled from and comprehensive approach which claims that "there is no single factor" leading to the process of addiction, and that different personality subsystems (environmental-behavioral, biological, cognitive, affective, spiritual, unconscious and systemic) may be involved[2-4]. Drug is considered to be any substance whose use/abuse can cause psycho-neuro-bio-socio-toxic consequences[5]. "Drug" is commonly used to refer to chemical substances which can cause dependence. The World Health Organization Expert Committee on Drug Dependence defines drug as a chemical, whether natural or synthetic, which, when introduced into a live organism by any route (inhalation, ingestion, intramuscular, intravenous), is capable of acting on the central nervous system, producing physical and/or psychological alteration, experience of new sensations or modification of a psychological state, i.e. is capable of altering the person's behavior.

Adolescent health is a key factor in the economic, social and political development of Latin American countries, upon which the success, development and competencies of the societies to which they belong depends[6].

Adolescence is considered to be a stage which begins at puberty and lasts until the beginning of adulthood, when the subject becomes autonomous, assumes responsibility for his own life, and achieves his identity. How this process evolves depends on the characteristics of the context (the person's particular situation regarding family, gender, location, social class, education, among others) and the time in history in which the adolescent lives[7]. During the construction of identity, the adolescent endeavors to differentiate him/herself, and this may include attitudes ranging from personal untidiness, lack of interest in cleanliness and neatness, challenging authority, direct provocation of adults, poor school performance, dropping out of school, and sleeping too much or loafing, to behaviors that place him/her at real risk,[8] such as premature sexual activity, running away from home, alcohol and/or drug abuse, eating disorders, and criminal acts, in an attempt to distinguish him/herself clearly from his/her past world and seek limits imposed by adults[9].

One of the systems affected by exposure to PAS is the stomatognathic system. It may be affected by lesions caused by the psychoactive substances themselves, or related to negligence in attention to oral hygiene, e.g. diseases such as gingivitis and extrinsic discoloration[10]. Substance users have many clinical odontological alterations such as xerostomia[11], alteration of saliva flow, reduction in saliva buffer capacity[12], erosion, abrasion, atypical caries and tooth loss[13]. Many factors can reduce saliva flow, including physiological situations with anticholinergic effect, medications[14,15], diseases of the salivary glands or systemic processes affecting them[16]. The reduction in saliva flow is explained in marihuana users as a parasympatholytic effect[17]. Di Gugno[18] considers that there are three main factors causing deterioration in PAS users: reduction in saliva pH and saliva flow, low concentration of inorganic phosphate, which hinders remineralization, and high intake of refined carbohydrates.

Also frequent are changes in the pain threshold and perception of taste, atrophy of salivary glands, erosions and ulcers of the oral mucosa.

Marihuana users have greater deterioration in the oral cavity than non-users, with increased risk of caries and periodontal disease[19]. Smoking marihuana is also carcinogenic and associated to dysplastic changes and precancerous lesions of the oral mucosa, increasing the incidence of squamous cell carcinoma, xerostomia, severe gingivitis, ischemic necrosis of palate, and bruxism[20]. Marihuana users are more prone to oral infections, possibly due to the alteration in oral immunology. Higher DMFT and bacterial plaque indices have been found in marihuana users than in non-users[17,21].

Drug addicts are careless of their general health and have behavioral disorders and infections associated to addiction[22], as well as high incidence of caries and periodontal diesase[23].

The aim of this study was to compare the oral health situation of adolescent and young adults who are recovering drug addicts to that of adolescents and young adults who do nor report PAS use.

MATERIALS AND METHODS

A retrospective observational Case-Control study was conducted on adolescents aged 15 to 25 years, of both sexes, paired according to sex, age, and educational vulnerability, following the classification by González, 2007[24,25], who classified the areas in Córdoba Capital district into five levels: cluster 1 – areas with medium high income; cluster 2 – areas with medium income; cluster 3 – areas with low

income and clusters 4 and 5, rural areas with few inhabitants per square kilometer.
The "Case" group was further characterized by considering their use of psychoactive substances based on clinical records prepared by the institution when subjects enrolled at the Recovery Treatment Program.
Pursuant to the Tokyo convention, written informed consent was obtained prior to participation in the study. For adolescents under 18 years old, the informed consent had to be signed by the tutor or guardian.

Inclusion criteria
Adolescents aged 15 to 25 years at the time of the interview. All subjects included in the Case and Control groups were social tobacco smokers and regular alcohol drinkers (at least once a week).

Exclusion criteria
Diagnosis of systemic disease (metabolic, infectious-contagious, autoimmune, HIV, transplanted patients, among others); reporting taking prescribed medication for over 1 year at the time of the interview; wearing orthodontic appliances or having had them removed up to 12 months prior to the interview.

Case group
60 institutionalized drug addict adolescents in recovery period, aged 15 to 25 years, who voluntarily joined the NGO "Programa CAMBIO". As a result of the care system and institutional approach, participants were subjects who had joined the program and undergone not more than one month's treatment to recover from addiction, with a recent history of exposure to PAS from 2007 to 2010. International criteria were followed to determine drug addict status[26], and the psychoactive abuse drugs were classified following the Diagnostic and Statistical Manual of Mental Disorders of the American Psychiatric Association –DSM IV TR[27].

Control group
60 adolescents who during the interview did not freely and voluntarily report PAS use, aged 5 to 25 years, of both sexes, who attended the school Colegio Nacional del Monserrat (formal teaching center) and the Centro de Orientación Vocacional (informal teaching center), both located in Córdoba city. In order to facilitate the validity of the self report on PAS use, a prior instance of personal interrelation was established, at which the subjects felt free from pressure to express their experience of possible exposure to PAS. For the Control group, subjects were paired according to sex, age and educational vulnerability with regard to the subjects in the Case group, which was formed first.

Clinical – odontological assessment
A clinical examination28 was conducted in classrooms assigned by the institutions, using non-conventional simplified methodology with an exploration kit consisting of mirror, tweezers, explorer and periodontal probe, under artificial light (surgical headlight). Observations were recorded on a card designed ad-hoc, which in addition to personal data and general health background, provided space for information on cariogenic-periodontopathic risk categories. For hard tissues: the following were recorded: number of teeth in the mouth, presence of caries (D), discriminating White Spot incipient caries (WS)29 and non-cavitated caries (nCD); fillings (F); extractions (M). WHO criteria were followed to diagnose lesions28. This information as used to calculate the DMFT and DMFS indices30. Considering that the DMFT index provides information limited to cavitated carious lesions, for a more reliable analysis of real conditions of the health-disease process, the variable "total caries" was used, which was the sum of all the evolutionary stages of the disease: WS, nCD and DT (component D in DMFT). Gingival-periodontal tissue was assessed using Löee Silness plaque index (PI)31 and Community Periodontal Index (CPI)28. *Saliochemical assessment*
Sialochemical assessment was performed on total mixed saliva samples, stimulated by chewing a 4cm x 4cm piece of Parafilm. It measured saliva flow in ml/min.; pH; and buffer capacity following Ericsson[32] and expressed as final pH. A portable manual pH-meter with temperature probe was used (Adwa).

Statistical analysis
Centralization and dispersion measures were used to describe the behavior of quantitative variables: mean ± SD and median for discrete variables. Non-parametric Mann Whitney-U test was used to assess the significance of the differences in behavior of the variables in the Case and Control groups[33]. Pearson's Chi squared was used to establish differences between proportions or associations among cate-

gorical variables. Contingency 2x2 tables (in dichotomic variables) were used to establish Odds Ratios (OR) and their respective confidence intervals (CI)[33]. Statistical significance was established as p = 0.05 for all cases. Data were processed using Infostat software version 2010[34].

RESULTS

The study was conducted on 120 adolescents, 91% male and 9% female, mean age 19±2 and 18±3 years, distributed in two groups (Case and Control) paired according to sex, age and educational vulnerability. Table 1 shows the distribution according to groups.

Characterization of the "Case" group: Subjects in the Case group began using alcohol at age 13 ± 0.5 years, and PAS at 16 ± 0.3 years, with marihuana being the most frequent substance used first (83%) followed by inhalants (11%) and psychopharmaceuticals without medical prescription (5%). The remaining 1% began directly with cocaine.

In the year prior to joining the Institution, (considered recent use), prevalence of psychoactive substances used (statistically significant, Chi squared <0.000) was: marihuana 58%, followed by a combination of other drugs 28% (polydrug use, including marihuana, cocaine and psychopharmaceuticals), cocaine 10%, and psychopharmaceuticals without medical prescription 4%.

Exposure time to PAS (time between first use reported by the subject and date he/she joined the recovery program), varied significantly (Chi squared <0.000). For adolescents and young adults in the Case group it was 1 to 14 years, with a median of 4 years and a mean of 4.4 ± 2.6 years. Twenty-two (22) (36.6%) subjects had been addicts for 4 or more years and 38 for less than 4 years (63.3%).

Frequency of use in the last year prior to joining the Institution was daily in 70% of the Case group.

Table 1: Distribution of gender and age in Case and Control groups.

GROUP	GENDER	Age Mean±SD
Case	FEMALE	17.83±1.47
	MALE	19.22±2.79
Control	FEMALE	17.83±2.04
	MALE	18.38±3.18

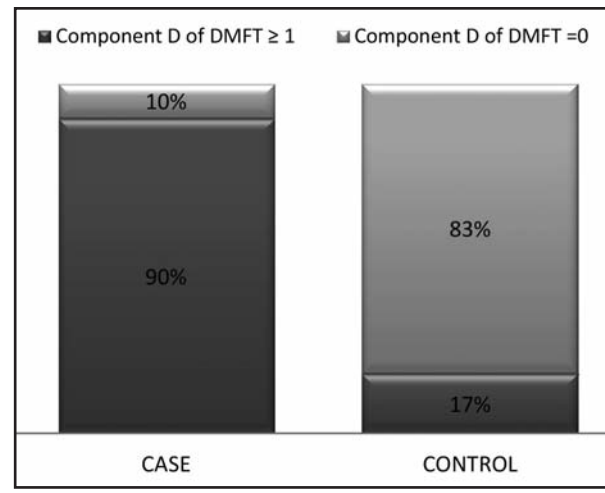

Fig. 1: Distribution of the D component of DMFT in the study groups.

Clinical-odontological assessment: No alteration was found for pain threshold, taste perception, atrophy of salivary glands, erosions and ulcers of the oral mucosa.

Table 2 provides the mean values for tooth status indicators regarding caries experience.

DMFT differed between groups, with the mean and median values for the Case group being twice as high as for the Control group, mainly due to the difference in the D component. Upon considering DMFS, the values were three times as high for the Case group.

Table 2: Central measurements of DMFT and DMFS indices, and their components.

		DMFT	D	M	F	DMFS	D	M	F
Case	MEAN±SD	8.58±4.34	4.42±3.38	0.69±1.5	3.48±3.64	15.4±10.4	6.87±6.81	3.33±7.4	5.2±5.38
	MEDIAN	9	4	0	2	14	5	0	3 a 4
Control	MEAN±SD	4.33±4.3	0.33±1.05	0.25±0.89	3.77±4.35	5.1±4.7	0.4±1.17	0.45±1.37	4.3±4.66
	MEDIAN	4	0	0	3	5	0	0	3 a 4
Wilcoxon's test (significance p<0.05)		p<0.0001	p<0.0001	p=0.0100	p=0.9957	p<0.0001	p<0.0001	p=0.0036	p=0.4445

With regard to the D component in DMFT (Fig. 1), 83% of the subjects in the Control group (n: 50) had D = 0 and 90% of the subjects in the Case group (n: 54) had D ≥ 1, with OR=45; CI 95% [15.74%; 128.65].
Table 3 shows dental status, with statistically significant differences between Cases and Controls.
CPI was compatible with gingival-periodontal health in 45% of the subjects in the Control group and 20% in the Case group. The distribution of CPI categories showed differences in frequencies: **CPI 2**=33%; **CPI 3**=0% for the Control group and **CPI 2**=57%; **CPI 3**=5% for the Case group (Fig. 2).
Mean PI values were 0.97±0.83 for the Case group and 0.8±0.52 for the Control group, Wilcoxon test p=0.4910 (significance p<0.05). Although the mean value for both groups reflected low risk (PI <1), it should be noted that BP plaque index indicated at risk (>1) for 33% of the Control group and 47% of the Case group.

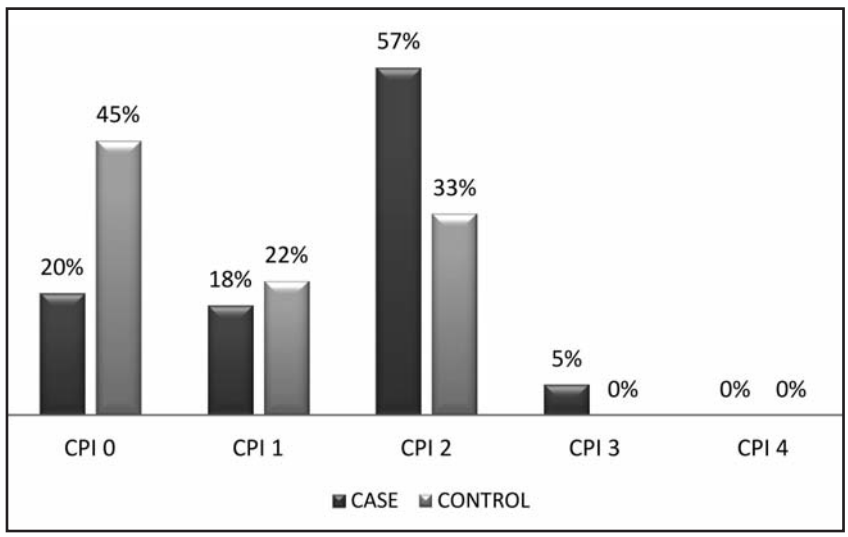

*Fig. 2: Distribution of Community Periodontal Index *categories in the study groups.*

Saliochemical assessment
Table 4 provides the mean values for the variables recorded in stimulated saliva: saliva flow, pH, and buffer capacity expressed as final pH[32].
Even though the mean value in both groups is compatible with health, 41.6% of the Case group and 50% of the Control group were assessed as at risk (salivary flow < 1 ml/m).
Wilcoxon's test for independent samples showed significant differences (p<0.05) between Case and Control for the variables Saliva Flow and Buffer Capacity, with no statistical significance for saliva pH between groups.

DISCUSSION

Drug addiction is a complex issue in the sphere of Public Health, because it is associated with a series of pathologies. This study found more males (91%) than females in the group of drug addicts undergoing recovery. Many studies conducted at different places and times[35-37] agree that addiction to psychoactive substances is more frequent in males, except for use of anxiolytics, sedatives and tranquilizers, which are more frequently used by females[38]. Considering that the participants in our study are undergoing recovery, we should take into account that several factors may influence their interest or decision to join a process to recover from addiction; thus, based on our study, we cannot state that addiction is more frequent in males.

Table 3: Situation of developmental status of caries in Case and Control groups.

		WS	nCD	DT	Total Caries
Case	MEAN ±SD	3.83±4.83	4.22±2.85	4.42±3.38	12.47±3.2
	MEDIAN	1 a 2	4	4	12
Control	MEAN±SD	2.43±4.57	2.08±2.87	0.33±1.05	4.84±2.83
	MEDIAN	0	1	0	4
Wilcoxon's test (significance p<0.05)		*p=0.0386*	*p<0.0001*	*p<0.0001*	

Table 4: Sialochemical variables in Case and Control groups.

		Saliva flow Vol/ min SE	Initial pH SE	Final pH SE
Case	MEAN±SD	1.42±1.08	6.96±0.33	6.73±0.29
	MEDIAN	1.24	7	6.75
Control	MEAN±SD	0.98±0.41	6.86±0.27	6.61±0.28
	MEDIAN	0.99	6.89	6.61
Wilcoxon's test (significance p<0.05)		*p=0.0449*	*p=0.0542*	*p=0.0151*

Because alcohol use is associated as a necessary antecedent to PAS use[39], in our study, all subjects had used alcohol, at least as weekend users. The starting age for alcohol use in the Case group was 13 years, significantly lower than the age provided in the latest national survey on PAS use conducted by SEDRONAR (Secretariat of Planning for Prevention of Drug Addiction and Trafficking) in 2010, which reports 16 years as the starting age for alcohol use[40]. Our results are closer to a study conducted in Córdoba city by Godoy J 2009[41], where use is "anticipated" in 11-year-old children.

Studies in other countries assessing the prevalence of caries in alcoholics report different results[42,43]. These are due to factors such as duration and type of alcohol abuse, oral hygiene, smoking, time since last visit to the dentist, sugar intake, and abnormal liver function, among others. Dasanayake 2010[44] concludes that the group "alcohol and drugs" was at greater risk of caries (38%) than the "alcohol only" group, concluding that the risk of caries among "alcohol only" abusers is significantly lower than among "alcohol and drug" abusers. That study suggests that alcohol needs to be taken into account with regard to caries evolution conditions, based on the biological model that would explain the association between alcohol and caries. Microbial oxidation of ethanol in the saliva of alcohol abusers would produce acetaldehyde[45], which can also alter cariogenic flora by reducing levels of Streptococcus[46]. Warnakulasuriya[47] has demonstrated that certain alcoholic beverages in the United Kingdom contain high levels of fluoride and people who drink three cans of beer per day receive the upper limit of daily fluoride recommended. In Argentina there are no available data on fluoride content among the components of alcoholic beverages.

Starting age for PAS use in the Case group was 16 years, marihuana being the preferred first substance, followed by inhalants and psychopharmaceuticals without medical prescription. Prevalence of PAS use in the past year in the Case group was Marihuana 58.32%, polydrug 28.33%, cocaine 10% and psychopharmaceuticals without medical prescription 3.33%, with oral intake as the most frequently used route of administration. Studies conducted in Spain at hospital detoxification centers [48] found that the variety of routes of administration has increased over recent years. The polydrug use detected matches data in the literature reporting that patients who are addicted to substances are not usually purists either with regard to the substances or to the form of taking them, and they combine different substances and alternate nasal, oral and more rarely, venous routes [49], in agreement with our results.

Although the literature reports frequent findings of variations in pain threshold,[50] taste perception, atrophy of salivary glands, erosions and ulcers of the oral mucosa[51-53], our study found none of those alterations. Incidence of lesions in the gingival-periodontal tissues increases with modality, duration, frequency and intensity of PAS use, together with lack of oral hygiene[54,55], as reflected by the fact that 47% of the Case group had at risk plaque index (PI> 1), in contrast to 33.33% of the Control group, although mean PI for both groups did not indicate risk. This may be due to the fact that the patients are institutionalized, and have therefore recovered more regular hygiene and diet habits. The risk shown by plaque index associated to gingival alteration detected in almost 50% of the Case group, it matches reports in the literature about the frequency of chronic gingivitis in patients who are addicts[56]. According to one study[57] the type of brush, and frequency and way of brushing differ significantly between drug users and controls without exposure to PAS. This variable was not considered in our study.

With regard to periodontal status, in our study, CPI was compatible with gingival-periodontal health in 45% of the Control group and 20% of the Case group, with different distributions: CPI^2=33%; CPI^3=0% in the Control group and CPI^2=57%; CPI^3=5% in the Case group. Considering that grade 2 reflects the presence of dental calculus and not necessarily the evolution of gingival-periodontal disease, there is lack of evidence to suggest a link between PAS use (primarily marihuana) and periodontal disease in the adolescent population in the Case group exposed to PAS, in agreement with studies conducted in Chile[58].

Persons who are still cannabis smokers can be classified as ''long-term users, and thus at amotivational risk" as described in Schwartz syndrome[59], characterized by lack of concern for personal hygiene and appearance, suggesting self-abandonment. In our study we may infer that the periodontal effects of short-term exposure to cannabis (participant ages in this study were 15 to 25 years)

may differ from the more serious effects of long term exposure to it in an older population.

With regard to dental disease, specifically caries prevalence, in the drug addict group, our research found similar results to studies conducted in Spain[60,61], Italy[62], Denmark[63], United States[64], France[65] and Australia[23].

It has been reported that caries experience in marihuana addicts is high[17,19,21]. In our Case group, DMFT showed a mean value of 8.58 ± 4.34 teeth with caries history, notably lower than values reported in other papers, such as DMFT = 18.3 in Aarhus, Denmark[63], 16.9 in Holland among addicts aged 20-40 years[50], 12.9 in Mercato San Severino (Salerno), Italy, for addicts aged 18 to 34 years[62], 12.8 in Barcelona, Spain in addicts aged 17 to 40 years[60], 13.03 in another group in Spain[61], among various other studies conducted in Australia[23], United States[64] and France[65]. Nevertheless, upon considering "total caries" in the Case group, which also includes cavitated caries, incipient lesions (WS) and non-cavitated caries (nCD), the mean value is closer to the values mentioned above.

A paper by Reece 2007[66] reports a cross-sectional study of 280 subjects aged 19 to 45 years, divided into a group of drug addicts and another group of non-drug addicts, showing that the addicts had a higher percentage of decayed and missing teeth than the non addicts. The pathology developed at an earlier age in addicts. Among subjects younger than 35 years old, 56.8% of addicts had the pathology compared to 5.4 % of the non-addicts. This agrees with our findings, in which the Case group had greater number and severity of caries (reflected by the higher number of caries with pulp complication) than the Control group.

Szymaniak 1990[67] studied tooth status in 30 drug addicts aged 21-34 years (duration of addiction 3 to 16 years) and compared the results to those from a similar group of subjects of the same age who were not drug addicts. The study found that drug addicts had twice as many decayed and missing teeth and four times fewer fillings than the controls. It concludes that drug addiction activates the carious process and the tendency to mutilation of the stomatognathic system.

Another study on addicts (mainly intravenous route users) in India[57], aged 18 to 48 years, found mean DMFT 4.84 for addicts and 3.73 for controls, which are lower than the values found in our study, possibly related to the type of use.

Our study found no significant difference in the F component between groups, so it is assumed that there has been adequate dental care availability, possibly before Case group subjects became addicted, since there is a marked difference in the D components and severity of lesions between groups.

For the M component, the Case group had higher frequency, which may be evidence of lack of timely dental care for restoration, conversely to what happens with the F component.

Drug abuse may reduce pain associated to dental caries, thus by the time PAS users are examined they are late in the process of the disease, in agreement with Charnock 2004[54].

In our study, the mean value for decayed teeth with cavitated carious lesions (D) and missing teeth (M) were higher in the Case group (5.11) than in the Control group (0.58), reflecting access to dental care and low self esteem during the addiction process in the Case group, in agreement with studies conducted in Spain[60].

Addiction to PAS may thus be considered to act at least as a factor which, from the socio-cultural to the individual psychological, has negative influence on timely visits to the dentist and thus, on receiving early care for the pathology.

In our study, the Cochran-Mantel-Haenszel test shows the variable Gender influenced behavior of presence of caries in both groups.

Sialochemical assessment showed that saliva flow differed significantly between Case (1.42 ml/min) and Control (0.98 ml/min) groups. The value for drug addicts was similar to those found in other studies in Brazil[68] in similar conditions on a population exposed to PAS under study, who were found to have mean values of 1.13 ml/min. It should be noted that in this population in Brazil, 64% had saliva flow >1, whereas in our study, only 41.1% did. Although various studies mention dry mouth as an effect of drugs[18,69,70], it was not observed in our study. Salivary glands might have normalized their functioning after PAS use stopped, restoring the salivary flow. Moreover, due to regulations at the institution "Programa Cambio", it was not always possible to know what type of medication the patients were using in order to make a more specific analysis of the effects of each drug prescribed by the physician at the institution. It is worth highlighting that because the institution is coordinated by psychologists, its therapy minimizes the psychiatric

medication administered to institutionalized patients, in contrast to other therapeutic communities where there is a tendency to substitution therapy with regard to psychiatric medication, which may substantially modify saliva flow rates.

The pH was similar in both groups (Case group: 6.96; Control: 6.86), with no statistically significant difference between groups, in a range compatible with health, in agreement with other studies on drug addicts in Spain,[60] where pH was 6.80.

Buffer capacity in both groups was compatible with healthy values (Δ pH 0.23 for the Case group and 0.25 for the Control group) in contrast to studies in Brazil[12,68] which found alterations in buffer capacity.

Although according to a study by Kumar in 2006 on 220 institutionalized psychiatric patients[71], caries index increases with age, our study did not analyze the age factor as predictive, as being a PAS addict was more important.

Some authors[50,62,72] have suggested that in this type of patients the degree of dental pathology is directly related to poor oral hygiene and the years of actively using psychoactive substances. The literature in general reports data on oral use of marihuana and cocaine in relation to lesions found in the oral component[19,21].

In our study, adolescents in the Case group reported that they use marihuana, usually smoked, together with alcohol and tobacco, which makes it impossible to discern whether the oral-dental component status in the Case group can be attributed exclusively to PAS.

There is not enough evidence of association between PAS addiction and caries. In our study, the conditions of oral deterioration in the Case group compared to the Control group cannot be attributed exclusively to exposure to PAS. PAS use is a complex phenomenon in which the psychological-emotional factor has an impact on personal care, including hygienic-dietary habits, generating an unfavorable context which makes it difficult to identify which effects on the oral component are attributable to PAS use. Further studies are needed on other therapeutic communities for drug addicts, and which look in greater depth at other socio-cultural aspects not considered in this study, in order to identify the factors that have the greatest incidence on the development of caries in drug addicts.

There is a wide gap between the oral-dental health status of drug addict adolescents undergoing recovery and adolescents who do not report use of psychoactive substances. Worldwide, there are few reliable epidemiological data on dental caries in alcohol and drug users, even though both alcohol and drug abuse, whether individually or combined, have harmful effects on health. Further research is needed to understand the true nature of the effect of these damaging exposures on various components of caries experience.

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PERCEPTION OF PROFESSIONALS IN THE ASSESSMENT OF CORONOID HYPERPLASIA BY COMPUTED TOMOGRAPHY

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ABSTRACT
Coronoid Hyperplasia (CH) is a non-neoplastic and relatively rare enlargement of the coronoid process that may limit mandibular movement as a consequence of the close association between the hyperplastic coronoid process and the anterior region of the zygomatic bone. Computed tomography (CT) is extremely useful for the observation of this association and plays an important role in diagnosing and planning surgical treatment. Once the CT scan is performed, the data can be viewed in many different arrangements, including multiplanar (MPR) and 3D rendering, although the resolution of the latter may not be as good as that of the former. Our aim is to analyze the importance of and preference for multiplanar and 3D reconstruction images for diagnosing and interpreting Coronoid Hyperplasia (CH), by comparing the opinions of oral surgeons and oral radiologists who analyzed both temporomandibular joints (TMJ) in 20 patients. Three images of each TMJ comprised the set of scans (MPR, 3D reconstructions with maximum intercuspation and 3D reconstructions with maximum mouth opening). After each analysis, the members of the two groups answered a questionnaire about the usefulness of each examination and classified the association between the head of mandible and mandibular fossa. Hypomotility was present in 55.2%. Both groups stated that both MPR and 3D reconstructions, particularly the latter, were fundamental for diagnosing CH and that they would request them in order to interpret CH correctly. The examiners were found to differ significantly regarding their opinion of MPR; only radiologists considered MPR to be less elucidative for the diagnosis of CH.

Key words: *temporomandibular joint disorders; computed tomography.*

PERCEPÇÃO DE PROFISSIONAIS NA AVALIAÇÃO DA HIPERPLASIA CORONÓIDE PELA TOMOGRAFIA COMPUTADORIZADA

RESUMO
A Hiperplasia Coronóide (HC) é um aumento não-neoplásico e relativamente raro do processo coronóide que pode limitar o movimento mandibular, como conseqüência da íntima relação entre o processo coronóide hiperplásico e a região anterior do osso zigomático. A tomografia computadorizada (TC) é extremamente útil para a observação dessa relação, desempenhando assim um papel importante no diagnóstico e planejamento do tratamento cirúrgico. Uma vez que a TC é realizada, os dados podem ser vistos em muitos arranjos diferentes, incluindo o multiplanar (MPR) e a reconstrução em 3D, no entanto, a resolução desta última pode não ser tão boa quanto a primeira. Nosso objetivo é analisar a importância e a preferência por reconstruções de imagens multiplanares (MPR) e 3D para diagnosticar e interpretar a Hiperplasia Coronóide (HC), comparando cirurgiões buco-maxilo-faciais com radiologistas orais. Ambas as articulações temporomandibulares (ATM) de 20 pacientes foram analisadas pelos 2 grupos. Três imagens de cada ATM compreenderam o conjunto de exames (MPR, reconstruções 3D em máxima intercuspidação e reconstruções 3D com abertura máxima da boca). Após cada análise, os grupos responderam a um questionário sobre a utilidade de cada exame e classificou a relação entre a cabeça da mandíbula e da fossa mandibular. A Hipomobilidade esteve presente em 55,2%. Ambos os grupos afirmaram que tanto MPR e as reconstruções em 3D, especialmente a última, foram fundamentais para diagnosticar CH e as requisitariam para interpretar a HC corretamente. Foi encontrada uma diferença significativa entre os examinadores sobre a MPR; apenas o grupo de radiologistas considerou que este exame não é tão elucidativo para o diagnóstico da CH.

Key words: *articulação têmporo-mandibular; tomografia computadorizada.*

INTRODUCTION

Temporomandibular joint (TMJ) dysfunction is a common disease that causes many people to seek treatment at dental care centers to restore proper, pain-free TMJ movement. However, it may be mistakenly diagnosed as a different pathology. Some patients in fact present Coronoid Hyperplasia (CH), which is a non-neoplastic, relatively rare enlarge-

ment of the coronoid process[1] that may limit mandibular movement as a result of the close association between the hyperplastic coronoid process and the anterior region of the zygomatic bone[2,3].

CH may be more common than is believed, but because it is mostly painless, patients only seek treatment if the inability to open the mouth is severe enough to compromise mastication[4-7]. However, the disease gradually becomes more severe during the second decade of life. It can be unilateral (with only one hyperplastic coronoid process) or bilateral.

In radiographs, a hyperplastic coronoid process is generally large and long, projecting into the infratemporal fossa, with normal trabecular bone[8]. Radiographs are thus inappropriate to diagnose it[9]. Computed tomography (CT), in contrast, is extremely useful for the observation of the association between the hyperplastic coronoid process and the zygomatic bone[4,10,11], and thus plays an important role in diagnosing and planning surgical treatment. Treatment consists of surgical correction of CH. Coronoidectomy is the method of choice, followed by intense physiotherapy [12,13].

Once the CT scan is performed, the data can be viewed in many different arrangements, including multiplanar (MPR) and 3D rendering, although the resolution of the latter may be lower than that of the former. The literature does not discuss which of these arrangements dental specialists consider most useful, or whether they would always use both of them to make a safe, correct diagnosis of CH. The purpose of this study was to analyze the importance of and the preference for MPR and 3D reconstruction images in diagnosing CH, by comparing the opinions of oral surgeons and radiologists.

MATERIAL AND METHODS

Sample

Archival images of spiral CT scans of 152 patients with signs and symptoms of TMJ dysfunction were analyzed. Said patients had been referred to the Dentomaxillofacial Imaging Department of a Dentistry and Medical Clinic, for CT scan of the TMJ, requested by specialized professionals (orthodontists and oral surgeons) with diagnostic purposes. This was a multicenter study which was approved by the Research Ethics Committee of the School of Dentistry of the University involved.

Among the 152 patients, 20 cases diagnosed with CH were selected. The diagnosis was initially made by an independent oral surgeon specialized in oral maxillofacial radiology, who made the diagnosis based on clinical (limitation of mouth opening) and imaging information. The diagnosis of CH was made when the coronoid process in patients with closed mouth exceeded the zygomatic process and in multiplanar images (MPR) whenever it exceeded the height of the condyle. All diagnoses were revised and confirmed by 3 different independent oral maxillofacial radiologists, who did not take part in the experiment. Age, sex and race were not considered when selecting the sample. The selected images were obtained through multiplanar (Fig. 1) and 3D reconstruction (Figs. 2 and 3).

CT acquisition

The images were acquired by a fourth generation Toshiba Auklet™ CT scanner (Tustin, CA, USA) with a matrix size of 512 X 512. Technical parameters were as follows: 120 kVp, 250 mA, 2 mm slice width, 1 mm pitch factor.

Eighty-eight axial 2 mm slices were obtained with 1 mm reconstruction intervals and a pixel size of 0.4688 mm. The images were acquired as follows: a) maximum intercuspation position; and b) maximum mouth opening (patients were instructed to open the mouth to their limit and immediately bite a mouth block - 40x30x20mm or 30x25x18mm Maquira, Maringá, Brazil - throughout the acquisition).

The acquired images were sent to an Alatoviewä workstation (Tustin, CA, USA), where volume reconstruction was carried out. Only multiplanar and 3D reconstructions were used in this study. The images were converted from DICOM to TIFF format, transferred to a conventional computer and stored on CD.

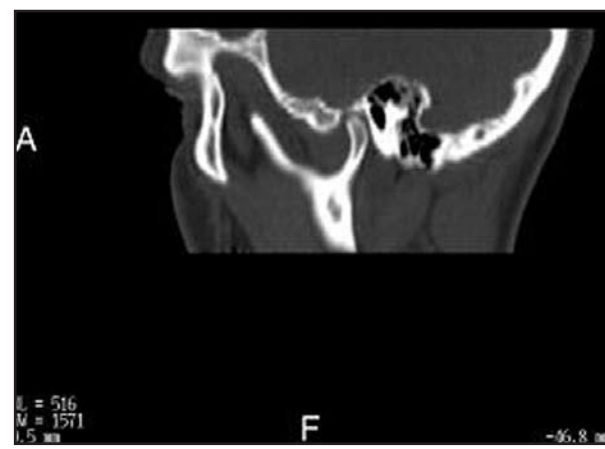

Fig. 1: Multiplanar Reconstruction: right side at maximum intercuspation position.

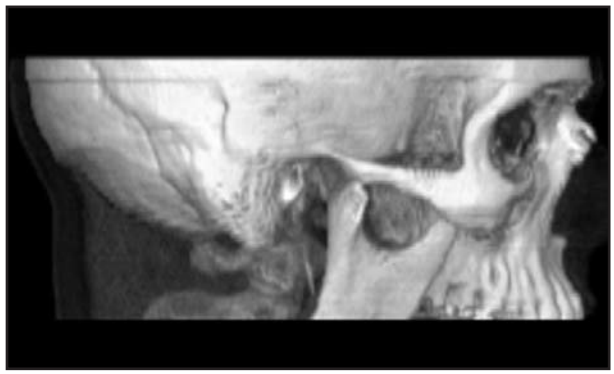

Fig. 2: 3D Reconstruction: right side at maximum mouth opening.

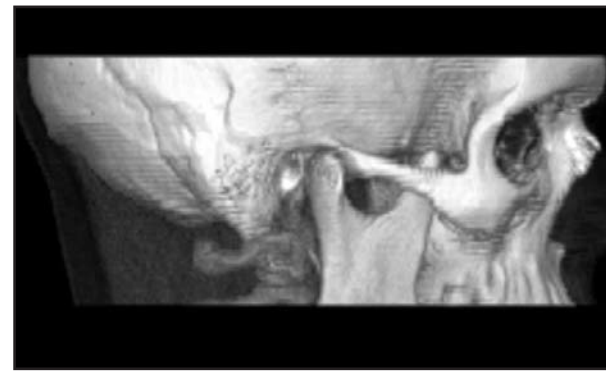

Fig. 3: 3D Reconstruction: right side at maximum intercuspation position.

Image analysis

Image analysis was performed by two groups of examiners with at least twenty years' experience in their specialties. One group included five oral maxillofacial radiologists and the other group included five oral and maxillofacial surgeons, all of who were renowned professors and PhDs in their areas.

Both groups received 20 sets of scans (20 cases), composed of multiplanar reconstructions (MPR at maximum intercuspation position, right side and left side) and 3D reconstructions (right side and left side at maximum intercuspation and maximum mouth opening). A total of three images of 40 TMJs were analyzed (Figs. 1, 2 and 3).

After analyzing the images of each of the 20 cases, each examiner answered a questionnaire about the comparison between MPR and 3D reconstructions. Each examiner thus answered 20 questionnaires. The questionnaire was composed of the following questions:

1) Do you think that 3D reconstructions were more elucidative than MPR to correctly diagnose and interpret these images of CH? Score 1 – Yes; Score 2 – No.
2) Do you think that MPR was more elucidative than 3D reconstructions to correctly diagnose and interpret these images of CH? Score 1 – Yes; Score 2 – No.
3) How important are these imaging methods, analyzed separately or simultaneously, to help establish a correct diagnosis and interpretation of CH? Score 1 – Very important; Score 2 – Not so important; Score 3 – Not important.
4) Would you request one or both of these imaging tests to analyze CH? Score 1 – Yes; Score 2 – No.
5) By interpreting 3D reconstructions of maximum mouth opening, it was possible to conclude that the patient had: Score 1 – Hypermobility; Score 2 – Hypomobility; Score 3– Normal jaw movement; Score 4 – It was not possible to establish a diagnosis.

In relation to the question 5, the following classification was explained for each examiner before evaluation:

Normal: the uppermost portion of the head of mandible is below the bottommost portion of the articular tubercle of temporal bone.

Hypermobility: the uppermost portion of the condylar process is beyond the bottommost portion of the articular tubercle of temporal bone.

Hypomobility: the uppermost portion of the condylar process does not reach the bottommost portion of the articular tubercle of temporal bone.

The data obtained were tabulated and submitted to statistical analysis by the Analysis of Means (ANOM), Kruskal-Wallis test and Fisher's exact test.

RESULTS

The answers to questions 1 and 2 were transformed into dichotomous variables: "yes" represented Score 1 (examiner agrees) and "no" represented Score 2 (examiner disagrees). Thus, the tables that represent questions 1 and 2 show the percentage of affirmative answers.

For the analysis of question 3, means or medians of the scores were used. The answer to question 4 was also transformed into a dichotomous variable: "yes" represented Score 1 (examiner would request imaging tests) and "no" represented Score 2 (examiner would not request imaging tests). The tables that

represent question 4 also show the percentage of affirmative answers.

Tables 1 and 2 show the proportion of affirmative answers to questions 1 and 2 and 4, as well as the mean score for the answers to question 3 for both groups.

For the analysis of question 5, a graph that expresses in percentage hypermobility, hypomobility or normal jaw movement (relation between the head of mandible and mandibular fossa) was used and showed that 55.2% of the cases had hypomobility, 24.1% had normal mobility and 20.7% had hypermobility (Fig.4). The Kruskal-Wallis test was used to compare the scores for both groups (oral radiologists and oral surgeons), revealing no significant difference between them either for the right side (p=0.934) or the left side (p=0.064). Diagnosis of mandibular movement was based on the relation between the head of mandible and the mandibular fossa, analyzed by both groups upon observation of 3D reconstructions.

The answers to each question provided by the five examiners in each group were compared by means of statistical analysis. ANOM was used to calculate a general average for the five examiners and an interval that indicated which examiners had similar opinions and which had different ones. ANOM compares group means to the overall mean, providing a graphic procedure for comparing a collection of means, rates or proportions to determine whether any of them differ significantly from the overall

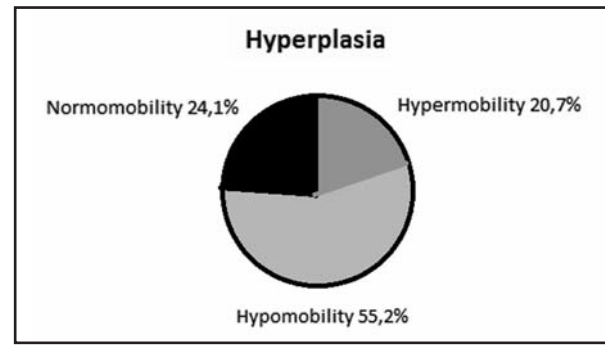

Fig. 4: Percentage of the jaw movement (relation between the head of mandible and mandibular fossa).

Table 1: Percentages of affirmative answers and scores for oral surgeons.

Surgeon	Question 1 (3D better)	Question 2 (MPR better)	Question 3 (need for the examinations)	Question 4 (indication for the examinations)
1	95	65	1.00	100
2	70	25	1.40	85
3	55	60	1.05	100
4	70	85	1.00	100
5	45	55	1.05	100
Mean	67	58	1.10	97
Median	70	60	1.05	100
SD	19	22	0.17	7

Table 2: Percentages of affirmative answers and scores for oral radiologists.

Radiologist	Question 1 (3D better)	Question 2 (MPR better)	Question 3 (need for the examinations)	Question 4 (indication for the examinations)
1	35	60	1.00	100
2	55	45	1.15	95
3	60	15	1.00	100
4	90	40	1.10	100
5	95	35	1.65	100
Mean	67	39	1.18	99
Median	60	40	1.10	100
SD	25	16	0.27	2

mean, rate or proportion. ANOM is a type of multiple comparison procedure.

The groups were compared (radiologists and oral and maxillofacial surgeons). Since questions 1 and 2 and 4 dealt with proportions, the proportion test was used to compare them. No significant difference (p=1.000) was observed between groups with regard to question 1 (3D better). Regarding question 2 (MPR better), the oral and maxillofacial surgeons had a higher percentage of affirmative answers than the radiologists. This was confirmed by the proportion test, which revealed statistically significant differences between groups (p=0.006).

For the analysis of question 3, the Kruskal-Wallis test was used to compare the scores, revealing no significant difference between them (p=0.194). For question 4, Fisher's exact test was used, revealing no significant difference between groups (p=0.621).

DISCUSSION

Diagnosis of CH is only possible by image examination. Most authors highlight the role of CT with coronal slices and sagittal reconstructions in the analysis of this disease. They also highlight the relation between the hyperplastic coronoid processes and zygomatic arches.[1,4,10-12,14-17] We confirm, based on this and other preliminary studies, that CT is essential for distinguishing CH from TMJ dysfunctions through different imaging exams. This is of particular importance because upon further investigation, many supposed TMJ disorders are found to be CH. Our data confirm this, as 7.6% of the "TMJ problems" were in fact CH pathologies.

In the present study, MPR and 3D reconstructions showing hyperplasia of the coronoid process were analyzed by experienced oral maxillofacial radiologists and oral maxillofacial surgeons, the specialists that deal with this type of pathology, and their preferences were compared.

Tables 1 and 2 show that both the oral surgeons and the oral radiologists stated that imaging methods, combined or not, were essential for diagnosing and interpreting CH correctly (Question 3), and that they would request these tests to analyze and diagnose the condition (Question 4).

The proportion test revealed no significant difference between groups with regard to Question 1. The percentage of affirmative answers was higher for 3D images than for MPR images. This means that both the oral surgeons and the oral radiologists consider that in most of the cases, 3D images were more elucidative than MPR images to diagnose and interpret CH correctly. This agrees the findings of Guimarães and Marie [18] (2005), who highlighted the use of 3D reconstructions for the analysis of the relation between hyperplastic coronoid processes and zygomatic arches and bones.

Regarding Question 2 (MPR better), the oral surgeons had a higher percentage of affirmative answers than the radiologists. This was confirmed by the proportion test, which revealed statistically significant differences between groups. We believe that the difference is due to the fact that oral surgeons are more familiar with this imaging method, since it is used in a variety of clinical situations in Oral and Maxillofacial Surgery.

Both groups considered the two imaging methods, combined or not, very important for analyzing CH, as shown by the percentage of answers to question 3 (need for these examinations). The Kruskal-Wallis test revealed no significant difference between groups. Participants would also request one or both of the imaging tests to analyze CH. Fisher's exact test revealed no significant difference between groups for Question 4 (indication of examinations).

When the oral surgeons and the oral radiologists analyzed the 3D reconstructions to evaluate mandibular movement, hypomobility was present in 55.2% of the cases analyzed (Graph 1) on the same side of the affected TMJ. This agrees with the findings of some authors, who report that limited mouth opening was often due to CH rather than to TMJ dysfunctions, as was believed at first.[2,9,16,19]

Imaging examinations are essential for analyzing clinical cases of limited mouth opening with no pain, as shown in this and in other preliminary research. These examinations would allow practitioners to analyze soft and bony structures of TMJ, as well as the relation between the coronoid process and zygomatic bone. By requesting such examinationss, specialists would avoid overlooking the possibility of hyperplasia of the coronoid process.

We conclude that both groups of examiners believe that the combination of MPR and 3D reconstructions are fundamental to correctly diagnose and interpret CH, especially the latter, and would request one or both examinations to diagnose it. A significant difference was found between the examiners regarding the use of MPR (p=0.006), with only the oral radiologists considering that it is not so elucidative for the diagnosis of CH.

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INFLUENCE OF POLISHING PROTOCOL ON FLEXURAL PROPERTIES OF SEVERAL DENTAL COMPOSITE RESINS

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ABSTRACT

The aim of this study was to determine the influence of the finishing protocol (FP) on flexural properties of several composites (CR). Twenty composite samples (25x2x2 mm) were prepared: G1 Helimolar®; G2 Filtek™ Z350;G3Tetric® N Ceram, G4 Point 4™, G5 Premisa™; G6 Esthet.X® HD, G7 ice, G8 Vit-L-escence®, G9 Grandio®, G10 TPH®3, G11 Amelogen® Plus, G12 Brilliant Enamel; G13 Filtek™ Z100 and randomly divided into four groups according to the finishing system: C control, J Jiffy®, SS Super Snap®, AA Astropol® /Astrobrush®. Each sample was polished for 10 seconds with each sequence instrument, and stored in distilled water for 24 hours, after which a three-point flexure test was applied to determine flexural strength (FS) and modulus (Flexural modulus). Data were analyzed using a two-way multivariate ANOVA and means were compared with Tukey's test. Results were: FS level CR p=0.000 with significant differences. FS level FPp= 0.093 with significant differences. In order: FM level CR p 0.00 with significant differences. FM level PS p=0.001; with significant differences. Under the study conditions, the polishing systems based on silicone rubber decreased the flexural properties of composite resins.

Key words: *composite resins; elastic modulus; dental polishing.*

INFLUENCIA DEL PROTOCOLO DE PULIDO SOBRE LAS PROPIEDADES FLEXURALES DE VARIAS RESINAS REFORZADAS

RESUMEN

El objetivo de este trabajo fue determinar la influencia del protocolo de pulido sobre las propiedades flexurales de varios composites. Se prepararon veinte probetas de composite (CR) (25x2x2 mm) en cada grupo: G1 Helimolar®; G2 Filtek™ Z350;G3Tetric® N Ceram, G4 Point 4™, G5 Premisa™; G6 Esthet.X® HD, G7 ice, G8 Vit-L-escence®, G9 Grandio®, G10 TPH®3, G11 Amelogen® Plus, G12 Brilliant Enamel; G13 Filtek™ Z100 y se dividieron aleatoriamente en cuatro grupos según el sistema de pulido (SP) utilizado: C control, J Jiffy®, SS Super Snap®, AA Astropol® /Astrobrush®. Cada probeta se pulió durante 10 segundos con cada secuencia de instrumentos, se almacenó en agua destilada durante 24 hs y se aplicó un ensayo de resistencia flexural de tres puntos a fin de determinar la resistencia (RF) y el módulo (MF) flexural. Los valores obtenidos fueron analizados con un ANOVA multivariado de dos vías y comparación de medias de Tukey. Los resultados obtenidos fueron: nivel de RF para CR p=0,000con diferencias significativas. RF nivel SP p= 0,093 con diferencias significativas. Para MF a nivel de CR p=0,000con diferencias significativas. MF a nivel SP p=0,000, con diferencias significativas. En las condiciones de este trabajo se puede concluir que el uso de sistemas a base de gomas siliconadas disminuyen las propiedades flexurales de las resinas reforzadas restauradoras. : composite resins; elastic modulus; dental polishing.

Palabras clave: *resinas reforzadas; módulo elástico; pulido dental.*

INTRODUCTION

Composite resin is universally accepted as an anterior direct restorative material, even for large restorations. In posterior restorations its application is becoming more popular, probably because of its high esthetic potential, acceptable longevity, tissue preservation potential, tooth structure bonding, low temperature conductivity and increased demand from patients [1]. Initially these materials had many disadvantages, such as high polymerization shrinkage; occasional patient-referred post-operative sensitivity and accelerated wear in restorations, mainly located in the molar area, leading to loss of occlusal and proximal anatomy. A meticulous

restoration technique, the right case selection, improvement in material formulations and better knowledge of their performance have enabled safe use of these materials.

When considering desirable properties for clinical performance, mechanical properties are described as very important, especially because they are closely related to the long-term success of these restorations. Improvement in mechanical properties in recent formulations has led to increased toughness and resistance to abrasion and attrition wear[2]. Mechanical properties depend mainly on composite microstructure and composition; therefore filler amount, size, morphology and distribution[3] are critical for composite selection. In addition, variations in matrix chemistry should be taken into account, since it has been reported that they could significantly affect flexural strength and modulus. Flexural modulus means material stiffness, which is important because it influences composite selection in high stress situations, such us medium-large sized restorations in proximal or occlusal locations, or for incisal angle replacement or when used to replace cusps [4].

In a three-year clinical study[2], 102 class 4 restorations made with 4 different composite formulations were evaluated and a correlation between mechanical properties and clinical performance was found, which is an association between wear, modulus and defect size.

Clinical procedures that may damage or prematurely harm restorations should be accurately performed because the primary cause of failure during the first five years is related to technique and material selection [5]. Optimal finishing and polishing of composites is one of the most important steps when performing a restoration, since it not only results in optimal esthetics, but also favors gingival health, restoration of marginal integrity over time and patient comfort [6,7], as well as increasing resistance to pigmentation and wear [8,9] and possibly influencing restoration longevity. Surface roughness creates a favorable microenvironment for bacterial adhesion and growth, which enables the development of secondary caries, gingival inflammation and restoration staining. Polishing aims to reduce surface roughness and lines progressively until they are smaller than visible light wavelength. A traumatic technique might overheat and damage a composite surface, [10-12] resulting in accelerated wear. This research group has published previous data evaluating how filler morphology, matrix composition and finishing protocol correlate with flexural properties and mass loss in several composites[13] . We concluded then that all variables evaluated correlate with a stronger weight on modulus.

There is no clear evidence of how different clinical polishing protocols might affect the flexural properties of composite resins reinforced with different fillers; thus, the aim of this study was to evaluate the effect of three polishing protocols on flexural properties of thirteen restorative composites.

MATERIALS AND METHODS

Thirteen groups of twenty samples of light cured composite resins, shade A2, from eight manufacturers, were prepared: G1 Heliomolar (Ivoclar-Vivadent, Schaan, Liechtenstein), G2 Filtek Z350 (3M/ESPE. St. Paul, USA), G3 Tetric N Ceram (Ivoclar-Vivadent, Schaan, Liechtenstein), G4 Point 4 (KERR®- Sybron dental Specialties, Orange, USA), G5 Premisa (KERR- Sybron dental Specialties, Orange, USA), G6 Esthet.X HD (DENTSPLY-Caulk, Milford, USA), G7 Ice (SDI Limited. Victoria, AU), G8 Vit-L-escence (Ultradent Products, INC. South Jordan, USA), G9 Grandio (VOCO America INC. Sunnyside, USA), G10 TPH3 (DENTSPLY-Caulk, Milford, USA), G11 Amelogen Plus (Ultradent Products, INC. South Jordan, USA), G12 Brilliant Enamel (Coltène Whaledent**,** Altstätten, Switzerland), G13 Filtek Z100 (3M/ESPE. St. Paul, USA). They were randomly divided into four groups of five samples each according to the polishing protocol to be applied: C control, J Jiffy (Ultradent Products, INC. South Jordan, USA), SS Super Snap (Shofu Dental Corporation, San Marcos, USA), AA Astropol /Astrobrush (Ivoclar-Vivadent, Schaan, Liechtenstein-batch H32042). Samples were prepared according to ISO Standard 4049 for flexural strength and ANSI/ADA standard 27, using a standardized 25x2x2 mm aluminum mold and verified by means of a digital micrometer 500 (Mitutoyo Corporation, Japan) with an accuracy of 0.01 mm. Composite was placed in 2 mm increments, each of which was light cured for 40 seconds using an Astralis 3 (Ivoclar-Vivadent, Schaan, Liechtenstein) unit at 600 mW/cm ^{2}. Samples were normalized using moist 400 grit sandpaper. One hour later each sample was submitted to the

Table 1: Flexural strength and modulus.

	Flexural strength in MPa	Flexural modulus in GPa
Equations	$\sigma=3Fl/2bh^2$	$E=l^3F/4bh^3d$

F maximum load (Newton). l distance between supports, millimeters. b width at the centre of the specimen, millimeters. h height at the centre of the specimen, millimeters. d deflection due to load, millimeters.

Table 2: Classification of the composite according to filler morphology.

Group and Composite	Filler Morphology
G1 Heliomolar	Pre-polymers
G2 Filtek Z350	Spherical-conglomerates
G3 Tetric N Ceram	Irregular + pre-polymers
G4 Point 4	
G5 Premisa	
G6 Esthet.X HD	Irregular
G7 Ice	
G8 Vit-L-escence	
G9 Grandio	
G10 TPH3	
G11 Amelogen Plus	
G12 Brilliant Enamel	Irregular + pre-polymers
G13 Filtek Z100	Spherical

Table 3: Descriptive statistics and multiple comparisons for flexural strength at composite level.

Composite	Mean	Typical deviation	T-HSD
G1 Heliomolar®	87.76	9.45	C
G2 Filtek™ Z350	96.77	21.48	BC
G3 Tetric® N Ceram	106.53	9.56	B
G4 Point 4™	104.16	18.40	B
G5 Premisa™	96.61	9.55	BC
G6 Esthet.X® HD	120.03	11.66	A
G7 ice	120.51	18.56	A
G8 Vit-L-escence®	119.38	14.39	A
G9 Grandio®	124.70	14.87	A
G10 TPH®3	120.80	10.37	A
G11 Amelogen® Plus	115.12	16.85	AB
G12 Brilliant Enamel	119.74	19.08	A
G13 Filtek™ Z100	135.21	18.56	A

Mean (MPa).T-HSD: Tukey´s test. Equal letters indicate homogeneous groups

polishing protocol, applying 10 seconds per step and following the manufacturer's instructions with a handpiece at 10,000 rpm NSK (NAKANISHI, Kanuma, Japan), being very careful to maintain water refrigeration and not to press too hard. Samples were stored in distilled water at 37ºC for 24 hours. Sample dimensions were determined, after which they were submitted to a three point flexural test (ISO4049/2000 - 27 ANSI/ADA specification) using a universal testing machine 1011 (INSTRON®, Norwood, USA) with a crosshead speed of 1 mm/min until fracture.

Data were recorded and processed applying the equations in Table 1 in order to determine flexural strength and modulus for each sample. Statistical analysis was performed using two-way ANOVA, and DHS Tukey test was applied for multiple comparisons. (Statistical Package for the Social Sciences 15.0®).

Two series of samples of each composite resin were prepared and metalized with gold and argon laser for SEM observation at the Advanced Microscopy Center, University of Buenos Aires, Argentina, with a Supra 40 Scanning Electron Microscope, Carl Zeiss, Germany, with a field emission gun. Micrographs were taken and composites were classified into five groups: spherical, spherical-conglomerates, irregular, irregular + pre polymers and pre-polymers. (Table 2).

RESULTS

Flexural strength: descriptive statistics (mean and standard deviation) are shown in Table 3. ANOVA for flexural strength (FS) showed statistical differences among groups $p<0.001$ (Table 4). HSD Tukey (Table 3) proved composites reinforced with spherical (G13 Filtek™ Z100) and irregular particles (G9, G7, G10, G6, G12, G8 and G11) to have higher flexural strength than composites with mixes of pre-polymerized/ irregular (G3, G4 and G5), spherical conglomerates (G2 Filtek™ Z350) and pre-polymerized particles (G1 Heliomolar®). When flexural strength was analyzed related to polishing system, ANOVA showed significant differences among groups $p<0.05$ (Table 4). Descriptive statistics are shown in Table 5 with mean and typical deviation expressed in MPa. HSD Tukey test showed that there was no significant difference among J, SS and AA and only AA differed significantly from C. Composite-polishing

system interaction on flexural strength showed p=0.416, indicating that flexural properties are independent from it.

Modulus: ANOVA performed for flexural modulus showed significant differences among materials p<0.001. Descriptive statistics (mean and standard deviation in GPa) (Table 5) were G13:15.03(1.09)= G9:14.50(1.50)> G11:9.79(0.52)= G10:9.76(1.27)= G2:9.72(2.20)≥ G12:8.89(0.4)= G7:8.56(0.73)≥ G4:7.98(1.01)= G6:7.88(0.46)= G8:7.57(0.50)= G3:7.19(0.66)≥ G5:6.53(0.71)> G1:4.65(0.38). When modulus was analyzed with relation to polishing system, ANOVA showed significant differences among groups, p<0.001 (Table 6). Descriptive statistics are shown in Table 7 with mean and standard deviation expressed in GPa.

Table 4: Tests of Between-Subjects Effects (ANOVA).

Source	Dependent Variable	Sum of Squares	Df	Mean Square	F	Sig.
Composite	FS	43520.192	12	3626.683	15.736	<0.001
	M	2020.912	12	168.409	273.815	<0.001
Polishing	FS	1964.956	3	654.985	2.842	0.039
	M	21.394	3	7.131	11.595	<0.001
Composite* Polishing	FS	8622.865	36	239.524	1.039	0.416
	M	105.083	36	2.919	4.746	<0.001
Error	FS	47937.216	208	230.467		
	M	127.930	208	0.615		
Total	FS	3414321.239	260			
	M	23721.978	260			
Corrected Total	FS	102045.229	259			
	M	2275.319	259			

FS: flexural strength. M: modulus df: degrees of freedom

Table 5: Descriptive statistics and multiple comparisons for flexural modulus at composite level.

Composite	Mean (GPa)	Typical deviation	T-HSD. p=.05
G1 Heliomolar®	4.65	0.38	F
G2 Filtek™ Z350	9.72	2.20	BC
G3 Tetric® N Ceram	7.19	0.66	DE
G4 Point 4™	7.98	1.01	D
G5 Premisa™	6.53	0.71	E
G6 Esthet.X® HD	7.88	0.46	D
G7 ice	8.56	0.73	CD
G8 Vit-L-escence®	7.57	0.50	D
G9 Grandio®	14.50	1.50	A
G10 TPH®3	9.76	1.27	B
G11 Amelogen® Plus	9.79	0.52	B
G12 Brilliant Enamel	8.89	0.40	C
G13 Filtek™ Z100	15.03	1.09	A

T-HSD: Tukey´s test. Equal letters indicate homogeneous groups

Table 6: Descriptive statistics and multiple comparisons for flexural strength at polishing level.

Group	Composite	Mean (GPa)	Typical deviation	T-HSD. p=.05
C	Control	117.54	20.33	A
J	Jiffy®	111.53	21.20	AB
SS	Super Snap®	111.92	20.57	AB
AA	Astropol/Astrobrush	110.48	16.66	B

T-HSD: Tukey´s test. Equal letters indicate homogeneous groups

Table 7: Descriptive statistics and multiple comparisons for flexural modulus at polishing level.

Group	Composite	Mean (GPa)	Typical deviation	T-HSD. p=.05
J	Jiffy®	8.65	2.50	C
SS	Super Snap®	9.16	3.10	AB
AA	Astropol/Astrobrush	9.06	3.25	B
C	Control	9.45	2.95	A

T-HSD: Tukey´s test. Equal letters indicate homogeneous groups

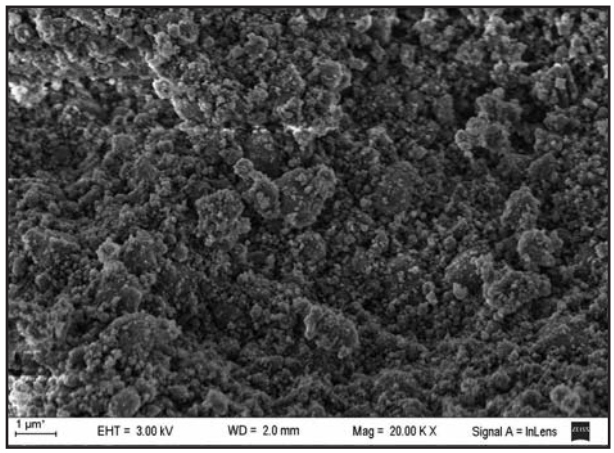

Fig. 1: SEM image at 10,000x of Filtek Z100 composite resin reinforced with spherical fillers.

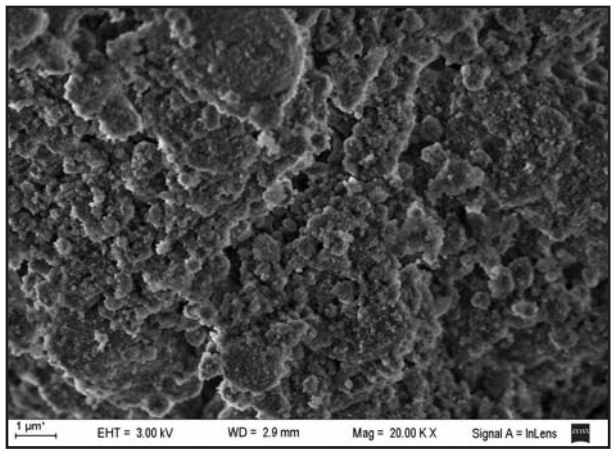

Fig. 2: SEM image at 10,000x of Filtek Z350 composite resin reinforced withspherical conglomerate fillers.

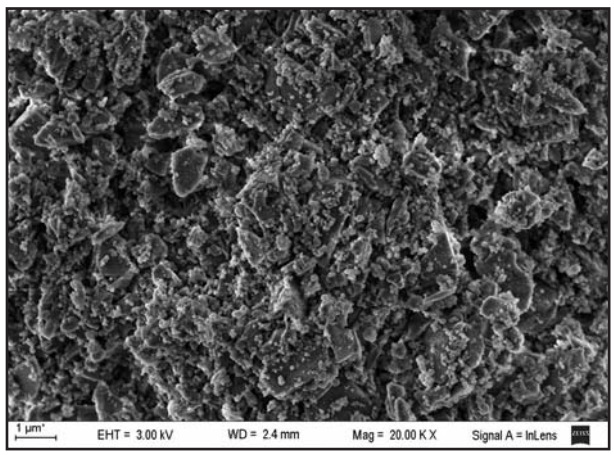

Fig. 3: SEM image at 10,000x of composite resin reinforced with irregular fillers. From right to left upper and lower images of Esthet.X® HD, Ice, Vit-L-escence, Grandio, TPH3 and Amelogen Plus.

Groups were as follows: C= SS≥ AA: > J. HSD Tukey test proved that all experimental groups showed lower modulus values than the control, although SS had no significant difference. Jiffy® had the lowest values and differed significantly. Composite-polishing system interaction on flexural modulus showed $p<0.001$ (Table 4), indicating that flexural modulus is dependent on it.

Figures 1-5 show SEM photographs taken of each type of composite and their classification in terms of filler morphology.

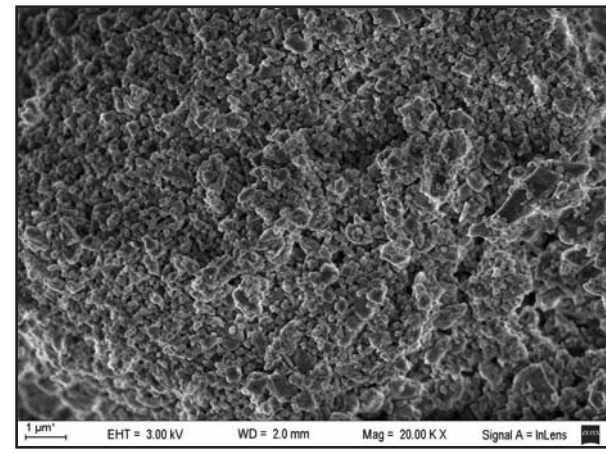

Fig. 4: SEM image at 10,000x of composite resin reinforced with irregular fillers + pre-polymer fillings. From right to left upper and lower images of Point 4, Premisa, Tetric N Ceram and Brillant Enamel.

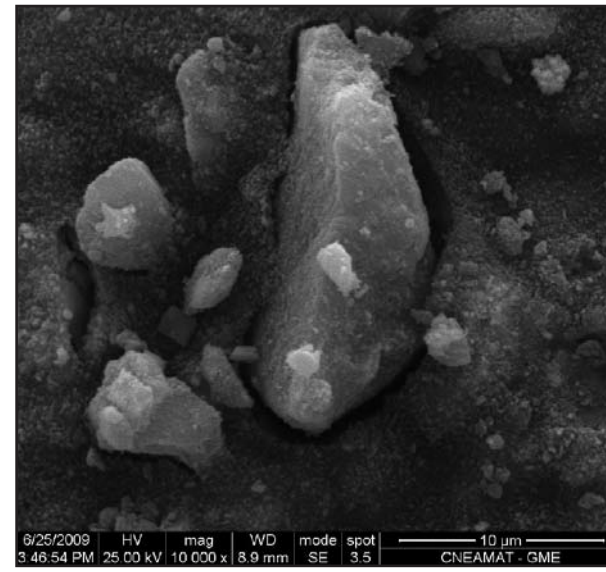

Fig. 5: SEM image at 10,000x of Heliomolar composite resin reinforced with pre-polymers.

DISCUSSION

Two out of the three polishing protocols evaluated in this investigation are based on silicone impregnated with varied grain size abrasives, mostly silicon carbide (Jiffy and Astropol). The third (Super Snap) is based on flexible paper discs impregnated with silicon carbide and aluminum oxide.

In order to calculate flexural performance, we carried out a three point stress test which applies one force in one sense that generates three types of loads (shear, tensile and compressive)[14]. Some authors consider that these results cannot be transferred to clinical situations, primarily because failure distribution is different. However, the model we applied in order to determine mechanical properties here is accepted worldwide because it is easy to reproduce, serves in terms of comparative purposes and represents material behavior in biomechanical efforts and determines critical performance during function [15,16].

In terms of FS, all evaluated composites comply with ISO Standard 4049. However, there were some differences among commercial brands. In our study, composites with spherical filling proved to be similar to composites with irregular particles. Small variations might be given by filler quantity and mean size because the manufacturing processes are essentially the same. Therefore, it can be speculated that recent formulations with optimized matrix containing nanometric spherical filler do not seem to improve FS. It could be considered that variations (although not significant) in FS occur because irregular particles concentrate stress at dead angles in the polymerized matrix. On the other hand, differences in monomer proportions might significantly change mechanical properties [4], where a slight TEGDMA increase might cause a strength reduction and a UEDMA increase would turn into an increase. Furthermore, BisGMA variations do not seem to affect the final result. It can thus be speculated that TEGMA content may be responsible for preventing Grandio high filling content and spherical particles in FilteK Z-100 from being significantly superior in mechanical properties to the other irregular particles composites. Finally, the lower part of the table shows the results for pre-polymerized + irregular reinforced composites (Tetric N Ceram, Point 4, Premisa), spherical conglomerate (Filtek Z-350) and pre-polymerized (Heliomolar). It could be explained by several factors such as their lower sized fillers, which increase the surface to be covered by the matrix, leading to less filler content. Also more silane could be present and diminish the polymerization degree, modifying their mechanical properties[18]. All composites, with the exception of Tetric N Ceram, have TEGMA in their composition and smaller particles are less effective in preventing crack propagation because they are easier to go around. Regarding composites with pre-polymerized particles, it could be speculated that the high conversion degree might affect bond strength with unpolymerized matrix, leading to lower mechanical properties. In Filtek Z-350, it can be inferred that although the filler keeps its spherical shape, sintered nanometric particles are not bonded enough to alter the course of the fracture, causing a conglomerate separation. Our group has previously reported a part of this research focused on filling morphology, matrix and polishing protocol correlation with flexural properties and surface loss [13]. We found that flexural strength, flexural modulus and surface loss were significantly correlated to the composition and morphology of these composite resins. The highest influence was found for modulus at 85.7%, while it was lower for resistance and surface loss, at 41% and 36.3% respectively. Although that paper also analyzed the influence of the matrix composition, we reported, for example, that spherical conglomerates negatively influence mechanical properties. On the other hand, independent spherical particles might turn into a more regular structure that could help distribute tension, thus increasing properties. This might suggest that properties are influenced not only by filler morphology, but also by filler distribution.

Finishing refers to the restoration´s adaptation to the tooth and aims to obtain good contour, occlusion, healthy relationship with adjacent teeth and a smooth surface [9], while polishing refers to the elimination of irregularities in order to create a surface which is as smooth as possible, to minimize gingival irritation, biofilm accumulation, pigmentation, and recurrent formation of caries[18-21]. We carried out the whole process using water cooling in order to avoid some factors that may affect resins when the procedure is performed without water cooling[22]. It was also finished a few minutes after initial curing, since lack of polymerization and surface roughness in newly cured composite resins [23] have been rejected in recent formulations [24,25].

There are more variables that might influence restoration properties, such as operator, time, humidity and matrixes were controlled, pressure applied to polish the samples. That pressure might increase tension in the sample, and even transmit pneumatic handpiece vibrations, which might induce some kind of initial defect in the material. All these variables were controlled by limiting all procedures to a single operator.

The efficacy of abrasive systems is related to the flexibility of the supporting material. Abrasive hardness in this case is very close in the different systems (2500 Knoop H Kg/mm^2 silicon carbide and 2100 Knoop H Kg/mm^2 aluminum oxide), instrument geometry and application . Differences between flexible discs and silicone rubber reside in the fact that discs wear filler particle and matrix in a similar way [7], whereas rubbers wear matrix by plastic deformation, leading to temperature increase and either leaving particles on the surface or removing them, both of which leave irregularities that translate into a less bright surface. They also create defects where the cracks initiate, leading the material to its strength limit. Moreover, physical stress caused by temperature increase might result in the formation of micro-cracks, micro-pores or interface spaces between matrix and filler, which might affect physical properties [9]. Our results differ from those reported by Gordan et al. 2003 [10], who found an increase in FS when polishing maneuvers were carried out.

CONCLUSIONS

Under the conditions of this study, flexural properties of composite resins were affected by the polishing systems tested. FS is higher in composites reinforced with spherical and irregular particles. Flexural strength is diminished when the Astropol/ Astrobrush system is used. Regarding stiffness, Filtek Z100 and Grandio proved to be superior to the other resins tested. However, both resins showed lower stiffness when systems based on silicone rubber such as Astropol/Astrobrush and Jiffy were used.

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INFLUENCE OF ADDITION OF 2-(3-(2H-BENZOTRIAZOL-2-YL)-4-HYDROXYPHENYL)ETHYL METHACRYLATE TO AN EXPERIMENTAL ADHESIVE SYSTEM

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ABSTRACT

The aim of this study was to evaluate the addition of 2-[3-(2H-Benzotriazol-2-yl)-4-hydroxyphenyl]ethyl methacrylate (BTAM) to an experimental adhesive resin. An experimental base adhesive resin was formulated with BisGMA, TEGDMA and HEMA, to which BTAM was added at 1, 2.5 and 5%, in weight. One group with no addition was used as control. The experimental adhesives were evaluated for antibacterial potential (against Streptococcus mutans), degree of conversion with FTIR, softening in solvent and microRaman interface analyses. Data were analyzed by Kruskal-Wallis, paired t test and ANOVA and Tukey, considering a 5% level of significance. The results showed antibacterial activity of 5% BTAM against S. mutans ($p<0.05$), however, no difference was found among BTAM groups ($p> 0.05$). The results of degree of conversion and softening of solvent showed no statistical difference between BTAM and control groups ($p>0.05$). The addition of 5% BTAM showed higher antibacterial activity than the negative control, and copolymerization with comonomer blend of adhesive resin and BTAM was detected at the dentin/adhesive interface.

Key words: *Anti-Bacterial Agents; Dentin-Bonding Agents; Polymerization.*

INFLUÊNCIA DA ADIÇÃO DE 2-(3-(2H-BENZOTRIAZOL-2-YL)-4-HYDROXIPHENIL)ETIL METACRILATO EM UMA RESINA ADESIVA EXPERIMENTAL

RESUMO

O objetivo do presente estudo foi avaliar a adição do 2-[3-(2H-Benzotriazol-2-yl)-4-hidroxifenil]etil metacrilato (BTAM) a um adesivo experimental. Uma resina adesiva base experimental foi formulada com BisGMA, TEGDMA e HEMA e a essa resina foi adicionado o BTAM nas concentrações de 1, 2,5 e 5%, em peso, além de um grupo controle sem adição. Os adesivos experimentais foram avaliados quanto ao potencial antimicrobiano contra Streptococos mutans, grau de conversão com FTIR, degradação em solvente e análise da interface com microespectroscopia Raman. Os dados foram analisados considerando um nível de significância de 5%. Os resultados obtidos no teste antimicrobiano contra S. mutans mostrou diferença estatisticamente significativa do grupo com 5% de BTAM em relação aos demais grupos e ao controle negativo ($p<0,05$). Os resultados de grau de conversão e degradação em solvente dos grupos com BTAM não apresentaram diferença quando comparado ao grupo controle ($p>0,05$). Foi possível observar a penetração do BTAM na dentina. A adição de BTAM na concentração de 5% mostrou atividade antimicrobiana comparado ao controle negativo, além de ter sido capaz de copolimerizar e penetrar na dentina.

Palavras chave: *Adesivos dentinários; Antibacterianos; Polimerização.*

INTRODUCTION

Longitudinal clinical trials show a high success rate for adhesive restorations[1,2]. However, new materials with improved properties need to be developed in order to further reduce the failure rate of adhesive procedures. Some of the desired features are reduction of polymerization shrinkage[3] and degradation in the oral environment[4], as well as the presence of antimicrobial properties[5].

Despite progress in monomer synthesis for low shrinkage and degradation, resin based materials with antimicrobial properties remain poorly explored. Materials with added chlorhexidine[6] and triclosan[7] have been tested. However, despite their

antimicrobial properties, no copolymerization is observed. The absence of copolymerization could increase leaching of these agents and degradation of the polymer[8]. A quaternary ammonium compound with a methacrylate functional group was used for composite resin development with no decrease in the antibacterial effect over time and no leaching of compounds[9]. However, other methacrylate antibacterial compounds could be used for developing dental materials.

Compounds with a triazole group are widely used as antifungal and antibacterial agents because they inhibit the synthesis of ergosterol – a fungal membrane constituent - preventing fungal growth[10]. The compound 2-[3-(2H-Benzotriazol-2-yl)-4-hydroxyphenyl]ethyl methacrylate (BTAM) has a methacrylate functional group that copolymerizes with the comonomer blend of the adhesive, preventing leaching and sustaining the antibacterial effect over time [5]. Thus, the aim of this study was to evaluate the influence of the addition of different concentrations of 2-[3-(2H-Benzotriazol-2-yl)-4-hydroxyphenyl]ethyl methacrylate on the properties of experimental adhesive resins.

MATERIALS AND METHODS

Formulation

The monomers used in this study were bisphenol A glycol dimethacrylate (BisGMA), triethylene glycol dimethacrylate (TEGDMA), 2-hydroxyethyl methacrylate (HEMA) and 2-[3-(2H-Benzotriazol-2-yl)-4-hydroxyphenyl]ethyl methacrylate (BTAM) (Fig. 1). The organic phase of the adhesive was prepared by mixing 50 wt% Bis-GMA, 25 wt% TEGDMA and 25 wt% HEMA. An antibacterial compound (BTAM) was added at four concentrations: 0, 1, 2.5 and 5 wt%. Camphoriquinone, DMAEMA and Diphenyl iodonium salt were used as initiator system. The formulations were mixed and ultrasonicated for 480 s. To perform monomer photo-activation, a light-emitting diode unit (Radii Cal, SDI LTD., Australia) was used. An irradiation value of 1200 mW/cm^2 was confirmed with a digital power meter (Ophir Optronics, USA).

Direct Contact Inhibition (DCI)

Three cylindrical samples of adhesive (3 mm in diameter and 1 mm in height) were produced for each group. The specimens were sterilized in hydrogen peroxide plasma. *S. mutans* (OMZ175) was grown aerobically in Brain Heart Infusion (BHI) broth (HiMedia Laboratories Pvt.Ltd, Mumbai, India) at 37ºC. Cells were harvested by centrifugation and re-suspended in fresh medium. Inocula were prepared by adjusting the cell suspension to a predetermined optical density (OD) of 0.02 at 600 nm. Using a 96-well plate, each specimen was placed in a well with 300 µl of BHI broth (HiMedia Laboratories Pvt. Ltd, Mumbai, India). Each well was inoculated with 20 µL of the *S. mutans* suspension. The negative control consisted of three sets of wells containing uninoculated fresh medium (300 µl). Immediately after the placement of inoculums and after a 24 hour period, 90 µl of each well content were diluted in saline to 10^{-8}. The 10^{-1}, 10^{-3}, 10^{-6} and 10^{-8} dilutions were plated on BHI Agar (HiMedia Laboratories Pvt.Ltd, Mumbai, India) using 25 µl aliquots of each dilution in duplicate. Plates were incubated at 37ºC, under anaerobic conditions. After 24 hours, colonies were counted visually, scaled by dilution factors and then transformed into colony forming units (CFUs) per milliliter. The groups were statistically compared to each other. The experiment was carried out under aseptic conditions.

Degree of Conversion

The degree of conversion of the experimental adhesive resins was evaluated using Fourier Transform Infrared Spectroscopy (FTIR) with a Vetrex 70 (Bruker Optics, Ettlingen, Germany) spectrometer equipped with an attenuated total reflectance device composed of a horizontal diamond crystal with a mirror angle of 45 degrees. A support

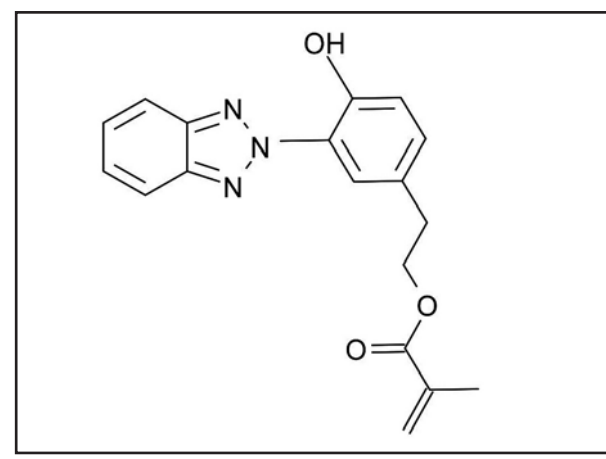

Fig. 1: Chemical structure of 2-[3-(2H-benzotriazol-2-yl)-4-hydroxyphenyl] ethyl methacrylate (BTAM).

was attached to the spectrometer to fix the light-curing unit and standardize the distance between the fiber tip and sample at 5 mm. Opus software (Bruker Optics, Ettlingen, Germany) was used a Blackman-Harris 3-Term apodization in a range of 4000 to 400 cm^{-1} and resolution of 4 cm^{-1}. With this setup, one spectrum was obtained prior to photocuring and one immediately after photocuring. The samples (3 µl) were directly dispensed onto the diamond crystal and light-activated for 40 s (n=3). The degree of conversion was calculated as described in a previous study[10], considering the intensity of carbon-carbon double bond stretching vibration (peak height) at 1635 cm^{-1}, and using the aromatic carbon-carbon at 1608 cm^{-1} from the polymerized and unpolymerized samples as an internal standard.

Softening in Ethanol

To determine degradation in solvent, the specimens produced during degree of conversion evaluation were used. Three specimens for each experimental adhesive (n=3) were embedded in acrylic resin and polished, after which they were stored and dried at 37°C for 24 hours. The specimens were subjected to a microhardness test in which five indentations (10 g/5 s), 100 µm apart from each other, were assessed using a digital microhardness tester (HMV 2, Shimadzu, Tokyo, Japan). The microhardness was calculated as described in a previous study[12]. The initial Knoop microardness number (KHN_1) was recorded, and the specimens were then subjected to softening in absolute ethanol for 2 hours at 37°C, after which the hardness test was repeated, and the post-conditioning hardness value measured (KHN_2). The percentage difference between KHN_1 and KHN_2 was calculated.

Interface Characterization

Four lower incisor bovine teeth were cleaned of organic debris and stored in distilled water at 4°C. The labial enamel was removed to expose the superficial dentin. A smear layer was produced by grinding the flat surface with a 600-grit silicon carbide (SiC) disc under water for 30 s. The dentin was etched with phosphoric acid for 15 s and washed for an additional 15 s. A commercial primer (Primer Scotch bond multi-purpose, 3M ESPE, St Paul, MN, USA) was applied, and the solvent was dried for 5 s with an air spray. Adhesive resin was applied according the experimental group and photocured for 20 seconds. A commercial composite resin (Z350XT, 3M ESPE, St Paul, MN, USA) was inserted in two increments of 2 mm and photocured for 40 seconds each to simulate tooth restoration.

The bonded specimens were stored in distilled water in a light-proof container at 37°C for 24 h. Sections (1 mm thick) were prepared by sectioning perpendicular to the flat adhesive-dentine surface. Micro-Raman spectroscopy was performed using a SENTERRA Raman Microscope (Bruker Optics, Ettlingen, Germany). The samples were analyzed using the following micro-Raman parameters: a 100 mW diode laser with 785 nm wavelength and spectral resolution of ~ 3.5 cm^{-1}. One-dimensional mapping was performed over a 150 µm line across the adhesive-dentine interface at 1 µm intervals using a computerized XYZ stage. These areas covered the composite resin, adhesive layer, hybrid layer, partially demineralized and unaffected dentine and were viewed and focused at x500 magnification. Accumulation time per spectrum was 5 seconds with 2 co-additions. Two mappings were performed per sample at random sites. Post-processing was performed in Opus software (Buker Optics) and consisted of analysis with modeling, which distinguished spectral components of the adhesive and dentine. One correspondent peak of each substance was used for integration. For the hydroxyapatite, 960 cm^{-1} was used, and for BTAM 998 cm^{-1} was used.

Statistical Analysis

The values of UFC were analyzed with Kruskal-Wallis. The results of the degree of conversion were evaluated with one-way ANOVA (BTAM concentration) and Tukey. For the analysis of softening in ethanol, a paired Student *t*-test (KHN1 and KHN2) and a one-way ANOVA for ΔKHN% were used. A level of significance of 0.05 was considered for all tests.

RESULTS

The values of direct contact inhibition are shown in Fig. 2. For the antibacterial analysis, no statistical difference was found between BTAM groups ($p>0.05$). However, a statistical difference was observed among negative control (uninoculated fresh medium) and groups with 5% BTAM ($p<0.05$). The mean values of degree of conversion ranged from 71.1 to 73.1 %. The control group presented the highest

mean values of degree of conversion ($p<0.05$). However, none of the groups with added 2-[3-(2H-Benzotriazol-2-yl)-4-hydroxyphenyl]ethyl methacrylate (BTAM) (1, 2.5 and 5 %) differed statistically ($p>0.05$). Microhardness values before (KHN_1) and after (KHN_2) ethanol immersion, percentage difference between KHN_1 and KHN_2 and degree of conversion are shown in Table 1. There was no statistical difference in initial microhardness values for any of the groups ($p>0.05$). After ethanol immersion, microhardness values were lower than the initial values for all groups ($p<0.05$). The percentage difference between KHN1 and KHN2 was higher in the group with 5 % BTAM than in the other groups ($p<0.05$). The spectra of pure BTAM (Fig. 3 A) and a representative image of each group from the interface characterization is shown in Figure 3 (B-H). The presence of BTAM can be observed across the hybrid layer. All groups with added BTAM exhibited the same behavior across the hybrid layer.

Table 1: Mean (± standard deviation) degree of conversion (DC), initial Knoop microhardness (KHN1), Knoop microhardness after solvent immersion (KHN2) and percentage difference between KHN1 and KHN2 (ΔKHN%).

Group	DC (%)	KHN1	KHN2	ΔKHN%
0%	73.1 (±0.5)^{A}	13.7 (±0.3)^{Aa}	5.1 (±0.3)^{b}	63.0 (±3.0)^{B}
1%	71.7 (±0.0)^{B}	13.3 (±0.4)^{Aa}	4.8 (±1.1)^{b}	64.1 (±7.1)^{B}
2.5%	71.1 (±0.3)^{B}	13.6 (±0.9)^{Aa}	4.6 (±1.2)^{b}	65.8 (±9.1)^{B}
5%	71.6 (±0.8)^{B}	11.9 (±2.7)^{Aa}	2.8 (±0.5)^{b}	75.6 (±4.8)^{A}

Different capital letters indicate statistical difference in the same column ($p<0.05$). Different lowercase letters indicate statistical difference in the same line, between KHN1 and KHN2 ($p<0.05$).

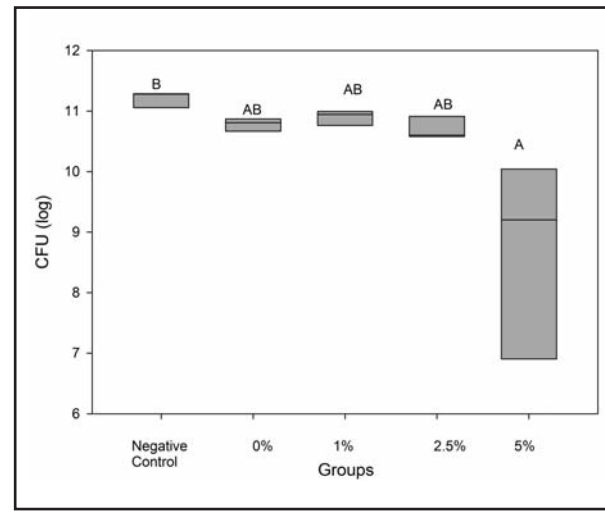

Fig. 2: Values of median and percentile 25 and 75 of microbiological analysis in CFU (log). Different capital letters indicate significant differences ($p< 0.05$).

DISCUSSION

The improvement of dental materials by the addition of different compounds is ongoing[12,13]. Substances that copolymerize with other methacrylate compounds are desirable. In this study, 2-[3-(2H-Benzotriazol-2-yl)-4-hydroxyphenyl]ethyl methacrylate (BTAM) showed copolymerization and antibacterial activity against *S. mutans* compared to a negative control.

The degree of polymer conversion is directly related to mechanical properties[14]. For adhesive resins, a high degree of conversion is related to high values of bond strength to dental tissues[15]. The groups with addition of BTAM showed lower values for degree of conversion than the control groups ($p<0.05$). The increase in the concentration of monofunctional monomers (BTAM) may explain the reduction of reactivity and consequently the reduction of the degree of conversion in the groups with addition of BTAM (Table 1). The values of the degree of conversion shown in this study are consistent with data in the literature[16,17]. The increase in the degree of conversion is not necessarily directly related to an increase in crosslink density[18]. Polymers with low crosslink density are more prone to degradation[19-21]. In this study, all groups showed reduction in microhardness values after two hours of ethanol immersion. However, the change in microhardness values was significantly higher in the groups with 5% BTAM than in the other groups ($p<0.05$). Polymers with high degradation during ethanol immersion may absorb more fluids due to the reduction of frictional forces between polymer chains[22], degrading the ester bond of methacrylate polymers and leading to a reduction of mechanical properties[23]. The reduction of frictional forces and degradation of ester bonds can be also detected during water immersion, although to a lesser degree than during ethanol immersion. The degradation caused by water can be detected in the oral environment and is related to color change and the indication for restoration replacement[19,23].

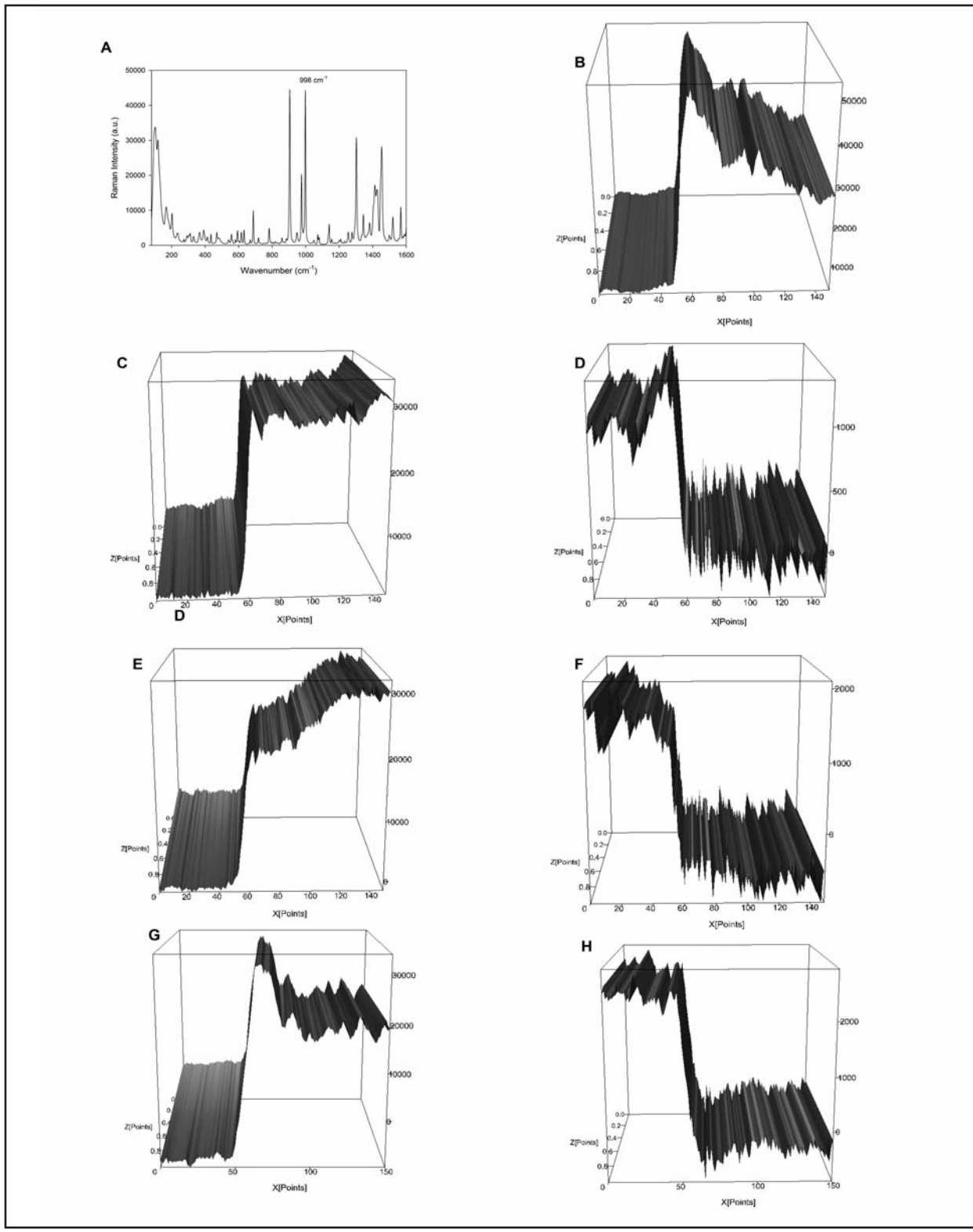

Fig. 3: Micro Raman characterization of 2-[3-(2H-benzotriazol-2-yl)-4-hydroxyphenyl] ethyl methacrylate (A) and interfaces between adhesive resin and dentin (B-H). Control group (0%) is represented in Figure 3B, integrate for phosphate peak (960cm-1). Integration of peak 998 cm-1 was not possible for control group, because of the absence of BTAM. Figures C, E and G represent the integration of phosphate (960cm-1) peak for groups with 1, 2.5 and 5% of BTAM, respectively. Figures D, F and H represent the integration of 2-[3-(2H-benzotriazol-2-yl)-4-hydroxyphenyl] ethyl methacrylate peak (998cm-1) for groups with 1, 2.5 and 5% of BTAM, respectively.

Penetration of experimental adhesive resins into demineralized dentin was observed by micro Raman spectroscopy. It may indicate the formation of a hybrid layer. The degree of conversion of adhesive monomers is important, because unreacted monomers close to hybrid layer may leach, causing damage to pulp cells or periapical tissues[24]. In this study, samples with added BTAM showed a reduction in the degree of conversion compared to the control group, although the values are comparable to commercially available adhesive resins. Despite the related antibacterial activity of triazole[25,26], in this study, experimental adhesive resins with 5% BTAM showed activity against *S. mutans*. The addition of BTAM at a higher concentration may present higher antibacterial effect, because the effect of triazole compounds is dose-dependent[27]. Further studies are needed at higher concentrations of triazole compound, evaluating activity against fungal contamination, since the development of adhesive systems with antimicrobial activity is desirable

Based on the results of this study, the addition of 5% BTAM may have potential for the development of adhesive resins with antimicrobial activity.

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EFFICIENCY IN BRACKET BONDING WITH THE USE OF PRETREATMENT METHODS TO TOOTH ENAMEL BEFORE ACID ETCHING: SODIUM HYPOCHLORITE VS. HYDROGEN PEROXIDE TECHNIQUES

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ABSTRACT

Bond failures are produced by the existence of biofilm on the tooth surface. Because biofilm is impermeable, it prevents contact in many areas, reducing the etching effect which selectively dissolves calcified tissues but does not seem to eliminate biofilm from the tooth surface, and thus the bond between the tooth and the bracket is not strong enough.

The aim of this study is to compare bracket bonding efficiency with two dental surface pretreatments: sodium hypochlorite vs. hydrogen peroxide techniques.

This was a cross-sectional, comparative, in vitro study. Seventy-five premolars extracted for orthodontic purposes were evaluated. They were divided into three groups of 25 teeth and assigned randomly toone of the pretreatment techniques (5.25%sodium hypochlorite or 3.5% hydrogen peroxide) or to a control group.

The most efficient pretreatment technique for bonding to brackets was sodium hypochlorite, with an average of 17.15 (kg/F). Significant differences were observed between groups (p=0.0001). The post hoc bond strength test showed statistically significant differences between the sodium hypochlorite technique and the control group (p=0.0001).

The sodium hypochlorite technique improves bracket adhesion to tooth enamel.

Key words: *dental enamel; dental etching; sodium hypochlorite.*

EFICIENCIA EN LA ADHESIÓN DE BRACKETS CON EL EMPLEO DE MÉTODOS DE PRE TRATAMIENTO AL ESMALTE ANTES DEL GRABADO ÁCIDO: TÉCNICA HIPOCLORITO DE SODIO VERSUS TÉCNICA PERÓXIDO DE HIDRÓGENO

RESUMEN

Las fallas de adhesión se producen por la existencia de la biopelícula en la superficie del órgano dental, ya que es impermeable y no permite el contacto en muchas áreas, de manera que disminuye el efecto del grabado ácido; el cual tiene la capacidad de disolver selectivamente los tejidos calcificados, pero no parece eliminar la biopelícula en la superficie dental, por lo tanto, no se lleva a cabo la suficiente fuerza de adhesión en la interfase diente-bracket.

El objetivo es comparar la eficiencia en la adhesión de los brackets con el empleo de dos métodos de pre-tratamientos de la superficie del esmalte, el hipoclorito de sodio vs. peróxido de hidrógeno.

Estudio comparativo, transversal, in vitro. Se evaluaron 75 premolares extraídos con fines ortodóncicos, tres grupos de 25 dientes, asignados aleatoriamente con alguna de las dos técnicas de pre-tratamiento al esmalte, hipoclorito de sodio al 5.25%, peróxido de hidrógeno al 3.5% y un grupo control.

La técnica de pre-tratamiento al esmalte más eficiente para la fuerza de adhesión a los brackets fue el hipoclorito de sodio, con una media de 17.15 (Kg/F), se observaron diferencias significativas inter-grupos (p= 0.001). Las pruebas post hoc para las fuerzas de adhesión mostraron diferencia estadísticamente significativa para la técnica de hipoclorito de sodio/ grupo control (p=.001).

La utilización de hipoclorito de sodio ayuda a mejorar la adhesión de los brackets en la superficie del esmalte.

Palabras clave: *esmalte dental; grabado dental; hipoclorito de sodio.*

INTRODUCTION

Problems with bonding, such as bracket detachment, are common in clinical practice, delaying the treatment and ultimately causing enamel demineralization[1].

Bonding quality is diminished by the presence of biofilm on the tooth surface, therefore it is important to use mechanical or chemical prophylaxis on teeth before etching the enamel, in order to remove the

biofilm and thus increase the surface energy of the substrate[2,3].

There are different opinions regarding whether enamel should be pre-treated, and many different preferences regarding the agent to be used for conditioning the enamel before any treatment[4,5]. The conventional technique for bracket placement consists exclusively of enamel etching, which can be achieved by demineralization with acid. Nowadays, pretreatment is recommended using physical abrasive methods such as pumice stone to eliminate biofilm and prevent continuous bracket detachment, or the use of sodium hypochlorite by depolarization or hydrogen peroxide to prepare the enamel surface[6-10].

The solvent and antimicrobial activity of sodium hypochlorite is principally due to its ability to oxidize and hydrolyze cell proteins, to release chlorine to form hypochlorous acid in the long term, and its osmotic ability to draw fluids out of cells[1,11-14].

Deproteinization is the removal of collagen from the previously conditioned surface by the use of substances capable of dissolving the protein content (NaOCI). It has been demonstrated as a way to minimize the sensitivity of the hybridization technique, consequently fostering adequate marginal seal without altering bond strength[2-4,13-16]. NaOCl is a non-specific proteolytic agent which removes organic components from the dentin, such as superficial destabilized collagen and the remnant smear layer from the etching, changing the chemical composition and leaving many exposed hydroxyapatite crystals in this deproteinized substrate[1,17,18].

Another enamel pretreatment method is hydrogen peroxide application, as a result of which oxygen and bleaching agents are retained in the enamel. Little is known about the effects of one application on the bonding to enamel [1, 19,20].

MATERIAL AND METHODS

This study was approved by the Master's Program in Stomatological Science in Orthodontics at the Faculty of Stomatology of the Benemeritus Autonomous University of Puebla and the Ethics Committee. The study was conducted at the dental biomaterials laboratory of the Faculty of Stomatology of the Autonomous University of Puebla, México, in February 2012.

It was a cross-sectional, comparative, *in vitro* study. Seventy-five premolars extracted for orthodontic purposes were evaluated. They were divided into three groups of 25 teeth, which were randomly assigned toone of the pretreatment techniques (5.25%sodium hypochlorite or 3.5% hydrogen peroxide)orto a control group.

A pilot test was performed before the definitive procedure, in order to adjust the shear test technique. The teeth indicated for orthodontic extraction were collected, kept in plastic containers of bidistilled water in a 41x35x30cmculture oven at 36°C, in order to replicateoral moisture and temperature conditions. The teeth were placed in transparent acrylic cubes (Nictone) with parallel walls, leaving the cervical third of the root free. Three groups of 25 teeth each were formed, by assigning them randomly to one of the pretreatment techniques: 5.25%sodium hypochlorite, 3.5% hydrogen peroxide, or to a control group with37% phosphoric acid, each for 15 seconds.

A thin layer of primer (3M McMark) was applied to the pretreated surface with a microbrushand spread with air from a triple syringe for about 3 seconds. A metallic MBT Gemini prescription bracket (3M Unitekal) was used and Transbond Xt resin (3M Unitek) applied on its mesh. A bracket was placed on the vestibular surface of each tooth using forceps (Ormco), and excess resin carefully removed. Theresin was immediately photopolymerized with a CuringLight XL 300 lamp (3M) for 20 seconds (10 on the mesial side and 10 on the distal side of the bracket).

Thetreated teeth were kept in the 41x35x30cm culture oven at 36°C inplastic containers with bidistilled water for 72 hours, after which the shear test was performed using a universal testing machine (Instron model 4465, InstronCorp.; Canton MA, USA) (Fig. 1) at a speed of 2.5mm per minute. The results were recorded and plotted in Kg/cm^2 by the machine software.

RESULTS

The results were analyzed by the statistical software SPSS version 20. Descriptive statistics, average, standard deviation of the numeric variables, percentages, proportions of the ordinal variables and inferential statistics ANOVA were performed.

Average bond strengths according to placement technique are shown in Table 1.

The models with sodium hypochlorite, hydrogen peroxide and control group are shown in Figs. 1 and 2.

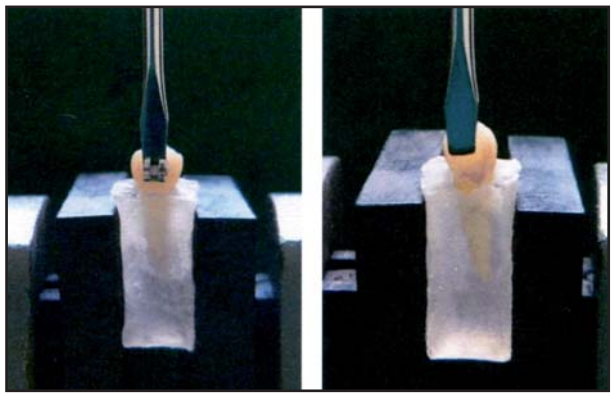

Fig. 1: Shear test using the Instron universal machine.

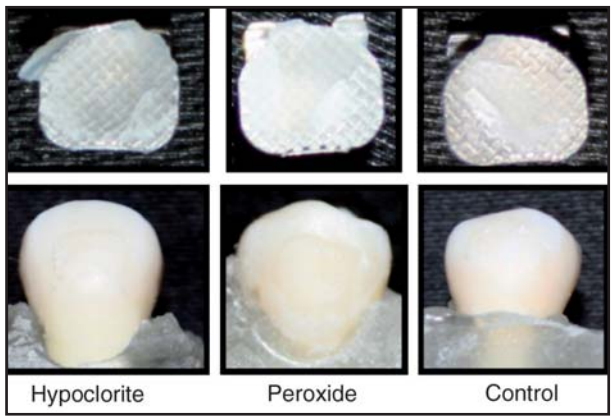

Fig. 2: Sample of pre-treatment according to the different enamel conditioning techniques.

The results of the analysis of variance (ANOVA) are shown in Table 2. Statistically significant differences are observed between groups.
Results of the *post hoc* test for bond strength showed that the 5.25% sodium hypochlorite pretreatment differs significantly from the other groups. Tables 3 and 4.

DISCUSSION

The group treated with sodium hypochlorite had the highest bond strength. Previous studies[1,3,18,20-23],have concluded that the use of 5.25% sodium hypochlorite for 30 seconds to eliminate the superficial collagen layer from the enamel surface as a pretreatment method improves bond strength[24]. Espinosa R et al.[23] ,showed that the use of 5% sodium hypochlorite for one minute followed by phosphoric acid, improves bond strength, a result which is consistent with this research. The sodium hypochlorite technique for one minute was found to be the most efficient[1].

Table 1: Average bond strength for both placement techniques.

Technique	s	Median(Kg/P)	SD	CI (95%)
Hypochlorite / Phosphoric Acid	25	17.15	3.28	15.79-18.50
Peroxide/ Phosphoric Acid	25	14.27	3.13	12.98-15.56
Phosphoric Acid	25	12.99	5.13	10.87-15.11

s=sample, Kg/P=kilograms/Power, SD=Standard Deviation, CI=Confidence Interval.

Table 2: Average differences between groups using ANOVA (analysis of variance).

	Sum of Squares	df	Quadratic Media	F	Significance (p)
Between groups	226.181	2	113.090	7.220	*0.001
Within groups	1127.771	72	15.663		
Total	1353.952	74			

df=degrees of freedom, F=statistic, p=significance

Table 3: Results of post hoc tests for membership.

	Group	Group	Significance
TestTukey	Hypochlorite	Peroxide	0.033
		Control	***0.001***
	Peroxide	Hypochlorite	0.033
		Control	0.492
	Control	Hypochlorite	***0.001***
		Peroxide	0.492
Scheffé	Hypochlorite	Peroxide	0.043
		Control	***0.002***
	Peroxide	Hypochlorite	0.043
		Control	0.525
	Control	Hypochlorite	***0.002***
		Peroxide	0.525
Bonferroni	Hypochlorite	Peroxide	0.037
		Control	***0.001***
	Peroxide	Hypochlorite	0.037
		Control	0.773
	Control	Hypochlorite	***0.001***
		Peroxide	0.773

Table 4: Results of *post hoc* tests for membership.

			Significance (p)
Dunnett´s t (bilateral)	Hypochlorite	Control	0.001
	Peroxide	Control	0.418

Our study observed lower bonding efficiency with peroxide; however, little is known of the effects on bonding to enamel when it is applied once, even when the amount is minimum in quantity, which turns out to be dependent on the time elapsed. Peroxide was used for its antiseptic and biofilm stripping action at a 3.5% concentration, which is why no article about the use of peroxide as pretreatment method for the bracket cementation was found.

Based on the results of this study, it may be concluded that 5.25% sodium hypochlorite as a pretreatment agent for the enamel significantly increases the bond strength for brackets and any type of resins on the surface of the enamel. Therefore, this method is recommended for better etching outcomes. Further studies are required to assess the use of hydrogen peroxide accurately before it is used as an alternative pretreatment method.

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EFFECT OF DEXAMETHASONE ON MANDIBULAR BONE BIOMECHANICS IN RATS DURING THE GROWTH PHASE AS ASSESSED BY BENDING TEST AND PERIPHERAL QUANTITATIVE COMPUTERIZED TOMOGRAPHY

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ABSTRACT

Long-term glucocorticoid administration to growing rats induces osteopenia and alterations in the biomechanical behavior of the bone. This study was performed to estimate the effects of dexamethasone (DTX), a synthetic steroid with predominant glucocorticoid activity, on the biomechanical properties of the mandible of rats during the growth phase, as assessed by bending test and peripheral quantitative computed tomographic (pQCT) analysis. The data obtained by the two methods will provide more precise information when analyzed together than separately. Female rats aged 23 d (n=7) received 500µg.kg^{-1} per day of DXT for 4 weeks. At the end of the treatment period, their body weight and body length were 51.3% and 20.6% lower, respectively, than controls. Hemimandible weight and area (an index of mandibular size) were 27.3% and 9.7% lower, respectively. The right hemimandible of each animal was subjected to a mechanical 3-point bending test. Significant weakening of the bone, as shown by a correlative impairment of strength and stiffness, was observed in experimental rats. Bone density and cross-sectional area were measured by pQCT. Cross-sectional, cortical and trabecular areas were reduced by 20% to 30% in the DTX group, as were other cortical parameters, including the bone density, mineral content and cross-sectional moment of inertia. The "bone strength index" (BSI, the product of the pQCT-assessed xCSMI and vCtBMD) was 56% lower in treated rats, which compares well with the 54% and 52% reduction observed in mandibular strength and stiffness determined through the bending test. Data suggest that the corticosteroid exerts a combined, negative action on bone geometry (mass and architecture) and volumetric bone mineral density of cortical bone, which would express independent effects on both cellular (material quality) and tissue (cross-sectional design) levels of biological organization of the skeleton in the species.

Key words: *mandible-bone-dexamethasone.*

EFECTO DE DEXAMETASONA SOBRE EL COMPORTAMIENTO BIOMECANICO DE LA MANDIBULA DE LA RATA EN FASE DE CRECIMIENTO, DETERMINADO MEDIANTE TEST DE FLEXION Y TOMOGRAFIA PERIFERICA CUANTITATIVA COMPUTARIZADA

RESUMEN

La administración crónica de glucocorticoides a ratas en fase de crecimiento induce osteopenia y modificaciones negativas del comportamiento biomecánico del hueso. El estudio presente fue realizado para estimar los efectos de dexametasona (DTX), esteroide sintético con actividad glucorticoide predominante, sobre las propiedades biomecánicas de la mandíbula de ratas durante la fase de crecimiento, estimación realizada mediante el ensayo de flexión a tres puntos, por un lado, y tomografía periférica cuantitativa computarizada (pQCT), por el otro. Los datos obtenidos mediante los dos métodos citados brindarán información más precisa cuando son analizados en forma conjunta que cuando son analizados separadamente. Ratas hembras de 23 d de edad (n = 7) recibieron 500µg.kg^{-1}/d por vía subcutánea durante 4 semanas. El peso y la longitud corporales mostraron una disminución del 51.3% y 20.6%, respectivamente, en las ratas tratadas con respecto a las controles (n = 7). El peso de la hemimandíbula derecha y el área mandibular (índice del tamaño del hueso) disminuyeron 27.3% y 9.7%, respectivamente. La hemimandíbula derecha de cada animal fue analizada biomecánicamente en el test de flexión a tres puntos. Se observó un significativo debilitamiento del hueso, demostrado por la disminución correlativa de la resistencia (a la fractura) y de su rigidez estructural (medida en la fase elástica de deformación) en los animales experimentales. La densidad ósea y el área de sección transversal fueron estimadas mediante pQCT. Las áreas de sección transversal, cortical y trabecular, mostraron una reducción significativa de entre 20% y 30%, así como la densidad ósea, su contenido mineral y el momento de inercia de la sección transversal. El BSI (índice de resistencia ósea), el producto de xCSMI y vCtBMD (medidos topográficamente), disminuyó un 56% en las ratas tratadas, valor semejante al 54% y 52% observado en la resistencia y rigidez mandibulares determinadas mediante el test de flexión. El análisis de los resultados obtenidos sugiere que DXT ejerce una acción negativa y combinada sobre la mandíbula, sobre su geometría (masa y arquitectura) y sobre su densidad mineral volumétrica del tejido cortical, acciones que expresarían efectos independientes sobre los niveles celular (calidad material) y tisular (diseño arquitectónico) de organización biológica del esqueleto en la especie estudiada.

Palabras clave: *mandíbula-hueso-dexametasona.*

INTRODUCTION

Long-term glucorticoid (GC) administration to growing rats can induce two main effects in long bones: 1) decrease in longitudinal bone growth, decreased bone formation and increased bone resorption, which may explain the decrease in bone mass (osteopenia)[1-6]; and 2) decrease in the bone biomechanical behavior, as shown by mechanical testing and tomographic studies[3,7-12]. GC administration increases bone resorption because it stimulates osteoclastogenesis by increasing the expression of RANK ligand and decreasing the expression of its decoy receptor, osteoprogeterin[6]. The strong effect of GC on bone formation is associated with a decrease in the number of osteoblasts and their function. The decrease in cell number is secondary to a decrease in osteoblastic cell replication and differentiation, and an increase in the apoptosis of mature osteoblasts[6]. We have previously reported[7] that the effects of GC on rat long bone biomechanics seem to reflect combined, negative action on diaphyseal geometry (mass and architecture) and volumetric mineral density of cortical bone, which may express independent effects on cellular (material quality) and tissue (cross-sectional design) levels of biological organization of the skeleton[13] in the species. Bones of the axial or appendicular skeleton show biomechanical properties associated with their condition of "weight-bearing bones". However, they are not only influenced by gravity (body weight) but also by the tensions generated during voluntary or reflex contractions of local muscles. In fact, muscle and bone are anatomically and functionally closely connected[14]. According to the mechanostat theory,[13] striated muscle is essential for bone development and maintenance, modeling and remodeling[13]. Both tissues are derived from somatic mesoderm and accumulate peak tissue mass synchronously, according to genetic information and environmental stimuli[14,15]. Excess GC thus results in progressive parallel loss of bone (osteopenia) and skeletal muscle (sarcopenia), with profound consequences for quality of life.

The mandible is both morphologically and functionally different from other bones of the axial skeleton. It also arises from a different embryonic germ layer (neuroectoderm), in contrast to bones of the axial or appendicular skeleton, which arise from the mesoderm. At the organ level, the bone of the mandible is dense with a high proportion of cortical bone. Loading of the mandible during mastication has an impact on the mass, density and microarchitecture of the mandibular alveolar bone[16, 17]. The mandible is not a "weight-bearing bone". However, since it is influenced by mechanical masticatory loading, it can be considered a "load-bearing bone" that presents similarities to the "weight-bearing bones" from the mechanical standpoint. Mandibular stiffness and strength, including the mechanical properties and distribution of bony tissue, are important because mandibular deformations, stresses and strains occur during static biting and chewing. In order to resist forces, bending and torsional moments, not only the material properties of the mandible but also its geometric design are important[16].

The inhibitory effect of GC on mandibular growth and bone biomechanical weakness in the rat when it is administered during the growth phase has been well documented[2,8,9,10,12]. Most of these studies used three-dimensional peripheral quantitative computed tomography (pQCT) to analyze the biomechanics of the mandible. Densitometric assessment shows that bone mass (the amount of mineralized material present in a bone) offers a certain association with bone strength *in vitro*[18, 19, 20]. However, bone strength and bone mass correlated over wide ranges ($r = 0.34$-0.89; R^2 0 0.12-0.79) in *in vitro* studies, confirming that much of bone strength or fracture risk variance remains unexplained by densitometrically assessed bone mass alone[20]. Moreover, a lack of correlation between DEXA-assayed BMD (areal bone mineral density) and bone strength or stiffness was reported in studies on bones from rodents of different sizes[21]. It is thus conceivable that the association between densitometry data and bone fragility may be less reliable than generally supposed for clinical studies[20].

To improve our knowledge of the real biomechanical effects of glucocorticoids on the rat mandible, dexamethasone (DTX), a synthetic steroid with predominant glucocorticoid activity, was chronically administered to rats during the growth phase, and the biomechanical behavior of the excised bone was assessed by bending test and peripheral quantitative computed tomographic analysis.

MATERIALS AND METHODS

Two groups (Control = C, and Experimental = E) of 7 female Sprague-Dawley rats aged 23 days were housed in cages under natural light cycle and controlled

temperature (23ºC), and fed a normal 1.0%Ca/0.8%P standard diet covering nutritional requirements to support normal mandibular growth[22]. The E group was administered 500 µg/kg per day of DXT (Dexamethasone; Sidus SA, Buenos Aires, Argentina) for 4 weeks, while the C group was injected with the vehicle. Body weight was recorded at the end of treatment in a Mettler P 600 scale to the nearest 0.1 g. Body length was measured from the tip of the snout to the base of the tail. Animals were euthanized by intramuscular ketamine (0.1 ml/100g b.wt) and xylasine (0.02 ml) injection at the end of the treatment period. The hemimandible of each rat was then removed, cleaned of adherent soft tissue, weighed, split at the midline suture, and stored at -20ºC wrapped in gauze soaked in Ringer's solution, as recommended by Turner and Burr[23]. Each bone was thawed at room temperature before analysis. Growth was estimated directly by taking measurements between anatomical points with digital calipers, following Eratalay et al.[2] with some modifications[24]. As an indicator of mandibular size, the *mandibular area* was calculated from a triangle formed between the most anterior inferior point of the inderdental spine and the angular process, the most posterior point of the angular process, and the most superior point of the coronoid process. The excised left hemimandible was measured by single-energy peripheral quantitative computed tomography on a developed, purpose-built scanner (XCT 960A), Stratec Medizintechnic, Pforzheim, Germany) equipped with an X-ray tube (38.5 kV) as the source of radiation. A region of interest was defined after recording a "scout" scan along the mandibular long axis. The third molar was identified in that scan and the reference line for the cross-sectional image was set approximately adjacent to the third molar. The resulting cross-sectional image of the mandible at this site was then encompassed manually by a square region of interest, allowing the software to detect automatically the outside edge of the bone, excluding the molar. This site was chosen because it does not include the incisor root. The threshold density was fixed at a linear attenuation coefficient of 0.900 cm^{-1} in order to distinguish between cortical and trabecular bone. The fixed threshold refers to mineralized cortical bone. Voxel size was set at 0.148 mm^3. Variables related to bone mass, density and architecture were determined as follows: 1) Cross-sectional area, as an indicator of the total area of the slice cross-section, 2) Cross-sectional area of cortical bone, as an indicator of *cortical bone mass*, 3) Volumetric cortical bone mineral content, as an indicator of *cortical bone mineral mass*, 4) Cross-sectional moment of inertia, as an indicative of *architectural fitness concerning bending strength*, and 5) Volumetric cortical bone mineral density, as an indirect indicator of *bone material quality*. Cortical area was calculated as the area encompassed by the endosteal and periosteal circumferences. Volumetric bone mineral density was calculated from the single slice cross-sections. The area moment of inertia was calculated along the mesiodistal and craniocaudal axes. The pQCT calculated the cross-sectional moment of inertia as the integral sum of products of each of the infinitesimal fractions into which the whole bone area can be divided and their respective square distances to the reference axis. The reference axis was horizontal and perpendicular to the longest axis. Therefore, we assumed a symmetrical cross-section. The right hemimandible was subjected to a three-point bending mechanical test[23] in an Instron test machine (model 4442, Instron Corp., Canton, MA, USA). Each bone was placed on two lower supports (11 mm span) with the lateral aspect facing down and centered along its length. Loads were applied transversally to the bone axis at a point immediately posterior to the posterior surface of the third molar at a rate of 5.00 mm/min. The resulting *load / deformation (W/d) curves*[23] enabled graphic determination of the main *structural* properties of the mandibles, which essentially measure the resistance to both deformation (*stiffness*) and fracture (*strength*). The structural properties are those corresponding to the whole bone as an organ. They are: 1) *load at the yielding point* (Wy, represents the end point of the elastic deformation of the bone and defines a threshold about which unrecoverable permanent deformation occurs (plastic deformation), marking the initiation of damage accumulation with the first appearance of the first microcracks that occur on the periosteal surface of the bone; it is a measure of the bone strength); 2) *structural stiffness* (represents the slope of the linear or elastic phase of the W/d curve and is a measure of the resistance of the bone to deformation, or bone rigidity); 3) *structural strength* (Wf, represents the value of the load at fracture and expresses directly the resistance of the whole bone to fracture). The data are expressed as mean ± standard error (SEM). The tomographic "Bone Strength Index" (BSI) was estimated as the product of CSMI and vCtBMD, Statistical analyses were performed

with the *t*-test using GraphPad Prism Software. The experiment was conducted in accordance with the principles outlined in the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes, and approved by the University of Buenos Aires Ethics Committee.

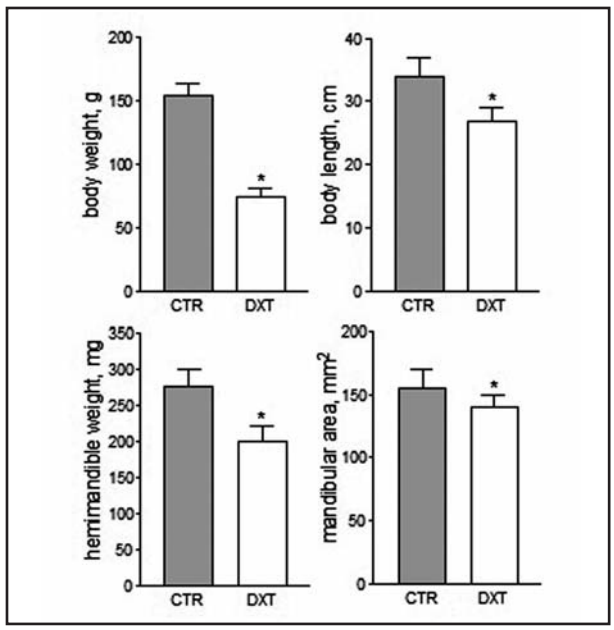

Fig. 1: Morphometric data (Mean ± SEM) from untreated (CTR) and dexamethasone-treated (DXT) rats. Asterisks above bars indicate p < 0.05..

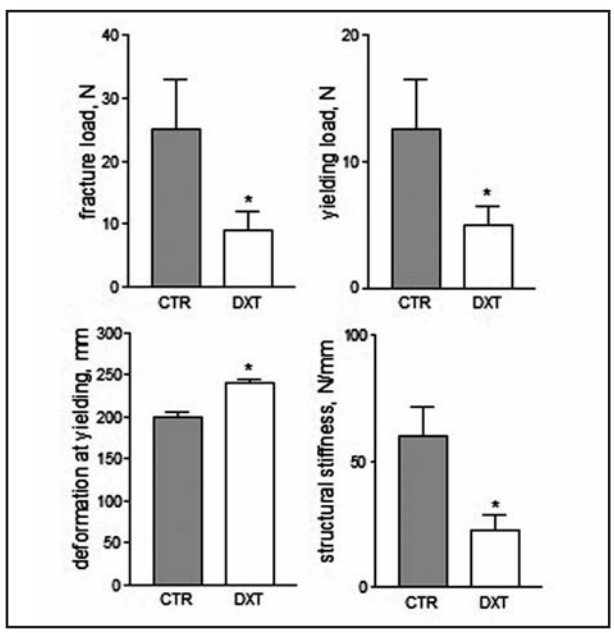

Fig. 2: Morphometric data (Mean ± SEM) from untreated (CTR) and dexamethasone-treated (DXT) rats. Asterisks above bars indicate p < 0.05..

RESULTS

As expected, E rats failed to attain normal weight gain compared with C, age-matched rats (Fig. 1). The 51.3 % reduction in final body weight found in DXT-treated rats was accompanied by a significant, 20.6% reduction in body length. Like body size, hemimandible weight and area (an index of mandibular size) were significantly lower (27.3 % and 9.7 %, respectively) in E than in C rats at the end of the experimental period (Fig. 1). Fig. 2 shows the structural properties of the mandible, as derived from the slope of the load/deformation curve in the linear region of the elastic behavior. The values for the fracture load, yielding load, and structural stiffness were significantly ($P < 0.001$) lower (36%, 40% and 38%, respectively)

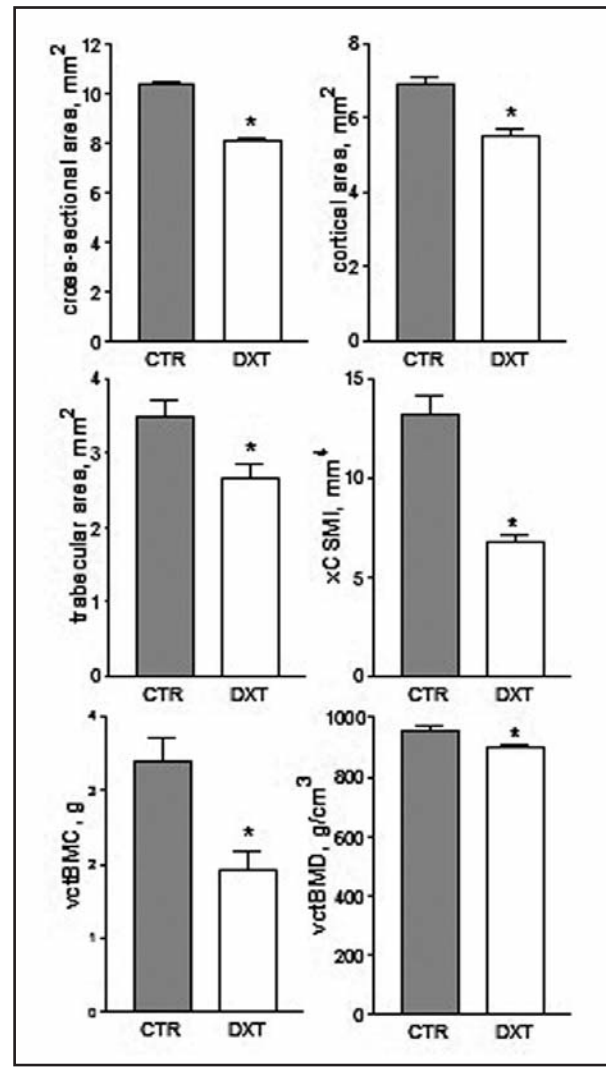

Fig. 3: Morphometric data (Mean ± SEM) from untreated (CTR) and dexamethasone-treated (DXT) rats. Asterisks above bars indicate p < 0.05..

in DXT-treated than in untreated rats, while the deformation at yielding was 20% higher in the former than in the latter. The "yielding load / fracture load ratio" did not differ significantly between E and C groups (0.55 ± 0.02 vs. 0.50 ± 0.02, $P > 0.05$), indicating that the elastic and plastic components of the load /deformation curve were not altered by treatment. The results of bone density and bone-cross sectional area as measured by pQCT are summarized in Fig. 3. I. Consistently with the effects on mechanical properties, the cross-sectional parameters of the mandible were reduced. Cross-sectional, cortical and trabecular areas were significantly lower (between 20% and 30%) in DXT-treated than in control rats. All the other cortical bone parameters including the density (vctBMD, 7%), mineral content (vctBMC, 43%), and cross-sectional moment of inertia (xCSMI, 49%) were significantly lower in the DXT-treated than in the untreated group.

DISCUSSION

This study demonstrated that one-month treatment of rats during their growth phase with 500 µg/kg of DXT administered daily impaired body mass growth in general and, directly related to the main purpose of the study, mandible growth and the biomechanical behavior of the bone. The corticosteroid dose chosen has been shown in a previous study[7] to induce negative effects in geometric properties and bone material quality of the femur, both of which play a significant role in determining the deleterious biomechanical consequences seen.

When the effect of DTX on the structural biomechanical properties of the mandible, considering the bone as a whole entity, were analyzed directly through the mechanical bending test, it was observed that the corticosteroid decreased the external load (yielding load) necessary to induce unrecoverable deformation of the bone, probably due to the appearance of microcracks on its periosteal surface. At this point, the deformation of the bone was 20% higher than that in the control bone. When the external load applied to the mandible was increased, it was also found that the microcracks progressed until the mandible fractured. Fracture occurred in the treated bone (fracture load) at a load level that was about 60% lower than that needed to fracture an untreated control bone. The load at yielding showed a very similar decrease percentage. As both parameters are very good indicators of bone strength, it seems acceptable to conclude at this point of the discussion that DXT markedly reduced the structural "strength" of the rat mandible under these conditions. The resistance of the bone to deformation in elastic conditions was also negatively influenced by DXT: the structural stiffness, which is an indicative predictor of bone rigidity, was about 60% lower in treated rats than in controls. By considering the results for mandibular structural strength and stiffness together, it is evident that the treated bone was weaker than that of untreated animals and, therefore, structurally incompetent.

In the pQCT analysis, DXT administration reduced the total area of the slice cross-section (-21.2%), the cortical bone mass (-20%), the trabecular bone mass (-30%), the cortical bone mineral mass (-43%), the architectural fitness concerning bending strength (-49%), and the volumetric cortical bone mineral density (-6%).

Analysis of the morphometrical and geometrical properties of the mandible by the two methods outlined showed that the DXT-treated bone was smaller than the untreated bone, with a significant reduction in cross-sectional area, cortical and trabecular bone mass and architectural design (xCSMI). At this point in the discussion, it is reasonable to conclude that the mandible of the DXT-treated growing rat was weaker than the untreated one because of its smaller bone mass and cross-sectional area, and inadequate spatial distribution of the resistive material over its cross-section.

However, the pQCT assessment of vCtBMD in this experiment allowed determination of the actual bone mineral concentration in cortical tissue. Its significant decrease induced by DXT administration could be taken as evidence of a negative effect of the glucocorticoid on a poor indicator of bone material quality in terms of mineralization, because other indicators of bone quality, such as tissue composition, amount of secondary mineralization, collagen cross-linking, mineral composition, particle size and distribution, presence of microdamage, cannot be estimated tomographically. In fact, vCtBMD is directly proportional to bone material stiffness or E, with a coefficient of determination ranging from 18% to 27%[25].

Ferretti et al.[20] showed in the rat femur that the product of the pQCT-assessed CSMI and vCtBMD, called BSI (Bone Strength Index) is an accurate and precise indicator of the actual mechanical quality of rat long bone tested by bending. It is interesting to note that in the rat mandible in our study, the BSI calculated and bone strength measured (fracture load) and the structural stiffness were 56%, 54%,

and 52% lower than control values, respectively, in response to DXT treatment. This reinforces the concept that BSI is a precise indicator of bone strength. Ferretti et al.[25] have estimated that the index is predictive of the actual fracture load with about 89% determining power. It should be noted, however, that the mechanical quality of a whole bone can only be directly assessed by destructive means, such as the one used in this study.

The biomechanical response of the mandible to DTX was similar to that found in the femoral shaft in our previous study[7] and suggests that corticosteroids exert a combined, negative action on bone geometry (mass and architecture) and volumetric mineral density of cortical bone, which would express independent effects on both cellular (material quality) and tissue (cross-sectional design) levels of biological organization of the skeleton in the species[13].

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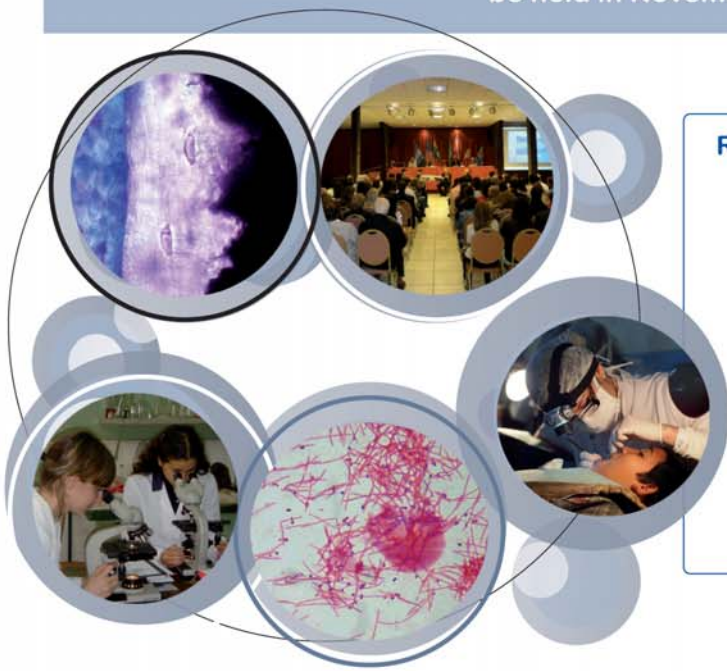

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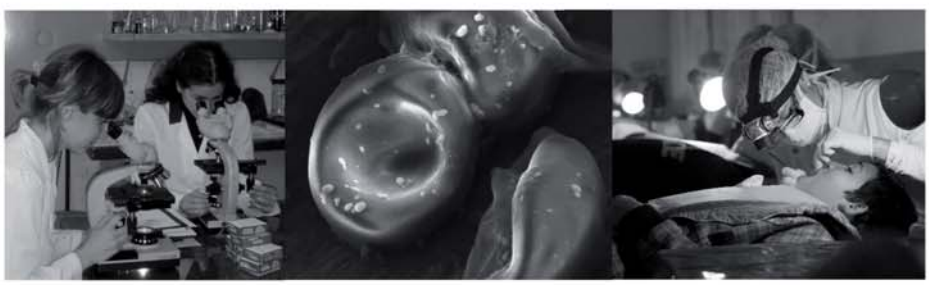

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‡ Resultados de un estudio de 6 meses para evaluar las mejoras en caries del esmalte usando el método QLFTM (Fluorescencia Cuantitativa Inducida por Luz) contra una crema dental regular sólo con fluoruro, ambas con 1450 ppm de fluoruro.

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