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

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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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THE INFLUENCE OF DISPLAY MODALITIES ON PROXIMAL CARIES DETECTION AND TREATMENT DECISION

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ABSTRACT

The aim of this study was to investigate the influence of digital radiographic display on caries detection and choice of treatment among undergraduate students. Forty images of extracted human teeth were acquired using a PSP digital system. The proximal surfaces were evaluated for the presence of proximal caries and choice of treatment by 36 undergraduate students, divided into three groups according to the semester they were taking. The images were evaluated in two forms of image display: laptop, and printed on acetate viewed on a lightbox. The accuracy of the different forms of image display on caries detection was evaluated by means of ROC curve analysis and its effect by mixed linear regression. Residue analysis was used to verify the adequacy of the treatment of choice for the chosen diagnosis. There was no significant effect either for the display modalities (p=0.058) or for the different undergraduate student groups (p=0.991). The Az was 0.539 for printed images and 0.516 for laptop. The decisions based on treatment of choice were consistent with the scores achieved for caries detection. Accuracy of caries detection using a laptop was comparable to accuracy using printed images. Treatment decision was not affected by image display modality. The semester of the dentistry course that undergraduate students were taking did not significantly increase the accuracy of their proximal caries detection.

Key words: *Dental Caries, Radiography, Dental-Diagnosis, Oral.*

INFLUÊNCIA DOS MEIOS DE APRESENTAÇÃO RADIOGRÁFICA NO DIAGNÓSTICO E TRATAMENTO DA CÁRIE

RESUMO

O objetivo deste estudo foi investigar a influência do meio de apresentação da imagem radiográfica digital no diagnóstico da carie e na decisão de tratamento realizado por alunos de graduação. Foram obtidas 40 imagens digitais de dentes humanos extraídos através do sistema digital PSP. As superfícies proximais dos dentes foram avaliadas quanto à presença de cárie proximal por 36 estudantes de odontologia, distribuídos em três grupos de acordo com o nível de formação. As avaliações foram efetuadas em um laptop e em imagens impressas com o auxílio do negatoscópio. A acurácia dos meios de apresentação quanto à detecção de cárie incipiente foi avaliada pela média das áreas sob as curvas ROC e seu efeito por uma análise de regressão linear mista. Para a tomada de decisão terapêutica foi realizada uma análise de resíduos para verificar sua adequação ao diagnóstico. Não houve efeito significativo nem para a modalidade de visualização (p=0.058) e nem para os grupos de alunos (p=0.991). A média das áreas sob as curvas Roc para o filme foi de 0.539 e de 0.516 para negatoscópio. A decisão de tratamento foi condizente com o diagnóstico efetuado. A acurácia do diagnóstico da cárie proximal realizado em tela de laptop é comparável ao realizado em negatoscópio. A tomada de decisão terapêutica não foi afetada pelo meio de apresentação da imagem radiográfica digital. A progressão do aluno no curso não melhora a precisão diagnóstica da cárie proximal.

Palavras chave: *Cárie Dentária, Radiografia Dentária, Diagnóstico Bucal.*

INTRODUCTION

Proximal caries still pose a challenge to dental care providers mainly because due to their location, they can only be detected clinically when a great extension of the proximal surface is compromised[1]. Bitewing radiography is used for detecting caries, but does not usually detect lesions in early stages, before cavitation. New, more accurate and more reproducible diagnostic methods are therefore needed to supplement early diagnosis and plan appropriate treatment for caries based on their low prevalence and extension and slow progression[2].

The use of dental radiographic films has largely been replaced by intraoral digital radiographic systems, most of which have been tested for efficiency in detecting caries[3-7].

Because digital images enable the acquired image to be viewed on a computer monitor, the evaluation of the different forms of image displays is of interest for dental health care providers and researchers. Haak et al.[8] evaluated the influence of digital image size on different types of monitors and found that the type of monitor has no effect on caries detection. Another form of image display still used by some professionals is a printed image, usually on acetate, which is similar to a conventional image[8].

Hellen-Halme et al.[9] suggest that in order to view proximal caries in digital images, ambient light conditions should be dimmed and brightness and contrast of the monitor adjusted to provide excellent image quality. Carmona et al.[10] claim that observer experience improves radiographic caries detection, reducing false negative results and increasing accuracy.

Based on the above, the aim of this study is to evaluate the influence of different image displays and the level of experience of undergraduate students on the diagnosis of proximal caries and treatment decisions by observing dental students from a Brazilian institution.

MATERIALS AND METHODS

This study was approved by the Ethics Committee of the Department of Dentistry at the State University of Paraíba (Protocol number CAAE 0384.0.133.000-11). Fifty extracted human teeth (10 canines, 20 premolars and 20 molars) were mounted on 10 silicone blocks, each of which held five teeth, simulating a normal condition. The canine was used to ensure proximal contact with the first premolar. The premolars and molars were either intact or had a small area of demineralization on their proximal surfaces.

Digital images were acquired using a GE 1000 (General Electric Company, Milwaukee, WI, USA) unit operating at 65 kVp and 10 mA. An acrylic plate 1.2 cm thick was placed adjacent to the models as a material equivalent to soft tissue. To ensure reproducible imaging geometry, the silicone blocks were stabilized on a customized acrylic device to provide a distance of 34 cm between the target and the image receptor, a centrally oriented X-ray beam and a distance of 2 cm between the teeth and the receptor.

The image receptor used in this study was size 2 intraoral PSP digital imaging (DenOptix, Gendex Dental Systems, Milan, Italy). Before exposure, each plate of the digital system was exposed to a lightbox for 130 s, as recommended by the manufacturer. The exposed phosphor plates were scanned using 300 dpi resolution and the files were exported and saved in tagged image file format (TIFF).

The acquired TIFF images were mounted in a PDF and printed using AGFA acetate film (Agfa Healthcare, Gevaert Group, Belgium) on a laser printer Drystar 5300 (Agfa Healthcare, Gevaert Group, Belgium). Each film contained eight images, and there were a total of five films.

Gold Standard

Histological sections (700 μm) served as a validating criterion for the presence and depth of the caries lesions. Before selection, the teeth were individually embedded in acrylic (Vipi, São Paulo, Brazil) and then sectioned in mesiodistal direction using a 200 mm diamond band. The sections were cleaned and glued to microscope slides using transparent varnish. Independent histological validation was performed by two previously trained observers under incident light (12.5 – 20X magnification) using a binocular microscope. If the observers' ratings varied, they were asked to perform a joint assessment to establish agreement.

Caries were defined as present when an opaque-white demineralization or brown discolored area was observed on the surface. For the histological surface, the following scale was applied: 0 = no enamel demineralization or narrow surface zone of opacity; 1 = demineralization limited to the enamel; 2 = demineralization involving the dentine.

Viewing Sessions

Prior to the examination sessions, the observers received explanations and practical instructions, and underwent calibration tests so that they would be familiar with the specific characteristics of the digital images used in the study. Viewers need to be trained with regard to the specific characteristics of images in each digital system so that diagnosis would not be compromised by the difference between systems. The professors were not required to undergo calibration because they are already familiar with the digital images used in this institution. The researchers who were not familiar with them underwent calibration but did not participate in the student training.

During calibration, the evaluation method was explained, and training and knowledge were verified. The researcher responsible for training the observers remained in the same room to answer any questions that might arise during the evaluation sessions. The digital images were displayed on a 17" color laptop monitor, size 1:1, and the printed images were displayed on a lightbox placed in a quiet room with dimmed lighting. The digital images could not be enhanced. All images were evaluated in a quiet and darkened room.

Each observer evaluated individually forty teeth, resulting in the evaluation of eighty proximal surfaces. The number of images evaluated at each session was determined by the observer and could not exceed 20 at a time.

Thirty-six independent observers, all undergraduate dentistry students at the State University of Paraíba (14 from the 7th semester, 12 from the 8th semester, and 10 from the 9th semester) were selected to evaluate the images. They were chosen according to their grades and success in radiology classes, and were considered able to diagnose dental caries. They were divided into groups based on the semester they were taking and coded as: Group A – 7th semester, Group B – 8th semester, and Group C – 9th semester.

The presence of proximal caries lesions was scored using a 5-point confidence scale: 1 = definitely not present, 2 = probably not present, 3 = unsure, 4 = probably present, and 5 = definitely present. After scoring the images individually for the presence of caries lesion, the observer scored the same image to indicate adequate clinical follow-up as: N – no treatment needed, P – Preservation, R – Restorative treatment.

Data Analysis

To measure the accuracy of the images displayed in different modalities and evaluated by different groups, the area under the Receiver Operating Characteristic (ROC) curves (Az) were calculated. Az was calculated for each observer in each modality evaluated. The possible effect of the observer group and image display modality on the ROC curves was evaluated using a mixed linear regression model, taking into consideration the possible structure of score correlation because each observer evaluated the dental surface in two forms of image visualization: printed on acetate viewed on a lightbox, and on a laptop monitor.

The possible association between caries detection and treatment decision was verified using the chi-square test. Adjusted residue analysis was used to identify the most significant sources of association presented in each contingency table for each group. All statistical tests adopted a 0.05 level of significance.

RESULTS

Of the 80 microscopically evaluated surfaces, 29 (36.25%) presented proximal caries lesions, of which 26 were restricted to the enamel, and only 3 reached the outermost dentin.

Table 1 shows the mean values for the area under the ROC curve, standard deviations, and confidence intervals (CI) for each group of observers for each image display modality. There was no statistical difference between display modalities ($p = 0.058$) or among the three observers groups (A, B, C) ($p = 0.991$).

Tables 2a and 2b show the adequacy of treatment choice to the caries detection scores chosen by group A for printed and laptop monitor image modality, respectively. The restorative treatment

Table 1: Means and Standard deviations of the areas under the ROC curves for the undergraduate student groups and image modalities.

		Modality			
		Printed		Laptop	
Group	N	Mean	Standard Deviation	Mean	Standard Deviation
A	14	0.538	0.070	0.515	0.091
B	12	0.542	0.068	0.516	0.079
C	10	0.536	0.052	0.515	0.068
Mean	36	0.539	0.063	0.516	0.079

Table 2a: Caries detection and treatment decision scores for the 14 observers in Group A for display modalities.

Display modality	Caries Detection	Treatment of choice			
		N*	P**	R***	Total
		n (%)	n (%)	n (%)	n (%)
Printed	Definitely not present	219 (92.8)	7 (3.0)	10 (4.2)	236 (100.0)
	Probably not present	68 (25.8)	188 (71.2)	8 (3.0)	264 (100.0)
	Unsure	18 (9.1)	155 (78.7)	24 (12.2)	197 (100.0)
	Probably present	1 (0.3)	176 (61.1)	111 (38.5)	288 (100.0)
	Definitely present	0 (0.0)	2 (1.5)	133 (98.5)	135 (100.0)
	Total	306 (27.3)	528 (47.2)	286 (25.5)	1120 (100.0)
Laptop	Definitely not present	251 (98.0)	4 (1.6)	1 (0.4)	256 (100.0)
	Probably not present	87 (33.3)	173 (66.3)	1 (0.4)	261 (100.0)
	Unsure	16 (14.4)	95 (85.6)	0 (0.0)	111 (100.0)
	Probably present	2 (0.6)	242 (71.4)	95 (28.0)	399(100.0)
	Definitely present	0 (0.0)	2 (1.3)	151 (98.7)	153 (100.0)
	Total	356 (31.8)	516 (46.1)	248 (22.1)	1120 (100.0)

*N = No treatment needed; **P= Preservation; ***R = Restorative treatment. Q-Square Test: $p < 0.001$.

Table 2b: Adjusted residues for Group A on display modalities.

Display modality	Caries Detection	Treatment of Choice		
		N	P	R
Printed	Definitely not present	25.407	-15.303	-8.446
	Probably not present	-0.652	8.961	-9.592
	Unsure	-6.309	9.768	-4.734
	Probably present	-11.919	5.510	5.873
	Definitely present	-7.596	-11.333	20.736
Laptop	Definitely not present	25.923	-16.266	-9.544
	Probably not present	0.613	7.480	-9.668
	Unsure	-4.141	8.799	-5.920
	Probably present	-14.772	11.198	3.123
	Definitely present	-9.087	-11.955	24.543

decision for surfaces scored for caries definitely present was practically unanimous (98.5% and 98.7%), whereas surfaces considered sound did not receive indication for treatment (92.8% and 98%) for either image display modality.

Tables 3a and 3b represent the correspondence between caries detection and treatment of observer group B on both image display modalities. In this group there is greater consistency between caries detection and restorative treatment decision when evaluating image modality (100%). None of the faces scored as sound or probably sound were scored for restorative treatment.

Tables 4a and 4b show the results for therapy chosen based on the diagnosis made by group C. Surfaces that the observers considered questionable were indicated for follow-up when evaluated in printed image modality by all observers, whereas for the laptop monitor modality, the result was 96.4%.

Table 3a: Caries detection and treatment decision scores for the 14 observers in Group B for display modalities.

Display modality	Caries Detection	Treatment of choice			
		N*	P**	R***	Total
		n (%)	n (%)	n (%)	n (%)
Printed	Definitely not present	403 (99.5)	2 (0.5)	0 (0.0)	405 (100.0)
	Probably not present	43 (26.2)	121 (73.8)	0 (0.0)	164 (100.0)
	Unsure	1 (0.8)	104 (84.6)	18 (14.6)	123 (100.0)
	Probably present	0 (0.0)	105 (67.3)	51 (32.7)	156 (100.0)
	Definitely present	0 (0.0)	0 (0.0)	112 (100.0)	112 (100.0)
	Total	447 (46.6)	332 (34.6)	181 (18.8)	960 (100.0)
Laptop	Definitely not present	361 (99.4)	2 (0.6)	0 (0.0)	363 (100.0)
	Probably not present	51 (37.0)	87 (63.0)	0 (0.0)	138 (100.0)
	Unsure	1 (0.9)	110 (97.3)	2 (1.8)	113 (100.0)
	Probably present	0 (0.0)	149 (73.0)	55 (27.0)	204 (100.0)
	Definitely	0 (0.0)	10 (7.0)	132 (93.0)	142 (100.0)
	Total	413 (43.0)	358 (37.3)	189 (19.7)	960 (100.0)

*N = No treatment needed; **P= Preservation; ***R = Restorative treatment. Q-Square Test: $p < 0.001$.

Table 3b: Adjusted residues for Group B on display modalities.

Display modality	Caries Detection	Treatment of Choice		
		N	P	R
Printed	Definitely not present	28.092	-18.970	-12.758
	Probably not present	-5.736	11.590	-6.779
	Unsure	-10.894	12.478	-1.281
	Probably present	-12.740	9.390	4.828
	Definitely present	-10.511	-8.187	23.360
Laptop	Definitely not present	27.536	-18.356	-11.962
	Probably not present	-1.555	6.760	-6.286
	Unsure	-9.631	14.054	-5.099
	Probably present	-13.985	11.898	2.944
	Definitely present	-11.217	-8.075	23.787

DISCUSSION

This study evaluated the influence of two different image display modalities on caries detection and treatment choice. Two methods for displaying the radiographic digital image were compared: printed on acetate viewed on a lightbox, and displayed on a laptop monitor. This study also aimed to evaluate whether students' undergraduate dental experience in radiology classes and clinical practice improved their capacity to detect incipient proximal caries lesions.

Most of the studies which have evaluated the influence of image quality on caries detection used a relatively small number of observers, typically oral radiologists or dental professionals with significant experience in caries detection[4,11,12]. In Rockemback et al. [13], one observer conducted the evaluation, while in other studies the number of observers varied from three[11,14,15] to 14[3] or 20[16]. Few studies used dental students as observers[16–18]. In our study, the evaluations were performed by 36 dental

Table 4a: Caries detection and treatment decision scores for the 14 observers in Group C for display modalities.

Display modality	Caries Detection	Treatment of choice			
		N*	P**	R***	Total
		n (%)	n (%)	n (%)	n (%)
Printed	Definitely not present	310 (98.4)	2 (0.6)	3 (1.0)	315 (100.0)
	Probably not present	64 (55.2)	52 (44.8)	0 (0.0)	116 (100.0)
	Unsure	0 (0.0)	67 (100.0)	0 (0.0)	67 (100.0)
	Probably present	1 (0.7)	91 (66.9)	44 (32.4)	136 (100.0)
	Definitely present	1 (0.6)	1 (0.6)	164 (98.8)	166 (100.0)
	Total	376 (47.0)	213 (26.6)	211 (26.4)	800 (100.0)
Laptop	Definitely not present	349 (98.0)	6 (1.7)	1 (0.3)	356 (100.0)
	Probably not present	23 (31.9)	49 (68.1)	0 (0.0)	72 (100.0)
	Unsure	2 (2.4)	81 (96.4)	1 (1.2)	84 (100.0)
	Probably present	0 (0.0)	108 (70.1)	46 (29.9)	154 (100.0)
	Definitely present	1 (0.7)	5 (3.7)	128 (95.5)	134 (100.0)
	Total	375 (46.9)	249 (31.1)	176 (22.0)	800 (100.0)

*N = No treatment needed; **P= Preservation; ***R = Restorative treatment. Q-Square Test: $p < 0.001$.

Table 4b: Adjusted residues for Group C on display modalities.

Display modality	Caries Detection	Treatment of Choice		
		N	P	R
Printed	Definitely not present	23.481	-13.403	-13.150
	Probably not present	1.907	4.797	-6.972
	Unsure	-8.053	14.196	-5.118
	Probably present	-11.866	11.667	1.736
	Definitely present	-13.454	-8.521	23.785
Laptop	Definitely not present	25.964	-16.104	-13.279
	Probably not present	-2.661	7.095	-4.724
	Unsure	-8.638	13.664	-4.867
	Probably present	-12.972	11.634	2.624
	Definitely present	-11.728	-7.506	22.518

students in order to investigate the influence of academic training and level of experience on radiographic proximal caries detection.

The teeth sample used in this study consisted of premolars and molars with carious lesion in the initial stages, which previous studies found to be more difficult to detect[3,9,11,12,19,20]. According to Shintaku, Scarbeczm and Venturim, the Az values are significantly higher when results are validated based only on radiographic and clinical evaluation[21,22].

To assess how the scores reflected on the diagnostic decision, Az values were calculated for each observer for each modality of image display. As in our study, Ferreira et al. evaluated enamel demineralization using radiographic film and digital and digitized images[23]. They evaluated the diagnostic performance of each observer and measured the efficiency of the methods by using the areas under the ROC curves. Otis & Sherman[22] evaluated caries diagnosis using printed photographic paper interproximal images and in the statistical analysis, calculated Az for each

observer and image format. Other similar studies evaluated observer performance by using ROC curve analysis[4-6,11,16,22,24].

Ludlow & Abreu[25] found that the accuracy values for caries lesions evaluated on laptop monitors were comparable to the accuracy values for printed images evaluated using the lightbox, in agreement with our study. All viewing sessions in our study were done in a quiet room with dimmed light for both image display methods; a setup which substantially increases diagnostic precision[9,12,26].

The Az projects total diagnostic accuracy and consequently represents the performance of the observer. In this type of analysis, perfect performance is represented by an Az value close to 1; nevertheless, values from 0.75 to 0.80 are considered acceptable for the detection of proximal caries involving dentin tissue. However, incipient caries are more difficult to diagnose because enamel decalcification needs to be higher than 30% for them to be detected in radiographic images, leading to lower Az levels than when evaluating dentin lesions[10]. In this study, the Az values ranged from 0.515 to 0.542, revealing lower diagnostic accuracy than is usually found in studies of this nature. Similar Az values were found in a study by Diniz et al.[16], in which the Az value for Brazilian undergraduate dental students was 0.53 for enamel caries detection. Hellen-Halme, Nilsson and Petersson[4] reported similar Az values, ranging from 0.542 to 0.557, even though the observers were one oral radiologist and six dental professionals, including dentists with more professional experience than the observers in our study, who were undergraduate dentistry students.

Adjusted residual analyses based on caries detection and therapeutic treatment decision for both forms of image display reflected alignment of academic teaching at the university with the new caries treatment philosophy. The strong tendency to prefer preservation over restorative treatment was clearly evident. The score showed that restoration was only selected for surfaces scored as caries definitely present. The theory of minimum intervention is based on preventive dentistry, which postpones filling or its substitution until strong evidence exists of caries lesion[27] and recommends maximum preservation of sound tooth structure, executing conservative preparation and providing higher resistance to the remaining dental structure. New developments in long-lasting restorative adhesive materials enable the use of conservative preparation limited to the size of the lesion and do not require additional time wearing of mechanical retention[28].

Rocha et al. found that, when in doubt, the undergraduate student prefers to classify the surfaces as intact during radiographic evaluation, increasing the number of false negatives, which immediately reflects on the therapeutic choice in the form of non-treatment or preservation[17]. On the other hand, the results found by Bervian et al.[18] revealed that a significant proportion of students chose restorative treatment for caries lesions restricted to the enamel surface, and this proportion was more evident among students attending private schools. As previously seen in other professional courses, the quality of dentistry education is related to the university and the course having an adequate pedagogical model . Despite the occurrence of major changes in higher education, the educational model for dentistry in Brazil still focuses mainly on technical training.

Our study found that when the diagnosis was either caries definitely present or definitely sound, the treatment decision was consistent with the diagnosis, which implies greater confidence of the observer during evaluation of the images. Definitely sound surfaces were not treated, while restorative treatment was indicated when caries were definitely present. The uncertain options, considered probably present or probably sound, or without any condition to diagnose -score 3-, led to follow-up, which is a safe, conservative therapy. These students should be trained to detect incipient proximal caries more effectively because the definitive diagnosis should not be jeopardized in favor of follow-up, as a neglected caries lesion can evolve. In conclusion, this study demonstrated that the different forms of digital image displays resulted in similar performance by observers and did not affect the precision of proximal caries detection or treatment decision. Student level in a dentistry course does not significantly increase precision with regard to proximal caries detection.

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A RETROSPECTIVE ANALYSIS OF REACTIVE HYPERPLASTIC LESIONS OF THE ORAL CAVITY: STUDY OF 1149 CASES DIAGNOSED BETWEEN 2000 AND 2011, CHILE

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ABSTRACT

The aim of this study was to determine the relative frequency and distribution of reactive hyperplastic lesions (RHL) of the oral mucosa at the Oral Pathology Institute of the School of Dentistry at the University of Chile.

This was a retrospective study of 1149 biopsies with histopathological diagnosis of RHL, performed between 2000 and 2011. The RHL were classified in 4 groups: fibrous hyperplasia (FH), pyogenic granuloma (PG), peripheral giant-cell granuloma (PGCG) and peripheral ossifying fibroma (POF).

Results: the most frequent RHL was FH (71. 1%), followed by PG (21.1%), PGCG (5 %) and POF (2.9%). RHLs were more frequent in women (70.7%). The most highly affected age group was the 50- to 59-year-olds (22%). The most frequent location for RHL was maxilla (24.7%), followed by cheek (20.6%), tongue (19.4%) and jaw (18.5%). The most prevalent RHL diagnosis was FH. The most frequently affected sex was female, the most frequent age range was 50-59 years, and the most frequent location, maxilla.

Key words: *gingival hyperplasia, giant cell granuloma, pyogenic granuloma, mouth mucosa.*

ANÁLISIS RETROSPECTIVO DE LESIONES REACCIONALES HIPERPLÁSICAS EN MUCOSA ORAL: ESTUDIO DE 1149 CASOS DIAGNOSTICADOS ENTRE EL 2000–2011 EN CHILE

RESUMEN

El objetivo de este estudio fue determinar la frecuencia relativa y distribución de lesiones reaccionales hiperplásicas (LRH) de la mucosa oral, presentes en el registro de biopsias del Servicio de Anatomía Patológica de la Facultad de Odontología, Universidad de Chile.

Este estudio, de tipo retrospectivo consistió en 1149 biopsias con diagnóstico histopatológico de LRH, entre los años 2000-2011. Las LRH se clasificaron en 5 grupos: Hiperplasia fibrosa (HF), granuloma piogénico (GP), granuloma periférico de células gigantes (GPCG) y fibroma osificante periférico (FOP). Los datos de edad y sexo de los sujetos, y de localización y tipo de lesión, fueron obtenidos del registro de biopsias de cada caso.

De las LRH, la lesión más frecuente fue HF (71,1%), seguido de GP (21,1%), GPCG (5 %) y FOP (2,9 %) respectivamente. Las biopsias de LRH fueron mas frecuentes en mujeres (70,7 %). El rango etario más afectado fue el de 50 a 59 años (22%). La localización de mayor frecuencia de LRH fue el maxilar superior (24,7%), seguida de mejilla (20,6%), lengua (19,4%), mandíbula (18,5%), labio inferior (9,9%) y labio superior (6,7%). En este estudio, de las LRH el diagnóstico más prevalente fue FH. El sexo más afectado fue el femenino, el rango etario el de 50 a 59 años y la ubicación más frecuente, maxilar superior. Estos resultados en general son concordantes con lo descrito en otros países.

Palabras-clave: *hiperplasia gingival, granuloma de células gigantes, granuloma piogénico, mucosa de la boca.*

INTRODUCTION

Reactive hyperplastic lesions (RHL) are a group of fibrous alterations of the connective tissue. They usually appear in the oral mucosa in response to injuries[1] such as local chronic irritation or trauma due to the presence of stone, restorations with irregular edges, low-grade traumatism and iatrogenic factors. The mucosa reacts to these irritant factors with local hyperplasia composed of mature collagen, fibroblasts, mineralized tissue, endothelial cells and giant multinucleated cells. These lesions are not considered neoplasms, but inflammatory hyperplastic reactions[2].

The accepted classification of oral mucosa RHL includes a wide range of lesions[2], including fibrous hyperplasia (FH), pyogenic granuloma (PG), fibrous epulis with ossification or peripheral ossifying fibroma (POF) and peripheral giant-cell granuloma (PGCG). The clinical and histopathological characteristics of each are described below.

FH is a solid mass, painless, nodular, with a smooth surface and normal color that can appear at any

location of the oral mucosa. Histologically, it is composed of connective tissue with dense collagen [2,3].
PG appears clinically as an erythematous mass, painless, smooth, lobulated, fast-growing and bleeding easily when touched. It is associated with trauma, poor oral hygiene and increase in hormonal levels during pregnancy. It generally appears in the gum but also in less usual places such as the lips, tongue and oral mucosa[3]. Histologically, it is composed of hyperplastic granulation tissue with a marked proliferation of endothelial cells covering the capillary channels and an infiltrate of mixed inflammatory cells[2].
POF appears only in the gum, beginning in the periodontal ligament. It is considered more an RHL than a neoplasm. Clinically, it appears as a nodular mass, pedunculated or sessile, painless, which usually comes from the interdental papilla, pink or red colored, and may be ulcerated[2]. Histologically, it is composed of cellular fibroblastic tissue and formation of mineralized products: bone, cement-like material or dystrophic calcifications[4].
PGCG is exclusively a lesion of the gum or the alveolar mucosa. It appears as an increase of nodular volume, painless, red to blue-red colored, pedunculated or sessile. Histologically, it consists of a proliferation of giant multinucleated cells and a proliferation of mesenchymal cells, associated with prominent vascularization, abundant hemorrhage and hemosiderin deposits at the periphery of the lesion. It can also present signs of chronic inflammation and areas of formation of reactive bone and even dystrophic calcifications[2-5].

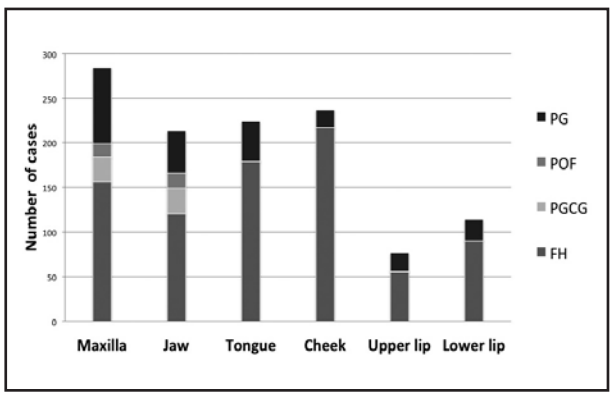

Fig. 1: Distribution of reactive hyperplastic lesions according to gender. FH: fibrous hyperplasia; PGCG: peripheral giant-cell granuloma; POF: peripheral ossifying fibroma; PG: pyogenic granuloma.

There are variations in the prevalence of RHL according to type of lesion, age, gender and affected site[1]. In its clinical appearance it is very similar to some neoplasms, making differential diagnosis difficult[6]. Appropriate knowledge of the distribution and frequency of RHL enables better clinical diagnosis of affected patients. These data have been analyzed in countries such as Iran[1], China[2], Canada[3], United States[4] and India[5]. However, there is no epidemiological record of oral reactional lesions in Chile or the rest of Latin America, so it would be of great interest to research the topic.
The aim of this study was to determine the relative frequency and distribution of the most common RHL of the oral mucosa by analyzing the 2000 to 2011archives of the Department of Oral Pathology at the School of Dentistry, University of Chile.

MATERIALS AND METHODS

We processed whole biopsies or specimens from the 2000 to 2011 archives of the Department of Pathology and Oral Medicine at School of Dentistry, University Chile. The study included samples with RHL histopathological diagnosis according to the revision in Buchner et al.[2] The samples were classified into 4 groups: fibrous hyperplasia (FH), pyogenic granuloma (PG), peripheral giant-cell granuloma (PGCG) and peripheral ossifying fibroma (POF). The specimens were analyzed based on their frequency and distribution according to patient age and gender. The anatomic location of the lesion was obtained from the biopsy register for each case. The Stata V10 software was used for data analysis.

RESULTS

Of the 6369 specimens diagnosed between 2000 and 2011 in the registers, 1149 (18%) had RHL. Out of the RHL, 817 (71.1%) were FH, 57 (5%) PGCG, 33 (2.9%) POF and 242 (21.1%) PG.
RHL affected 629 (72.2%) females and 520 (27.8%) males. Regarding the distribution of the RHL by gender, for FH, 238 cases (29.3%) were male and 575 (70.7%) female. For PGCG, 33 cases (51.6%) were male and 31 (48.4 %) female. For POF, 15 cases (42.9%) were male and 20 (57.1%) female. For GP, 49 cases (20.7%) were male and 188 (79.3%) female (Fig 1).
Out of all the RHL patients, 37 (3.2%) were 0-9 years old, 83 (7.2%) were 10 to 19 years old, 98 (8.5%) were 20 to 29 years old, 146 (12.7%) were 30 to 39

years old, 225 (19.5%) were 40 to 49 years old, 253 (22%) were 50 to 59 years old, 208 (18.1%) were 60 to 69 years old, 83 (7.2%) were 70 to 79 years old and 16 (1.4%) were over 80 years old (Fig. 2).
The most frequent location of RHL was maxilla, with 284 cases (24.7%), followed by cheek (oral mucosa) with 237 cases (20.6%), tongue with 224 (19.4%), jaw, which includes the gum and/or lower alveolar process, with 213 cases (18.5%), lower lip with 114 (9.9%) and upper lip with 77 (6.7 %) (Fig. 3).

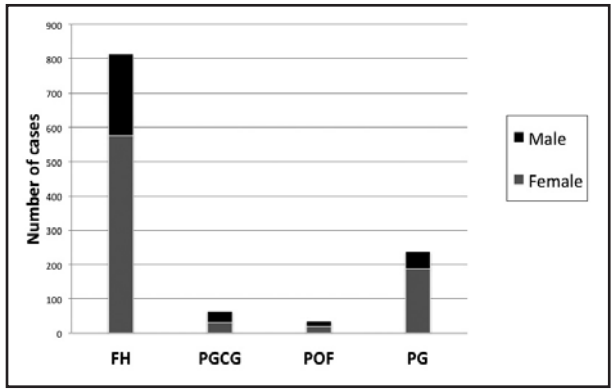

Fig. 2: Distribution of reactive hyperplastic lesions according to location. FH: fibrous hyperplasia; PGCG: peripheral giant-cell granuloma; POF: peripheral ossifying fibroma; PG: pyogenic granuloma.

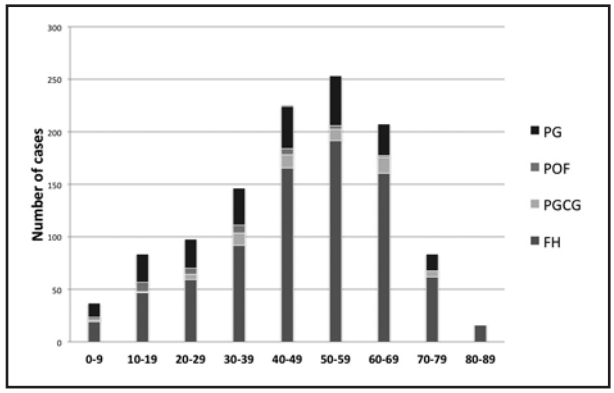

Fig. 3: Distribution of reactive hyperplastic lesions according to age group. FH: fibrous hyperplasia; PGCG: peripheral giant-cell granuloma; POF: peripheral ossifying fibroma; PG: pyogenic granuloma.

DISCUSSION

The aim of this study was to determine the relative frequency and distribution of the most frequent RHLs in the 2000 to 2011 archives of the Department of Oral Pathology at the School of Dentistry, University of Chile.

RHL frequency

Out of the 6369 specimens, 18.04% had RHL. When comparing this data with other studies, the percentage of RHL compared to total specimens varies from 5% in China [7], to 6. 4% in Canada [8], 6.7% in Israel[2] and 48% in Iran[1]. Among other reasons, these variations could be explained by the different criteria used in each country or location regarding what lesions should be biopsied. Moreover, many RHLs may have been clinically diagnosed but not biopsied, which does not necessarily lead to appropriate diagnosis and treatment.
Our study found a higher percentage of FH (71.1% of total RHL) than any of the other studies that used similar methodology and RHL classification. The results are closest to ours are Daley et al.[8] in Canada (61.2%) and Zang et al.[7] in China (61%).
Although most studies [2, 7–11] report FH as the most prevalent RHL, authors such as Stableinet et al.[12], Ababneh et al.[13] and Zarei et al.[7] report PG as the most prevalent. The percentage of PG with respect to RHL varies from 11.9% in Canada [8] to 42.8% in USA[12]. However, most authors report percentages between 20 and 30% [2, 9, 14, 15], in agreement with our results (21.1%). It should be highlighted that in all the studies, including ours, PG was more frequent in women.
Our percentage of POF compared to total RHL was lower (2.9%) than that of most of the other studies, which report percentages varying from 7.2% [13] to 40% [15]. The latter study even categorizes POF as the most frequent RHL.

In our study, PGCG accounts for 5% of all RHL. The frequencies described in other studies range from 18.7% in Israel[2] to 1.5% in China[7].

RHL distribution according to gender

PGCGs were more frequent in males (51.6%), but all the rest of the lesions were more prevalent in females, with 70.7% for FH, 79.3% for PG, and 57.1 % for POF. Buchner et al.[16] found similar results in both adults and children, where RHL are also more common in females, except for PGCG. Although Buchner describes differences between genders which are similar to ours, his FH percentage for females is lower than in our study (57.7% vs. 70.7%). Zarei et al.[17], Salum et al.[18], and Kfir et al.[9], also report a higher RHL frequency in females, except for PGCG, which was higher in males but

without significant differences. Other studies also showed significant predominance of RHL in females [1, 5, 7, 14, 17, 19], with the exception of Anneroth et al.[20] in Sweden, who found no difference in FH percentage between genders.

RHL distribution according to age group.

The highest RHL prevalence was found in the group of 50- to 59- year-olds. The oldest and youngest groups had the lowest prevalence, with 3.2% in 0- to 9-year-olds and 1.4% in patients over 80 years old.

FH (23.6%) and PG (19.9%) were the most frequent among 50- to 59-year-olds, while PGCG (23.7%) prevailed in 60- to 69-year-olds. These data match those reported in Greece[21], England[22], China[7], Canada[8], Sweden[20] and USA[9], where a similar distribution of these lesions was found according to age range. POF (23.8%) was most frequent in 10- to 19-year-olds, in agreement with Buchner et al.[2], with PG and POF being the most frequent in this age range, with 18.3% and 24.5%, respectively. Other studies [4,8, 9, 23,] also report POF as the most frequent lesion in this age range.

In general, our study matches the results reported in most papers on the frequency of RHL in different age ranges, except for PG, which at 19.9 %, mainly affects subjects aged 50 to 59 years, whereas most other papers[7-9, 11, 18, 20] place it before the age of 30 years.

Distribution according to location

Our study found similar percentages of FH distribution according to location in maxilla and jaw (19.1% and 14.7%, respectively) as studies published in Israel[2] and USA[13]. However, it is essential to note that 48.4% of FH were located in soft tissues, with 21.9% on the tongue and 26.6% on the cheek. Naderi[6] reports similar frequencies, with 54.8% for mandible and maxilla, 20.3% for tongue and 24.9% at other sites. Ala Aghbali[1] found a 83.9% for maxilla and jaw and only 12.8% for cheek and 2.6% for tongue.

As for PGCG, studies carried out in Sweden[20], Greece[21], Iran[5] and Denmark[24] report a higher frequency in the jaw than in the maxilla. Our study shows an equal distribution at both sites (50%-50%), which resembles studies in USA[9,23] and England[22].

Regarding PG, our results agree with those reported in Israel[2], USA[9], India[11] and Brazil[18], with this lesion being more prevalent in the maxilla than in the jaw. Higher prevalence in the jaw was only reported in China[7]. Although our study reports greater presence of PG in the maxilla (35.1%) than the jaw (19.4%), we also found 7.7 % of PG in cheek and 18.6 % in tongue.

Our study found slightly greater presence of POF in the jaw (51.5%), which differs from findings in Israel[2], USA[4, 9] and China[7], where greater prevalence was reported in the maxilla.

The differences in frequency in the several series detected when comparing our study to studies in other countries may be explained by the socio-economic and cultural variations, available resources and type of department where the research was conducted. These types of variables should be evaluated in the future in order to associate them with the frequency of the diagnosed pathologies and thus help improve the epidemiological approach in each country carrying out similar studies.

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PATIENTS' PERCEPTION OF INSTALLATION, USE AND RESULTS OF ORTHODONTIC MINI-IMPLANTS

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ABSTRACT

The purpose of this study was to evaluate patient acceptance and perception of pain with regard to orthodontic mini-implants. The study was conducted on 58 individuals undergoing orthodontic treatment, who had orthodontic mini-implants placed as anchorage devices. Data were collected using a questionnaire containing 6 questions evaluating perception of pain during mini-implant placement and during use, difficulty with cleaning, unaesthetic appearance, difficulty with eating and benefits observed. Data were tabulated and analyzed using Fisher and Spearman's Correlation Coefficient tests. It was found that 94.8% of the patients reported that they would be willing to undergo treatment with mini-implants again. Of the negative aspects evaluated, the most significant was discomfort during placement, while the least significant was difficulty with eating. Patients' perception of aspects related to mini-implants was shown to be independent of the quantity of these devices placed. Although the patients evaluated some aspects of mini-implants negatively, the mean score for benefits observed was very high, indicating good patient satisfaction with treatment.

Key words: *Orthodontics, Orthodontic Anchorage Procedures, Pain.*

PERCEPÇÃO DOS PACIENTES QUANTO A INSTALAÇÃO, USO E RESULTADOS DOS MINI-IMPLANTES ORTODÔNTICOS

RESUMO

O objetivo do presente estudo foi avaliar a aceitabilidade e percepção dolorosa dos pacientes em relação aos mini-implantes ortodônticos. Este estudo foi realizado com 58 indivíduos em tratamento ortodôntico que tiveram a instalação de mini-implantes ortodônticos como recurso de ancoragem. O instrumento de coleta de dados foi um questionário contendo 8 perguntas que avaliaram a percepção dolorosa durante a instalação e uso dos mini-implantes, dificuldade de higienização, aspecto anti-estético, dificuldade de alimentação e os benefícios observados. Os dados foram tabulados e analisados pelos testes de Fisher e de Coeficiente de Correlação de Spearman. Os resultados demonstraram que 94,8% dos pacientes relataram que se submeteriam novamente ao tratamento com mini-implantes. Dos aspectos negativos avaliados, o mais significante foi o incômodo e dor durante a instalação, enquanto o menos significante foi dificuldade de alimentação. A percepção dos pacientes sobre os aspectos relacionados aos mini-implantes mostrou-se independente da quantidade desses dispositivos instalados. Conclui-se que apesar da avaliação dos mini-implantes pelos pacientes ter apresentado alguns aspectos negativos, o escore médio dos benefícios observados foi bastante alto, indicando boa satisfação dos pacientes com o tratamento.

Palavras-chave: *Ortodontia, Procedimentos de ancoragem, Dor.*

INTRODUCTION

Anchorage is essential to achieving the objectives of orthodontic treatment.[1] The main advantage of mini-implants is that they allow the movement of several teeth without loss of anchorage. [2-4] Other advantages include the small size, minimal anatomic limitations, more comfortable surgery for patients, immediate loading and low cost.[5] They provide an excellent alternative, as extra-oral appliances depend on patients' cooperation, thus limiting treatment.[6] There are many studies on mini-implants in the literature, particularly discussing their properties and clinical applications. Nevertheless, even with the popularization of orthodontic treatment with mini-implants, there are few studies on the tolerance, acceptability and opinion of patients with regard to treatment with them.

Based on this premise, the aim of this study was to evaluate patients' perception of mini-implants with respect to pain, aesthetics, hygiene and benefits observed, and thereby fill this gap in the literature.

MATERIALS AND METHODS

The research was conducted on 58 individuals aged 30 to 50 years (Mean: 35 years 5 months), who were undergoing orthodontic treatment with mini-implant placement at the Orthodontic Clinic of the Southwest Bahia State University. Initially, all the patients presented skeletal Class II and Class I malocclusion, in which the proposed treatment involved premolar extractions. A total of 132 self-drilling mini-implants were placed, all of the self-tapping type, measuring 1.6mm thick and 8mm long (SIN - Sistema de Implantes Nacionais, São Paulo, Brazil). All mini-implants were inserted by the same orthodontist.

The mini-implants were placed after infiltrative local anesthesia with a little less than 1/4 of the anesthetic cartridge. A lancet was used to demarcate the cortical bone at the site defined as ideal for mini-implant placement, and the mini-implants were inserted directly into the bone using a manual driver. All the mini-implants were placed between maxillary second premolars and first molars. They were loaded using a nickel titanium spring with 100 g force measured with a dynamometer.

Data were collected by means of a questionnaire containing questions about pain perception during mini-implant placement and use, difficulty with cleaning, unaesthetic appearance, difficulty with eating and the benefits. Patients were asked to use a scale of 0 to 10 to rank their perceptions. One question asked whether the subject would be willing to undergo mini-implant placement again, and another asked how many mini-implants were placed. The questions were asked at different times. The question about perception of pain during placement was asked immediately after implant placement. The questions about pain perception while using mini-implants, difficulty with cleaning, unaesthetic appearance, and difficulty with eating were asked 28 days after placement. The question about the benefits observed was asked 6 months after placement (Figure 1). The research was conducted in compliance with the criteria established by Resolution CNS 196/96 of the Ministry of Health (Brazil, 1996), so the questionnaire was only administered after approval by the Research Ethics Committee of the Southwest Bahia State University, Protocol Number 125/2011.

The Spearman correlation coefficients were calculated to evaluate the relationship between the visual analog scale scores of the various aspects investigated. The scores were compared according to the number of mini-implants placed, using the Mann-Whitney test. The level of significance adopted was 5% ($\alpha = 0.05$).The data were tabulated and analyzed using the statistical software BioEstat (version 5.0, Belém-PA, Brazil).

RESULTS

The number of mini-implants placed in patients participating in the research ranged from 1 to 5, with mean ± SD = 2.3 ± 1.1. Of the 58 participants, 55 (94.8%) reported that they would be willing to undergo further treatment with mini-implants, 2 (3.4%) would not be willing to do so and 1 (1.7%) did not answer the question. The descriptive statistics for the Visual Analog Scale scores for the six aspects investigated are presented in Table 1. The factor with the most negative impact on mini-implant placement is discomfort and pain during placement, followed by the difficulty with cleaning. The factors that had the least negative impact were, in order, difficulty with eating and unaesthetic appearance. The mean score for benefits observed was very high, indicating good satisfaction with the end result of the treatment.

Table 1: Descriptive statistics of visual analog scale, according to the aspects evaluated.

Feeling with regard to	Mean	Standard Deviation	Min-Max
Discomfort and pain (during placement)	3.03	2.30	0.0 - 8.3
Discomfort and pain (during use)	1.56	2.16	0.0 - 8.0
Difficulty with cleaning	2.12	2.61	0.0 - 8.0
Unaesthetic appearance	0.81	1.87	0.0 - 10.0
Difficulty with eating	0.77	1.61	0.0 - 10.0
Benefits observed	8.56	2.21	0.0 - 10.0

IDENTIFICATION

Gender

☐Male ☐Female

Age _____

Please provide your answer on the following scale by marking the level that represents how you feel regarding:

→ **Discomfort and pain (during placement) – immediately after placement.**

→ **Discomfort and pain (during use) – 28 days after placement.**

→ **Difficulty with cleaning – 28 days after placement**

→ **Unaesthetic appearance – 28 days after placement**

→ **Difficulty with eating – 28 days after placement**

→ **Benefits observed – 6 months after placement**

→ **How many mini-implants were placed? ___**

→ **Would you be willing to undergo mini-implant placement again? () Yes () No**

Fig. 1: Assessment Questionnaire.

Table 2 shows the correlation coefficients (r) between the visual analog scale scores of the aspects analyzed. There was significant correlation between the variable "discomfort and pain (during use)" and the variables "difficulty with cleaning" and "difficulty with eating". Significant correlation was also found between "difficulty with cleaning" and "difficulty with eating".

Table 3 compares the visual analog scale scores of the aspects analyzed according to the quantity of mini-implants placed. There was no statistically significant difference for any of the study questions between patients receiving up to two mini-implants and patients receiving more than two.

DISCUSSION

The purpose of this study, as was the case with the few available in the literature[7, 8], was to evaluate patients' perception of mini-implants regarding pain, aesthetics, difficulty with cleaning and eating, and benefits observed. The scale used for evaluation enabled a clearer understanding of level of pain and difficulty related to mini-implants, in addition to the degree of benefits obtained.

The mean score for benefits was 8.56 and the percentage of patients who would be willing to undergo mini-implant placement again was 94.8%, so the rate of satisfaction with the treatment can be considered high. These results agree with Brandão and Mucha[9] (90%), Blaya*et et al.*[7](100%), Lee *et al.*[10] (77.8%) and Gündüz*et et al.*[11] (94.81%).

Discomfort and pain during mini-implant placement was the negative factor most often reported by patients, although since the mean is low, it is unlikely to be a limitation to this procedure. This finding is very close to other studies by Lee et al.[10], in which 72.2% of the subjects reported little or no pain, and by Kuroda et al.[12], in which only about 25% of the subjects reported pain at the time of self-tapping mini-implant placement. In the study by Baxmann *et al.*[13], the percentage of patients that felt little or no pain was lower, at approximately 40%.

In addition to discomfort and pain during mini-implant placement, the most relevant side effects, in decreasing order, were difficulty with cleaning, discomfort and pain during use, unaesthetic appearance and difficulty with eating. Other studies[12, 14]

Table 2: Spearman's Correlation Coefficient between the visual analog scale scores of the aspects evaluated.

Feeling with regard to	Discomfort and pain (during use)	Difficulty with cleaning	Unaesthetic appearance	Difficulty with eating
Discomfort and pain (during placement)	0.16	0.23	0.09	0.06
Discomfort and pain (during use)		0.37*	0.12	0.31*
Difficulty with cleaning			0.22	0.41*
Unaesthetic appearance				0.21
Difficulty with eating				

*p < 0.05

Table 3: Mean and standard deviation of the visual analog scale scores of the aspects investigated, according to the number of mini-implants placed.

Feeling with regard to	Quantity of mini-implants placed		p-Value
	≤ 2	> 2	
Discomfort and pain (during placement)	3.01 ± 2.49	3.07 ± 1.90	0.643
Discomfort and pain (during use)	1.59 ± 2.35	1.51 ± 1.71	0.445
Difficulty with cleaning	2.26 ± 2.76	1.80 ± 2.29	0.649
Unaesthetic appearance	0.90 ± 2.16	0.60 ± 1.00	0.742
Difficulty with eating	0.73 ± 1.79	0.85 ± 1.15	0.249
Benefits observed	8.42 ± 2.41	8.87 ± 1.73	0.464

have mentioned negative effects as being difficulty with speech, cleaning and chewing.

Correlation analysis suggests that patients reporting discomfort and pain during the use of mini-implants would probably also report difficulty with cleaning and/or eating. Similarly, patients reporting difficulty with cleaning may also have difficulty with eating, and vice-versa.

Biofilm control in the peri-implant region is essential for maintaining orthodontic mini-implants within patterns of normality, and is directly related to their successful use[15, 16]. As the variable "difficulty with cleaning" was observed in two of the three significant correlations previously mentioned, it is important for the dental surgeon to know and convey a mini-implant cleaning protocol to patients[16].

The quantity of mini-implants placed showed no relationship with the negative aspects evaluated by the patients, suggesting that the patients' perception with regard to treatment does not depend on the number of mini-implants placed.

The data gathered in this study show that the mini-implant is the greatest revolution in anchorage for orthodontics over the past 15 years, making treatments more predictable, aesthetic and comfortable.

CONCLUSION

From the information collected and analyzed, it may be concluded that mini-implants are recommended for clinical use, since the patients reported a low degree of discomfort and pain during their placement and use, little difficulty with cleaning, minimal complaints of aesthetic compromise and little difficulty with eating. Moreover, the great majority said that they would be willing to undergo mini-implant placement again, thus showing that the benefits outweigh the possible risks or discomfort.

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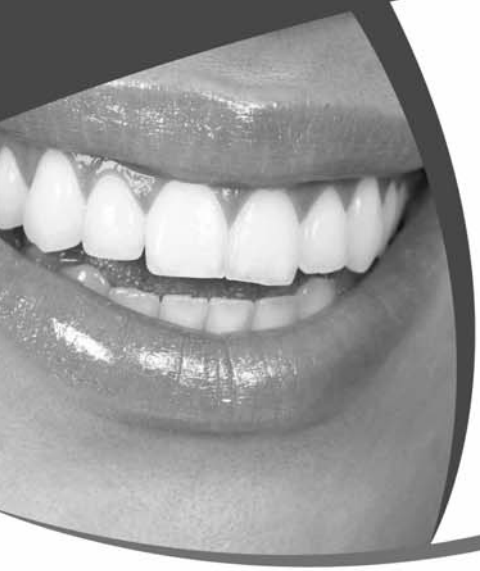
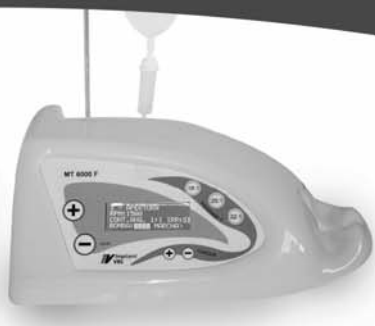


Implante **HC**

Avios **Primera Cirugía**



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EVALUATION OF TWO HUMAN DENTAL PULP STEM CELL CRYOPRESERVATION METHODS

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ABSTRACT

Dental pulp is a promising source of mesenchymal stem cells for use in cell therapy and regenerative medicine. Methods for storing stem cells with minimum compromise of cell viability, differentiation capacity and function should be developed for clinical and research applications. The aim of this study was to evaluate whether human dental pulp stem cells (hDPSCs) isolated and cryopreserved for 1, 7 and 30 days maintain viability and expression of specific stem cell markers. Human dental pulp stem cells were isolated from 23 healthy patients aged 18 to 31 years. Dental pulp was enzymatically dissociated, and CD105+ cells were separated using the Miltenyi™ system. The hDPSCs were cryopreserved using the Kamath and Papaccio methods. Post-cryopreservation viability was measured by flow cytometry (7AAD) and by the expression of the phenotype markers CD105+/ CD73+, CD34-/CD45-. The Papaccio method showed greater cell viability for cells that had been frozen for 30 days (59.5%) than the Kamath method (56.2%), while the Kamath method provided better results for 1 day (65.5%) and 7 days (56%). Post-cryopreservation expression of the markers CD105+/CD34- was greater after 1 and 7 days with the Kamath method and CD105+/CD45- were expressed after all 3 cryopreservation times. There was greater expression of CD73+ in the hDPSCs after 1 and 7 days with the Kamath method, and after 30 days with the Papaccio method.

These results suggest that hDPSCs express mesenchymal stem cell markers after cryopreservation. However, cryopreservation time may affect marker expression, probably by altering the spatialconfiguration of cell membrane proteins or by compromising cells at a certain level of differentiation.

Key words: *Cryopreservation, Mesenchymal stem cells, Phenotype, Cell viability, Dental pulp, Regenerative medicine.*

EVALUACIÓN DE DOS MÉTODOS DE CRIOPRESERVACIÓN DE CÉLULAS TRONCALES DE PULPA DENTAL HUMANA

RESUMEN

La pulpa dental es una fuente promisoria de células madre mesenquimales para ser utilizadas en terapia celular y medicina regenerativa. El desarrollo de métodos que permitan almacenar las células madre con mínimo compromiso de la viabilidad celular, capacidad de diferenciación y función es necesario para aplicaciones clínicas e investigación. El objetivo de este estudio fue evaluar si las células troncales de pulpa dental humana (hDPSCs) aisladas y criopreservadas durante 1, 7 y 30 días conservan la viabilidad y expresión de marcadores específicos de células troncales pos crio-preservación. Para esto, las hDPSCs se aislaron de 23 pacientes sanos entre 18 y 31 años. La pulpa dental se disoció enzimáticamente, y las células CD105+ se separaron mediante el sistema Miltenyi™. Posteriormente, las hDPSCs se criopreservaron utilizando el método de Kamath y de Papaccio, se evaluó la viabilidad pos crio-preservación por citometría de flujo (7AAD) y la expresión de marcadores CD105+/ CD73+, CD34-/CD45-. El método de Papaccio, mostró mayor viabilidad celular a los 30 días (59,5%) comparado con el método de Kamath, a 1 día (65,5%) y 7 días (56%) respectivamente. Se observó mayor expresión de los marcadores CD105+/CD34- a 1 y 7 días pos-criopreservación con el método Kamath y CD105+/CD45- a los 3 tiempos de criopreservación. CD73+ presentó mayor expresión en las hDPSCs a las 24 horas y 7 días con el método de Kamath, y al mes con el método Papaccio.

Estos resultados sugieren que las hDPSCs expresan marcadores de células troncales mesenquimales postcriopreservación. Sin embargo el tiempo de criopreservación podría modificar la expresión de los marcadores probablemente por alterar la configuración espacial de las proteínas de membrana celular; o por comprometer a las células a cierto grado de diferenciación.

Palabras clave: *Criopreservación, Células troncales mesenquimales, Fenotipo, Viabilidad celular, Pulpa dental, Medicina regenerativa.*

INTRODUCCIÓN

The discovery of stem cells and their potential has led to the development of new cell therapy strategies[1,2]. The availability and cryopreservation of stem cells have thus become the subjects of intensive research.

Human dental pulp stem cells (hDPSCs) have high potential for proliferation, self-renewal and

differentiation into odontoblasts, osteoblasts, chondrocytes, myocytes, adipocytes, neurons and corneal epithelial cells[1,3,4,5]. Moreover, the ease of obtaining healthy temporary or permanent teeth when they are extracted for treatments such as orthodontics makes dental stem cells an attractive source for autologous transplant as well as for the creation of specialized biobanks providing a reliable supply for applications in regenerative medicine and dentistry. Methods should be developed for storing hDPSCs which involve minimum loss of cell viability and preserve their differentiation potential and function.

The success of hDPSC isolation declines over time. When cell cultures are kept for a long time, certain complications and limitations may arise, such as 1) reduction in their differentiation potential, 2) senescence and apoptosis as a result of serial passages, 3) potential genetic alterations and 4) high costs[5,6]. These are the reasons why cryopreservation is proposed as the most effective method for maintaining cells available in the long term for future clinical applications in transplants[7,8,9]. However, the effects of cryopreservation on the properties of human dental pulp stem cell (hDPSCs) are not clear. Zhang et al.[10] reported that after being stored in liquid nitrogen for two years, hDPSCs maintain their potential for proliferation and differentiation into specific cell lineages[1] although the post-cryopreservation hDPSC isolation success rate may decline due to the formation of ice crystals during the freezing process. The use of cryoprotectants such as dimethyl sulfoxide (DMSO) is therefore suggested to prevent crystals formation[11].

The cryopreservation process may cause cell damage which could be associated to cryoprotectant toxicity or osmotic imbalance upon subjecting the cryoprotectant to freezing and thawing. Several studies have thus related the stress factors in cryopreservation to the activation of cell death signaling cascades[7]. DMSO is the most frequently used cryoprotectant in different methods of cryopreservation. It penetrates cells and forms hydrogen bonds with the water molecules, preventing the flow of water from the cytoplasm, and minimizing dehydration and formation of intracellular ice. However, the most effective cryopreservation protocol for maintaining post-cryopreservation cell viability has not yet been established. Thus, the aim of this study was to evaluate the effect of two cryopreservation methods, one reported by Papaccio *et al.* [12] and the other by Kamath[13], which use the same cryoprotectant (DMSO), to assess the viability and phenotype of mesenchymal stem cells obtained from human dental pulp after undergoing cryopreservation for 1, 7 and 30 days. Selecting the best method for maintaining these hDPSC features is important for their use in biobanks in order to ensure the quality of cryopreserved cells.

MATERIALS AND METHODS

Collection and transportation of extracted teeth

Extracted teeth were obtained from the Dental Clinic at El Bosque University from patients aged 18 to 31 years, with tooth extraction indicated for therapeutic purposes such as included or impacted third molars, or for orthodontic purposes, without caries or periodontal disease. The study was approved by the institution's research ethics committee, and prior informed consent was obtained from the patients. Immediately after extraction, the teeth were placed in sterile phosphate buffered saline (PBS) and transported in ice to the laboratory for processing.

Human dental pulp stem cell (hDPSC) processing and culture

To obtain the dental pulp, the teeth were disinfected with sodium hypochlorite followed by several rinses in sterile phosphate buffered saline (PBS). A high-speed rotary instrument with a sterile Zekrya bur was used to cut them at the cementoenamel junction under constant manual irrigation with sterile PBS. The crown portion was removed manually once the pulp chamber had been approached in order to avoid damaging the tissue. The tissue was placed in DMEM culture medium supplemented with 100U/mL penicillin, 100 µg/mL streptomycin and 2.5 µg/mL amphotericin. Then the tissue was enzymatically dissociated in a solution of 3mg/mL collagenase and 4mg/mL dispase. Cell number and viability were checked in a Neubauer chamber using trypan blue exclusion test. After obtaining an average $1x10^7$ cells, they were subjected to magnetic separation using Miltenyi MiniMACS system and CD105+ antibody conjugated magnetic microbeads, following the manufacturer's instructions. The cell fraction that passed through the column was cultured for use as negative control in flow cytometry tests. The eluted fraction was the enriched fraction of CD105+ hDPSCs. The CD105+ hDPSCs were cultured in 24-

well dishes according to experimental design (Table 1), and kept in NH CFU-F medium (Miltenyi Biotec) at 37°C and 5% CO_2 until 70% cell confluence was achieved.

HDPSC cryopreservation

Once cell confluence was obtained, the hDPSCs were cryopreserved (2.5x10^6 cells) for 1, 7 and 30 days using Method 1, reported by Papaccio et al.[12]: 10% DMSO + 90% fetal bovine serum (FBS), and Method 2, adapted from Kamath *et al.*[13]: 10% DMSO + 70% FBS + 20% NH Stem cells Medium.

Evaluation of hDPSC viability and phenotype after cryopreservation

To determine the efficiency of the cryopreservation protocols, following the freezing times, the cells were thawed in a water bath at 37°C, followed by centrifugation at 2000 rpm. Cell viability was evaluated using flow cytometry with 7-AAD (7 Aminoactinomycin D), a stain which is excluded by viable cells and has high affinity for DNA in dead cells, and phenotype markers expression CD105+/CD73+/CD34-/CD45- in the FACS CANTO II flow cytometer (Becton & Dickinson). These markers are used to characterize mesenchymal stem cells according to the consensus of the International Society for Cellular Therapy (ISCT)[14]. When the culture reached 90% confluence after cryopreservation, the hDPSCs were rinsed in phosphate buffered saline (PBS), trypsin was added at a concentration of 0.25%, and they were centrifuged at 1,500g for 4 minutes, after which the cells stained with trypan blue were counted in inverted microscope with Neubauer chamber. Four Eppendorf tubes were used for the experiment: (1) contained 2.5 x 10^5 unmarked cells, (2) was isotope control containing CD105 marked with phycoerythrin (PE) conjugated with IgG1 (MACS Miltenyi Biotec) and CD45 marked with fluorescein isothiocyanate (FITC) marked with IgG2 (MACS Miltenyi Biotec), (3) contained 2.5 x 10^5 cells with CD105 antibodies marked with PE conjugated with IgG1 (MACS Miltenyi Biotec), CD34 marked with FITC conjugated with IgG2a (MACS Miltenyi Biotec), CD45 marked with peridinin chlorophyll protein (PERCP) conjugated with IgG2a (MACS Miltenyi Biotec); and (4) contained 2.5 x 10^5 cells with the CD73 antibody marked with (PE) conjugated with IgG1 (MACS Miltenyi Biotec) and 7-AAD. They were all incubated for 2 hours before reading in the flow cytometer.

Statistical Analysis

The T-test was used for parametric data and U-Mann Whitney test for non-parametric data. A value of $p \leq 0.05$ was considered statistically significant.

RESULTS

A heterogeneous hDPSCs cell population was obtained from extracted human dental pulp subjected to enzymatic dissociation. The heterogeneous cell suspension was then subjected to magnetic separation using Miltenyi MINIMACS technology to isolate hDPSC CD105+ cells. Under inverted microscope, the cells obtained in fraction CD105+ (hDPSCs) were fibroblast-like, non-refringent and had well-defined spherical nuclei (Fig. 1A). Fibroblast colony-forming units (CFU-F) typical of mesenchymal stem cells were also observed (Fig. 1B). The cells obtained in fraction CD105- were fusiform, fibroblast-like, with cytoplasmic prolongations. The nucleus/cytoplasm ratio was 1/3, suggesting that they may be fibroblasts (Fig. 1C).

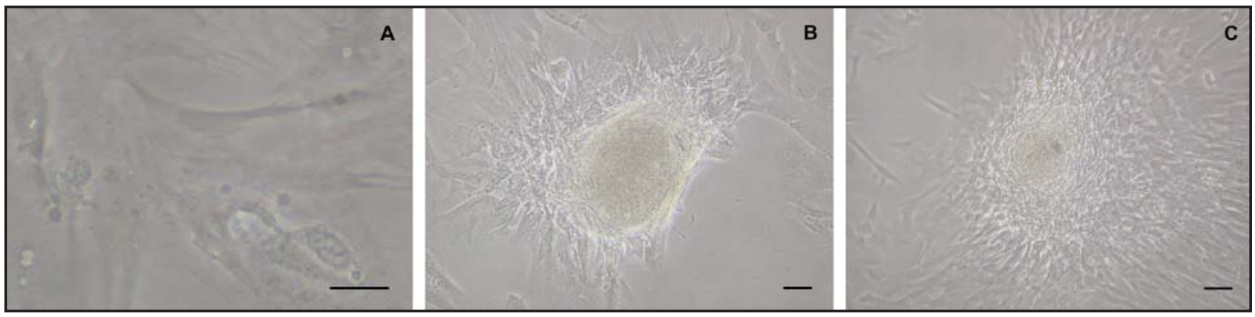

Fig. 1: In vitro morphology of cells obtained by magnetic separation using the Miltenyi system. A. Pulp fibroblasts of the CD 105- fraction (20x). B. Human dental pulp stem cells hDPSC CD105+ (40x). C. hDPSC CD 105+ colony forming unit (20x). Bar 20 μm.

Comparison of the number of viable cells before and after cryopreservation showed that in spite of the fact that viability declines with freezing time, the Kamath method provided better results for number of viable cells (Table 1).

The viability of hDPSC cells after cryopreservation was evaluated by flow cytometry using the fluorescent 7AAD stain. Unstained cells (7AAD-) are viable and stained cells (7AAD+) are dead. For cells that were cryopreserved for 30 days, the Papaccio method showed greater percentage of viability (59.5%) than the Kamath method (56.2%). Nevertheless, the Kamath method provided better viability results for cells cryopreserved for 1 and 7 days (65.5% and 56%, respectively) (Fig. 2).

With regard to immunophenotyping by flow cytometry, for the Papaccio method we observed that cells show greater expression of the markers CD105+/CD34- after 1 and 7 days of freezing (99.9%) than after 30 days (89%). The values for the Kamath method (95.8%, 97.8% and 94.5% after 1, 7 and 30 days, respectively) differed significantly ($p = 0.05$; Fig.3). There was greater expression of CD105+/CD45- with the Papaccio method for all 3 cryopreservation times (95.4%, 96% and 93.2%, respectively) than with the Kamath method (93.5%, 94.2% and 81.3%, respectively). The differences were significant at $p = 0.05$ (Fig.3). Moreover, post-cryopreservation hDPSCs did not express the markers CD34-/CD45-, suggesting that they are not hematopoietic stem cells.

The CD73 marker was found to have less expression after 1 day with both cryopreservation methods, with 84% and 79.6%, respectively. At 7 days they were 97.6% and 94.8% respectively, and at 30 days, expression declined to 72.8% with the Papaccio method, in contrast to 94.8% with the Kamath method ($p= 0.05$; Fig.3).

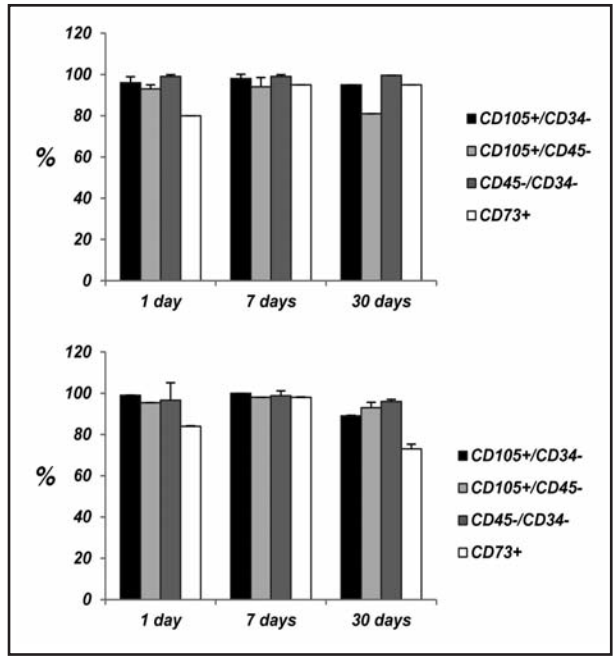

Fig. 2: Percentage of post-cryopreservation hDPSC which express the phenotypes CD105+/CD34-, CD105+/CD45-, CD45-/CD34- and CD73+. A. Kamath cryopreservation method, B. Papaccio cryopreservation method (p= 0.005).

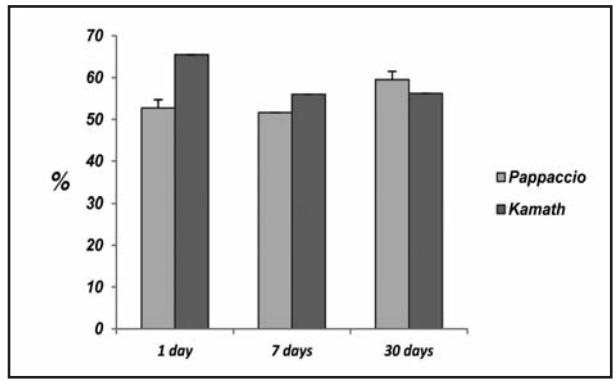

Fig. 3: Percentage of viable post-cryopreservation hDPSC using 7AAD dye-exclusion stain, for the Papaccio and Kamath methods.

Table 1: Cell counting ofhDPSCwithout cryopreservation and following the postcryopreservation methods (n=18).

	Kamath Method		Papaccio Method	
Cryopreservation time	**Average ± SDM without cryopreservation**	**Average ± SDM with cryopreservation**	**Average ± SDM without cryopreservation**	**Average ± SDM with cryopreservation**
1 day	266500 ± 143411	165417 ± 126709	207500 ± 38891	80000 ± 0
7 days	305833 ± 155168	221417 ± 96981	165000 ± 21213	75000 ± 35355
30 days	378000 ± 211644	3144 ± 1596	963750 ± 1253347	381 ± 539

Data are expressed as means +/-standard deviation

DISCUSSION

Freezing mesenchymal stem cells in stable state is an essential requisite for their application in tissue engineering and regenerative medical applications. The standardization of cryopreservation protocols for these cells has enabled the creation of banks to make them available for future applications in transplants or therapies required to ensure patient survival.

The purpose of cryopreservation is to reduce cell metabolic activity for long periods of time while at the same time preserving viability, phenotype and differentiation potential[9]. However, it may induce alterations in chemical, thermal and electrical properties of cell membranes, organelles and molecular structures involved in cell-cell/cell-extracellular matrix interactions.

In this study, mesenchymal stem cells were obtained from human dental pulp from permanent teeth. It was evaluated whether they preserve their characteristic phenotype and viability after cryopreservation in liquid nitrogen for 1, 7 and 30 days, comparing the Papaccio method[12] (10% DMSO and 90% FBS) and the commercial Kamath method[13] (10% DMSO, 70% FBS and 20% culture medium).

Ideal cryopreservation and subsequent recovery of viable cells depend on various parameters: (1) the processing time and storage temperature after isolation; (2) the sampling protocol; (3) the cryopreservant selected; (4) the freezing and thawing protocols and (5) the long-term storage temperatures[8].

In this study, the pulp tissue samples were transported at 4°C in DMEM culture medium supplemented with 100U/mL penicillin / 100 µg /mL streptomycin and 2.5 µg/mL amphotericin to eliminate any contaminant proceeding from the oral cavity[14]. The pulp tissue was processed for cell culture within 3 hours after extraction. In a previous study, Temmerman*y* et al. stored the tissue after extraction for 24h at 4°C in culture medium supplemented with antibiotics[12], which might have reduced the recovery of viable tissue cells[15].

The hDPSCs were obtained from dental pulp by enzymatic dissociation with collagenase/dispase for 2 hours with constant stirring at 37ºC, as reported by Woods et al.[9] It has been suggested that this procedure may compromise the integrity of cell membranes[8]. Although explant culture is more convenient than enzymatic digestion, the cells migrate slowly from the tissue fragments until they attain confluence after approximately 2-3 weeks[16]. Moreover, enzymatic digestion enables CFU-F colony-forming units to be obtained after 1 - 2 weeks in culture, making it the most efficient method[17,18].

Gronthos et al. and Batouli et al. used the enzymatic digestion method and showed that hDPSCs differentiate into odontoblast-like cells which form the dentin matrix *in vivo,* suggesting that this method does not alter the differentiation potential of stem cells[18,19]. In contrast, it was shown that with the explant culture method, cells are potentially able to differentiate into odontoblasts and form mineralized nodules *in vitro*[20-23]. To conclude, enzymatic digestion is an efficient method for isolating hDPSCs which meet the typical criteria for postnatal somatic stem cells[24], such as high proliferation rate, clonogenic nature and co-expression of specific markers[18]. In our study, enzymatic digestion and subsequent magnetic separation did not affect cell confluence, which was achieved at 2 weeks in culture, compared to the study by Temmerman et al.,[15] where confluence was achieved at 23 days. In addition, colony-forming units (CFU-F) were observed, as reported by Polisetty et al.[25]

Cryopreservation is the process of cooling and storing cells, tissues or organs at temperatures beneath -80°C, and usually as cold as −196°C, to maintain their viability[26]. The cooling process involves complex phenomena of water crystallization and changes in intracellular and extracellular solute concentrations, which may be harmful to cell survival. In addition, it has been reported that cell exposure to low temperatures induces stress leading to cell death[26 - 28].

The main steps for the cryopreservation of most cell types are usually: (1) isolating cells, (2) adding cryopreservant, (3) inducing ice crystals in the cell suspension after a certain cooling rate (-1 to -10ºC/min), (4) long-term storage at cryogenic temperatures (usually in liquid nitrogen), (5) rapid thawing at 37ºC, (6) removal of cryoprotectant by centrifuging, and (7) seeding cells to enable their growth in culture[29, 30].

Cell damage due to cryopreservation may be due to a combination of the following: (1) cryoprotectant toxicity[31,32]; (2) osmotic damage due to exposure

of cryoprotectants to freezing-thawing[29,33]; (3) formation of intracellular ice during cooling[33] and (4) re-crystallization of intracellular ice during warming[34]. Several studies have associated cryopreservation stress factors to the activation of cell death signaling cascades[26].

Conventional techniques for freezing stem cells, which include slow freezing, rapid thawing and vitrification have proved refractory for these cells, which exhibit low survival rates[35].

The low efficiency of stem cells to cryopreservation has been partly attributed to the fact that they need to be in close physical contact with one another within the colony to enable cell-cell signaling[36]. These observations show that this type of cell is highly sensitive to cryopreservation.

Heng et al. suggested for the first time that apoptosis, rather than necrosis, is the primary mechanism for the loss of viability in human embryonic stem cells during freezing/thawing with conventional slow cooling protocols[37]. They showed that most cells (~98%) are viable immediately after thawing (determined by trypan blue exclusion) and that cell viability declines gradually over time in culture at 37ºC[38]. DMSO (Me2SO) has been used as a cryopreservant for hematopoietic stem cells (HCP)[39] and is currently used in conventional protocols. For therapeutic stem cell applications, cryopreservation is performed immediately after isolation[40], using 1–2 M DMSO concentrations in a freezer with a controlled cooling rate of -1 °C / min, storage and/or transportation below -135°C, preferably between -150 and -196°C, and rapid thawing at 37°C[41–45]. The DMSO penetrates the cells and forms hydrogen bonds with the water molecules, blocking the flow of water from the cytoplasm and minimizing dehydration and the formation of intracellular ice during cryopreservation[46-49]. In addition, both the bovine fetal serum and the NH culture medium in the Papaccio and Kamath methods may protect cells from oxygen free radicals which are formed during freezing[47].

In this regard, it has been noted that cryopreservation can in fact compromise hDPSC viability, regardless of the freezing method used. However, it has been observed that the Kamath method provides higher values for cell viability on the first and seventh days (65.5% and 56%, respectively), while the Papaccio method does so at 30 days of freezing (59.5%) (Fig.3). Woods et al.[9] determined that dimethyl sulfoxide at concentrations of 1 to 1.5 M is a better cryoprotectant than ethylene glycol and propylene glycol at the same concentrations. In fact, this concentration is equivalent to the 10% DMSO employed in both cryopreservation methods used in this study.

With regard to the expression of stem cell markers, it was found that the cells preserve their undifferentiated cell phenotype regardless of the freezing method. However, a reduction in the expression of CD73+ was observed after 7 and 30 days with both freezing methods.

The CD73 marker or 5'-ectonucleotidase is considered as a lineage marker for mesenchymal stem cells and is believed to be related to cell adhesion mechanisms, because it has been found co-expressed with integrin α2 type molecules, which has characterized CD73 as a cell adhesion mediator in MSCs. The presence of CD73- cells suggest that they lose their capacity to differentiate into cardiac myocytes, although they do keep their potential to differentiate into adipocytes and osteoblasts *in vitro*[49, 50.]

These results suggest that although hDPSCs lose cell viability after cryopreservation, they keep mesenchymal stem cell marker expression. However, this expression depends on freezing time, probably due to the alteration of the 3-D shape of cell membrane proteins or because cells are compromised at a certain level of differentiation.

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SUBGINGIVALLY APPLIED MINOCYCLINE MICROGRANULES IN SUBJECTS WITH CHRONIC PERIODONTITIS. A RANDOMIZED CLINICAL AND MICROBIOLOGICAL TRIAL

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ABSTRACT

The aim of this study was to evaluate clinical and microbiological effects of subgingival minocycline microgranules when used as an adjunct to scaling and root planing in subjects with Chronic periodontitis.

Twenty-six non-smoker volunteers participated in the study. Four opposite sites, clinically standardized, with bleeding on probing (BOP) and pocket depth (PD) ≥ 6 mm were selected. Baseline BOP, PD and Clinical attachment level (CAL) were measured and microbiological samples were collected from the study sites and analyzed using PCR. Porphyromonas gingivalis (Pg) Tannerella forsythia (Tf), Treponema denticola (Td) and Aggregatibacter actinomycetemcomitans (Aa) were detected. One side of the mouth was randomly allocated to the experimental treatment: scaling and root planing plus minocycline microgranules (Test group= T) and the other side of the mouth to scaling and root planing alone (Control group= C). At days 30 and 90, clinical and microbiological examination was repeated.

After 30 days BOP was reduced to 81% in C and to 12% in T and at day 90 to 58% in C and to 8% in T (p< 0.05). PD was significantly reduced in both groups (C: 4.8mm, T: 4.2mm) favoring T at days 30 and 90 (p< 0.05). CAL reduction at day 30 showed no difference between groups. At day 90, CAL reduction was higher in T (p< 0.05). At days 30 and 90 Pg, Tf, Td and Aa was reduced in both groups. Pg reduction was significantly greater in group T. At day 90 frequency of sites with Td decreased in T and increased in C (p< 0.05).

No adverse effect was observed.

This study showed that minocycline microgranules adjunct to scaling and root planing resulted in grater reduction of BOP and PD, higher CAL gain, increased probability of Pg suppression and retarded recolonization of Td than root instrumentation alone.

Key words: *Periodontitis, Minocycline, Porphyromonas gingivalis, drug delivery systems.*

MICROGRÁNULOS DE MINOCICLINA SUBGINGIVAL EN SUJETOS CON PERIODONTITIS CRÓNICA. ESTUDIO CLÍNICO Y MICROBIOLÓGICO ALEATORIZADO

El objetivo de este estudio fue evaluar el efecto clínico y microbiológico de microgránulos de Minociclina, colocados subgingivalmente como coadyuvante del raspaje y alisado radicular en pacientes con Periodontitis crónica severa.

Participaron 26 sujetos voluntarios con Periodontitis crónica, no fumadores. Se seleccionaron 4 sitios contralaterales con Sangrado al Sondaje (SS) y Profundidad al Sondaje (PS) ≥ 6 mm. Condición Basal (CB): se registró SS, PS y Nivel de Inserción (NI). Se determinó mediante PCR presencia de Porphyromonas gingivalis (Pg), Tannerella forsythia (Tf), Treponema denticola (Td) y Aggregatibacter actinomycetemcomitans (Aa). Un lado de la boca fue aleatoriamente asignado al tratamiento experimental: grupo T, el otro al tratamiento control: grupo C.

Al día 30 y 90 se repitieron los exámenes clínicos y microbiológicos. Día 30: el SS se redujo al 81% en el grupo C y al 12% en el grupo T (p<0,05).Estas diferencias se mantuvieron al día 90 (C: 58%, T: 8%) (p<0.05).Día 30 y 90: hubo disminución de la PS en ambos grupos, siendo significativamente mayor en el grupo T (p<0,05).En ambos grupos hubo disminución significativa del NI (p<0,05), no hubo diferencias entre los grupos al día 30 y sí al día 90. A los 30 y 90 días en ambos grupos se redujo la prevalencia para Pg, Tf ,Td y Aa. A los 30 y 90 días la reducción de sitios con Pg fue mayor en el grupo T (p=0,002). A los 90 día Td disminuyó en el grupo T y aumentó en el grupo C (p=0,023).No se observaron efectos adversos.

Los resultados mostraron que la aplicación subgingival de microgránulos de minociclina adjunta al raspaje y alisado radicular produjo una reducción mayor del SS, la PS y el NI que el raspaje y alisado solo,aumentó la probabilidad de suprimir Pg y retardó la recolonización con Td.

Palabras clave: *Periodontitis, Minociclina, Porphyromonas gingivalis, sistema de aplicación de fármacos.*

INTRODUCTION

The role of certain bacteria residing in the subgingival biofilm, such as P*orphyromonas gingivalis, Tannerella forsythia, Aggregatibacter actinomycetemcomitans* and *Treponema denticola,* in the development and pathogenesis of periodontitis is well established [1-3].

Conventional periodontal mechanical therapy without adjunctive chemotherapy is often sufficient to suppress bacterial pathogens and attain periodontal health [4,5]. Adjunctive local or systemic chemotherapies can be used to try to enhance results or improve outcomes at sites not responsive to conventional therapy [6].

Several studies have indicated the effectiveness of systemic antibiotics adjunctive to scaling and root planing, particularly in terms of pocket depth reduction and attachment level gain, not only in Aggressive periodontitis, but also in advanced chronic periodontitis[7,8]. Inherent to systemic administration of antibiotics are problems such as the risk of increasing bacterial resistance as well as encountering potentially unpleasant side effects and problems with patient compliance.

As an alternative to systemic therapy, local delivery of antibiotics into periodontal pockets has been suggested. Local drug administration can avoid many of the side effects associated with systemic antibiotic therapy by limiting the agent to the periodontal pocket [9,10]. When only few sites in the mouth are affected, local delivery may be especially valuable.

Minocycline HCL is a semi-synthetic tetracycline, effective against *Porphyromonas gingivalis, Prevotella intermedia, Eikenella corrodens, Campylobacter rectus* and *Aggregatibacter actinomycetemcomitans* [11-13]. In addition to its antibacterial properties, it is inhibitory for collagenase, produced by *P. gingivalis*, neutrophils and fibroblasts from periodontal tissues [14]. Like all tetracyclines, minocycline exhibits substantivity, adsorption and subsequent desorption from dentine, while maintaining antimicrobial activity [15].

The first studies on local administration of minocycline used an ***ointment*** **(Periocline® - Cynamid®)**. Satomi et al [16] evaluated the concentration in gingival fluid of this minocycline ointment, and found 1000 ug-ml one hour after subgingival administration, which is 100 times the MIC90 required for periodontal pathogens. Concentrations in gingival fluid over 100 ug- ml were maintained over the first 6 hours. After 72 hr. 3.4 ug / ml of minocycline were found. This concentration exceeds the MIC 90 for *T.denticola, A. actinomycetemcomitans, P.gingivalis, P. intermedia* and *F. nucleatum* [13]. In clinical studies the addition of this ointment in three or four sessions after scaling and root planing, showed benefits in the reduction of deep pockets in a 3-month period [17-19] and in a 15-month period [20]. Microbiologically, Nakagawa et al. [17] found grater reduction of Gram-negative periodontopathic bacteria and van Steenberghe et al. [18,20] reported greater reduction of *P. gingivalis* and *P. intermedia.*

A ***gel*** **(Dentomycin®)** failed to provide additional benefits to the effect of scaling and root planing alone. Graca et al.[21] studied a small number of patients, Kinane & Radvar[22] evaluated residual pockets, McColl et al.[23] applied the gel during the maintenance phase, Timmerman et al.[24] found benefits at 1 and 3 months but not at 18 months, Jain et al.[25] found benefit only in pocket depth reduction at 6 and 9 months.

The third product developed for sustained release of minocycline is a ***dry powder*** **(*microspheres*) (Arestin®).** It was evaluated as an adjunct to scaling and root planing in numerous studies[26-33] for periodontal maintenance[34] and treatment of peri-implant disease[35] with, clinical and microbiological benefits.

The aim of this study was to evaluate the clinical and microbiological effect of ***minocycline microgranules*** applied subgingivally as an adjunct to scaling and root planing, in deep periodontal pockets of patients with Chronic periodontitis.

MATERIALS AND METHODS

Study design

This study was designed as a randomized clinical-microbiological trial, split mouth, single-blind with 3-month follow- up. The protocol was approved by the Ethics Committee of Maimonides University and the study was conducted according to the principles outlined in the Declaration of Helsinski on experimentation involving human subjects. Participants were volunteers and signed informed consent at enrollment.

Subject population

Subjects from the Periodontics Service of the Commercial Workers Union, Quilmes, Buenos

Aires were recruited voluntarily. Subjects were included if they were over 35 years of age, non-smokers, and showed clinical and radiographic evidence of moderate or severe Chronic periodontitis according to the criteria described by Armitage [36], and had at least 2 non- contiguous sites with probing pocket depth ≥ to 6 mm and ≤ to 8mm in each side of the mouth. Subjects were excluded from the study if they required antibiotic pre-medication for the performance of periodontal examination and treatment, suffered from any other systemic diseases (cardiovascular or diabetes), were pregnant or lactating female, had received antibiotic treatment in the previous 3 months, were taking long-term anti-inflammatory drugs, were allergic to minocycline, were not able to provide consent to participate in the study, or did not accept the proposed treatment plan.

Experimental design (Fig.1)

Pre-experimental: A clinical-radiographic examination was carried out to determine the periodontal conditions of the subjects. Thirty-two patients who met the inclusion criteria were enrolled in this study. Subjects went through motivation sessions and received instructions in oral hygiene. Two non-contiguous sites on each side of the mouth, with probing pocket depth ≥ to 6 mm and ≤ to 8mm and bleeding on probing were chosen per subject, excluding teeth with furcation lesions. During this phase full- mouth scaling and root planing was carried out, except for the selected sites.

Treatment: After baseline examination, every two sites per patient were randomly assigned to receive one or the other of the following treatments: control treatment: only scaling and root planning, or experimental: scaling and root planing and subgingival application of minocycline microgranules.

Each microgranule contained 0.35 mg minocycline hydrochloride incorporated to a bio-resorbable polymer (microcrystalline cellulose, sodium croscarmellose, hydroxypropylmethylcellulose phthalate 50, hydroxypropylmethylcellulose, light mineral oil, orange Opaspray). Eight microgranules per site were placed subgingivally, equivalent to 2.8 mg of minocycline. After instrumentation of the experimental sites, the area was isolated with cotton rolls, and a curette moistened with water was used to place the microgranules into each pocket with gentle apical movements (Fig. 2). Patients were instructed not to brush or use any interproximal hygiene implement on the study teeth for 24 hours. The treatment was conducted by one experienced periodontist (C.R.) throughout the study.

Clinical and microbiological examination

At days 30 and 90, clinical examination and microbiological sampling were repeated at the selected sites. After the examination on day 30, professional supragingival prophylaxis was carried out and oral hygiene measures were reinforced. A single trained, calibrated researcher (V.Ch.), to whom the assigned treatment was unknown, examined all the patients clinically and microbiologically.

Clinical examination: Clinical parameters were assessed using a Marquis CP 12(Hu-Friedy) periodontal probe. The following parameters were registered: (I) plaque was recorded by assigning a binary score to each surface (1 for plaque present, 0 for absent) (II) bleeding on probing to the bottom of the pocket, after 15 seconds (BOP): 0 (absent)- 1 (present), (III) probing depth (PD)mm: from the gingival margin to the bottom of the periodontal pocket, (IV) Clinical attachment level (CAL) from the cemento-enamel junction, crown margin or restoration to the bottom of the pocket. Measurements were rounded to the nearest millimeter.

Subgingival sample collection: After the clinical parameters had been recorded, the area was isolated with cotton rolls and supragingival plaque was removed with sterile gauze. Two sterile paper points number 30 or 35 were placed in each site for 15 seconds and immediately placed in separate Eppendorf tubes containing 200 µl of phosphate-buffered sterile saline.

Microbiological procedures: To amplify the bacterial ADN from A*ggregatibacter actinomycetemcomitans* (Aa)*, Tannerella forsythia* (Tf), and *Porphyromonas gingivalis* (Pg), a Multiplex PCR was carried out. Specific forward primers for each bacteria and a conserved reverse primer were used, following Tran & Rudney [37] (Table 1).

PCRs were carried at a final volume of 31µl. The reaction mixture consisted of 18 µl molecular biology quality water, 4 µ sample, 0.5 µl primer *Aa* forward 50 nM, 0.8 µl primer *Pg* forward 50 nM, 1 µl primer *Tf* forward 50nM, 0.5 µl conserved reverse primer 50nM, 0.125µl dNTPs 10 nM, 1 U Taq DNA polymerase (Taq-Free, Inbio-Highway), 5µl Buffer Green 5 X (Promega) and 1 µl Cl_2Mg 25

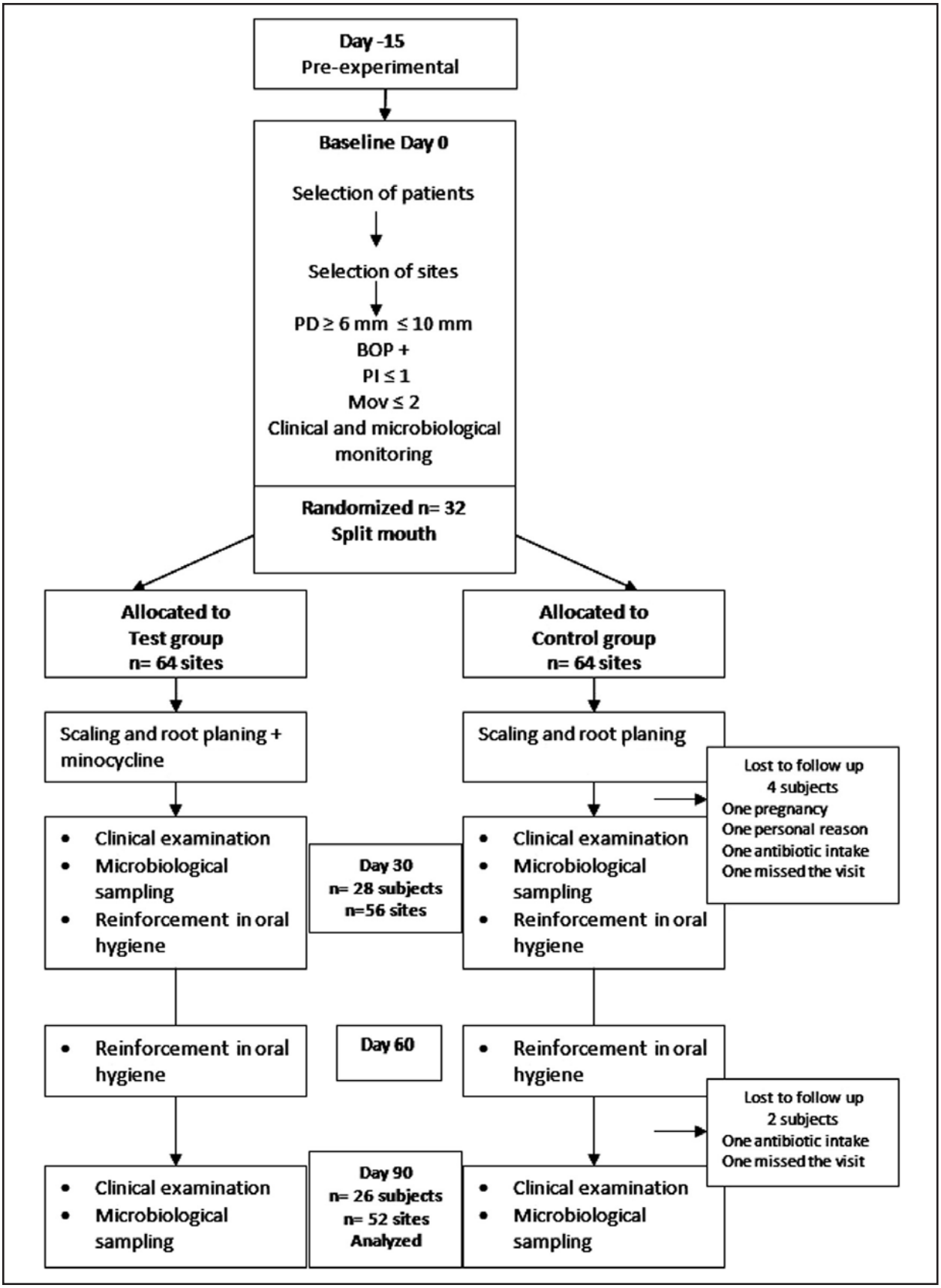

Fig. 1: Study design.

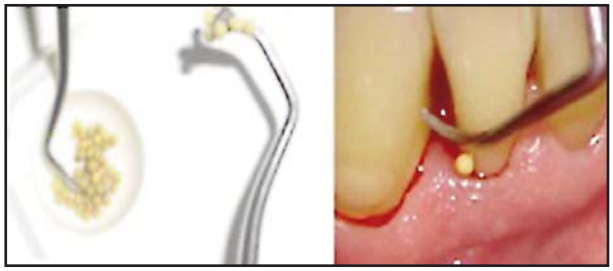

Fig. 2: Clinical application of minocycline microgranules.

mM. The expected product lengths were 360 bp for *Aa*, 745 b for *Tf* and 197 bp for *Pg*.

For detection of *T. denticola (Td)* forward and primer reverse was utilized according to Ashimoto [38], obtaining an amplicon of 300 pb. The reaction mixture, with a final volume of 29 µl, consisted of 18 µ molecular biological quality water, 4 µl sample, 1 µl forward + reverse primer 50 nM, 0.125 µl dNTPs 10 mM, 1 U Taq DNA polymerase (Inbio-Highway),

5 µl reaction Buffer Green 5 X (Promega) and 1 µl Cl_2Mg 25 mM.

The cycling conditions consisted of 35 cycles (1 minute at 94°C, 1 minute at 61°C and 1 minute at 74° C) and an initial denaturation step of 15 minutes at 94°C preceded the amplification cycles. Amplification products were separated in agarose gels (Invitrogen) with red gel coloring (Genbiotech). The gels were observed under light translumination. Amplicones lengths were confirmed by comparing with a molecular weight marker (100pb DNA Ladder, Genbiotech).

Statistical analysis

For the analysis of PD and CAL data, analysis of variance (ANOVA) was applied, through a repeated measures design. PD and CAL data from both sites per subject receiving the same treatment were averaged. The Mauchly test was applied to study the sphericity assumption. The normality of the residuals was studied by the Shapiro-Wilks test. Post hoc pairwise comparisons between groups were carried out through the Tukey test.

The McNemar test was used to compare presence of BOP and of pathogens. The presence of BOP or of the pathogen at one of the two sites on the same side of the mouth (same group), was considered as a positive result. Tests with $p < 0.05$ were considered significant. All statistical analyses were performed using InfoStat software (version 2013, Grupo InfoStat, FCA, Universidad Nacional de Córdoba, Argentina).

RESULTS

Of the 32 subjects initially enrolled, 26 completed the study (11 males, 15 females; age range: 39-56 years; mean: 45.6 years). Eight subjects were lost to follow-up due to reasons unrelated to the study and were not included in the analysis (Fig.2).

Clinical parameters

At initial examination no statistically significant difference was observed among groups for any of the parameters evaluated ($P>0.05$).

Bleeding on probing (Table 2): 100% of the sites of both groups bled on probing at baseline. At day 30 after treatment, this percentage was reduced to 81% in the control group and 12% in the test group. Reductions in the percentage of sites that bled on probing at days 30 and 90 were statistically significant in favor of the test group ($p < 0.05$).

Probing pocket depth (Table 3): Pocket depth showed no differences between the test and control groups at baseline. At day 30 both groups showed statistically significant probing depth reduction when compared to the respective baseline values ($p < 0.05$), however, the test group showed significantly greater improvement than the control group at days 30 and 90 ($p < 0.05$).

Clinical attachment level (Table 4): In both groups a significant reduction ($p<0.05$) was observed at day 30, with no significant difference between groups. At day 90 differences favoring the Test group were found ($p < 0.05$).

Microbiological results (Table 5)

Baseline data showed no difference between groups regarding the presence of the four pathogens studied: more than 70% of the subjects in both groups were positive for *Pg, Tf* and *Td,* detection frequency of *Aa* was less than 40% in both groups.

At 30 and 90 days the percentage of sites positive for Pg was reduced in both groups, with the reduction being significantly greater in the experimental group.

The percentage of positive sites for *Tf, Aa* and *Td* was reduced at day 30 in both the control group and in the experimental group, and no significant differences was found at this time. At day 90, no

Table 1: Primers used for amplification of gene ARNr 16S *A. actinomycetemcomitans*, *P. gingivalis*, *T. forsythia*, *T. denticola*.

Bacteria	Primer	Amplicon
A. actinomycetemcomitans (Aa)	5'-TACAGGGGAATAAAATGAGATACG-3'	360 pb
P. gingivalis (Pg)	5'-ATTGGGGTTTAGCCCTGGTG-3'	197 pb
T. forsythia (Tf)	5'-TACAGGGGAATAAAATGAGAT CG-3'	745 pb
Reverse (Aa, Pg, Tf,)	5'-ACG TCA TCC CCA CCT TCC TC-3'	
T. denticola (Td)	5'-TAA TACCGAATGTGCTACTTTACAT-3'	300 pb
T. denticola (Td)	5´-TCAAAGAAGCATTCCCTCTTCTTCTTA-3'	

Table 2: Bleeding on probing (BOP). Change in the frequency of bleeding sites from day 0 to day 90.

Day 0		Day 30		Day 90	
Control	**Test**	**Control**	**Test**	**Control**	**Test**
26 (100%)	26 (100%)	21 (81%)	3 (12%)	15 (58%)	2 (8%)
p > 0.20		p = 0.0001		p = 0.0001	

BOP was positive when present in at least one of the study sites on the same side (group) of the mouth. n=26 subjects.

Table 3: Probing pocket depth (PD) (mm) at Baseline, 30 and 90 days.

		Day 0		Day 30		Day 90	
Group	**n**	**Mean**	**S.E.**	**Mean**	**S.E.**	**Mean**	**S.E.**
Control	26	6.3^{A}	0.08	4.8^{B}	0.22	4.7^{B}	0.24
Test	26	6.6^{A}	0.10	4.2^{C}	0.19	3.8^{C}	0.21

S.E: Standard error. Different letters indicate significant difference between groups ($p < 0.05$)

Table 4: Clinical attachment level (CAL) (mm) at Baseline, 30 and 90 days.

		Day 0		Day 30		Day 90	
Group	**n**	**Mean**	**S.E.**	**Mean**	**S.E.**	**Mean**	**S.E.**
Control	26	6.1^{A}	0.65	5.4^{B}	0.89	5.1^{B}	0.96
Test	26	6.5^{A}	0.78	5.1^{BC}	1.14	4.8^{C}	1.12

S.E.: Standard error Different letters indicate significant differences ($p < 0.05$)

Table 5: Number and percentage of positive sites for the pathogens studied at 0, 30 and 90 days.

	Day 0			Day 30			Day 90		
Pathogen	**Control**	**Test**	**p**	**Control**	**Test**	**p**	**Control**	**Test**	**p**
Pg	26 (100%)	26 (100%)	>0.20	16 (62%)	3 (12%)	0.002	13 (50%)	1 (4%)	0.002
Aa	8 (31%)	10 (38%)	>0.20	6 (23%)	7 (27%)	>0.20	3 (12%)	5 (19%)	>0.20
Tf	19 (73%)	20 (77%)	>0.20	1 (4%)	2 (8%)	>0.20	1 (4%)	0 (0%)	>0.20
Td	24 (92%)	25 (96%)	>0.20	11 (42%)	11 (42%)	>0.20	17 (65%)	8 (31%)	0.023

Pathogen presence was positive when present in at least one of the two study sites on the same side (group) of the mouth.
P. gingivalis (Pg), T forsythia (Tf), A actinomycetemcomitans (Aa), T denticola (Td).

differences was found for *Tf* and *Aa* however, the percentage of *Td* positive sites decreased in the Test group between day 30 and day 90 and increased in the control group,thus the difference between groups was significant .

Percentage of sites with clinically and microbiologically relevant changes (Table 6)

Both treatments caused a significant reduction in deep pockets, increasing the number of shallow sites. The percentage of pockets that were reduced after treatment from ≥ 6 mm to ≤ 4 mm was significantly greater in the Test group (65.4 % vs 46.2 %), and the number of remaining deep sites (≥ 5mm) was greater in the Control group (53.8% vs 34.6%). Residual pockets (≥ 5 mm) in the Control group showed bleeding (19/28) more frequently than in the test group (1/18). *P. gingivalis* was found in residual pockets of the Control group (15/28) and not detected in the Test group (0/18).

DISCUSSION

The development of local controlled release systems has allowed the use of low dosage of antimicrobials in localized sites of periodontal destruction, reaching high concentrations, and reducing the risk of adverse effects and possible bacterial resistance[9].

In periodontics, minocycline is more effective for local, rather than systemic use. Even though periodontal pathogens are sensitive to minocycline in vitro, when it was orally administered, the concentration in gingival fluid did not show predictable concentrations [39].

Numerous studies concluded that local administration of 2% minocycline hydrochloride ointment or 1 mg minocycline microspheres in deep periodontal pockets provides clinical and microbiological benefits improving the effect of scaling and planing alone [40,41].

Our findings showed that minocycline microgranules applied along with scaling and root planing, at sites with probing pocket depth between 6 and 8 mm, in non-smoker patients with Chronic periodontitis, produced greater reduction in probing depth, and percentage of bleeding sites, greater attachment gain and greater reduction of *Porphyromonas gingivalis* .

Evidence shows that a favorable clinical response after scaling and root planing is associated with reduction in the species belonging to the red complex: *Pg, Tf* and *Td* [5]. In agreement with these findings, in our study, both groups showed a reduction in the red complex bacteria, with the expected clinical benefit. The differences in the clinical response favoring the test group, considering that *Td* and *Tf* reduction was similar in both groups can be explained because Pg was suppressed at a larger percentage of sites.

This clinical and microbiological correlation can better be understood by analyzing the data according to the percentage of closed pockets post treatment, 66% in the test vs 36.5 % in the control group, difference that was maintained at day 90 in accordance with the sustained absence of *Pg.* This beneficial ecological conditions achieved in

Table 6: Number /Percentage of sites with relevant clinical and microbiological changes at 30 and 90 days: Pockets changing from ≥ 6 mm at baseline to ≤ 4 mm after treatment and residual pockets ≥ 5 mm.

	Day 30			Day 90		
	Control	Test	p	Control	Test	p
n of initial sites ≥ PD 6 mm	52	52		52	52	
n (%) of sites changing from ≥ 6 mm at baseline to ≤ 4 mm after treatment *	19 (36.5%)	32 (66%)	<0.05	24 (46.2%)	34 (65.4%)	< 0.05
*Pg positive sites	4/19	1/32		2/24	1/34	
Percentage residual pockets ≥ 5mm ∞	33 (63.5%)	20 (34%)	< 0.05	28 (53.8%)	18 (34.6%)	< 0.05
∞ BOP positive sites	23/33	2/20		19/28	1/18	
∞ Pg positive sites	18/33	3/20		15/28	0/18	

the minocycline group at the immediate post-treatment, can probably explain the observed delay in recolonization of *Td,* compared with the rebound in the Control groups.

It is difficult to compare our results with other studies, because besides the difference in the pharmaceutical form of the drug there are also differences in the concentration, frequency of application and in the study periods.

Other products containing minocycline applied lower doses: Periocline® (2% ointment) and Dentomycin® (2% gel) provide 0.5 to 1 mg of minocycline per site (25 to 50 mg of ointment or gel is placed in each pocket, of the product that contains 10 mg of minocycline in 500 mg of vehicle). Arestin® cartridge (microespheres) contains 1mg of minocycline and 3 mg of powder vehicle which is utilized to fill only one pocket. In our study 8 microgranules were placed per site, equivalent to 2.5 mg-3 mg per pocket.

Despite this variability, our microbiological results in relation to Pg suppression, are consistent with the results of other studies using Arestin® [26,30] or Periocline® [18,20]. The clinical results are also consistent, many studies report a larger number of sites with probing depth ≤ 4 mm and absence of bleeding after adjunctive minocycline treatment [17-20,26-33].

No adverse side effect with local minocycline is described in the literature, and no adverse side effect has been found with this alternative form of application.

Our study did not evaluate smokers, but it is worth noting that other authors have found clinical and microbiological benefit with minocycline (Arestin®) in smokers [31].

Benefit achieved with systemic or local antimicrobials adjunct to scaling and root planing has been reported in the literature for deep pockets[42]. Upon considering the clinical relevanceof our results, it should be taken into account that the sites evaluated had pockets between 6 and 8mm at baseline.

This study proposes ***microgranules*** of minocycline HCL, a new method for subgingival administration of Minocycline. These are 0.8 mm diameter spheres, each containing 0.35 mg of the drug incorporated to microcrystalline cellulose. Due to their size, they can be placed individually in the periodontal pocket using a moistened instrument to which they adhere by surface tension.

In view of the results of this study we can conclude that microgranules could be considered an alternative form for local subgingival application of minocycline, as an adjunt to scaling and root planning, in deep periodontal pockets of patients with Chronic periodontitis.

Further studies should be conducted on periodontal maintenance and the treatment of perimplant disease.

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EX VIVO MICROLEAKAGE COMPARISON BETWEEN GLASS IONOMERS USED AS PIT AND FISSURE SEALANTS

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ABSTRACT

The aim of this study was to evaluate in vitro the marginal microleakage of two glass ionomer materials used as pit and fissure sealants. Thirty healthy premolars extracted for orthodontic treatment were randomly assigned to two groups (n=15) and respectively sealed with two glass ionomers (Group I, Fuji VII and Group II, Fuji IX). All teeth were preserved in artificial saliva (NAF) for 10 days, thermocycled (250 cycles; 5ºC, 37ºC and 60ºC), isolated, and immersed in 2% alcohol gentian violet blue solution for 24 h. After washing, teeth were included in acrylic resin and sectioned longitudinally in a bucco-lingual direction with a Struers-Minitom cutting device. Samples were analyzed for leakage using an optical microscope (Olympus BX-60M). TheWilliams and Winter semi-quantitative ranked scale was used to score dye penetration. In Group I the grades were distributed as follows: Grade 1, 1 sample and Grade 3, 14 samples (Mean 2.87 Median 3, SD 0.52). In Group II: Grade 0: 4 samples, Grade 1, 3 samples, Grade 2, 2 samples and Grade 3, 6 samples (Mean 1.67, Median 2, SD 1.29). Fisher's exact test showed statistically significant differences between materials (p=0.006). From these results, we conclude that Fuji IX had better marginal sealing than Fuji VII when used as a pit and fissure sealant.

Key words: *leakage, pit and fissure sealants, glass ionomer.*

COMPARACIÓN *IN VITRO* DE FILTRACIÓN MARGINAL ENTRE IONÓMEROS VÍTREOS SELLADORES

RESUMEN

El objetivo del trabajo fue comparar la eficacia como sellador de dos ionómeros vítreos. Se emplearon 30 premolares sanos extraídos por razones ortodóncicas. Se dividieron en dos grupos iguales (n=15) .Grupo I: Fuji VII y Grupo II: Fuji IX. Los dientes, fueron conservados en saliva artificial (NAF) durante 10 días. Luego, fueron termociclados (250 ciclos) a 5ºC, 37ºC y 60ºC. Posteriormente se sumergieron en violeta de genciana durante 24 hs. Una vez lavadas las muestras se seccionaron y evaluaron con el criterio de Williams y Winter, utilizando un microscopio óptico (Olympus BX-60M) para valorar la penetración del colorante. Los grados obtenidos para cada grupo fueron: en Grupo I (Fuji VII), Grado 1, 1 muestra y Grado 3, 14 muestras (Media 2,87, Mediana 3 y SD 0.52). En el Grupo II (Fuji IX) los grados se distribuyeron así: Grado 0, 4 muestras, Grado 1, 3 muestras, Grado 2, 2 muestras y Grado 3, 6 muestras. (Media 1.67, Mediana 2 y SD 1.29). La Prueba exacta de Fisher (p=0,006) demostró diferencias estadísticamente significativa entre materiales. Se concluyó que el mejor sellado marginal fue obtenido utilizando el material Fuji IX comparado con Fuji VII cuando es utilizado como sellador.

Palabras Clave: *Filtración, Sellador de fisuras, Ionómero vítreo.*

INTRODUCTION

During the last three decades the prevalence of caries has declined worldwide[1]. However, this decline has not been uniform on different tooth surfaces. Pits and fissures in human molars have been recognized as sites that are susceptible to dental caries[2]. Lack of post-eruptive maturation and contact with the antagonist favor the development of carious lesions[3]. We also know that pit and fissure caries account for the majority of carious lesions, 93.4% and 79.8%, respectively, in 12-year old children[4,5]. This high percentage can be attributed to the complex morphology of pits and fissures, which are ideal for retaining bacteria and food debris, making removal extremely difficult [6]. Their extreme vulnerability has prompted researchers to look for methods to prevent these situations [7]. Fig. 1 shows the typical appearance of a tooth surface.

Methods for preventing dental caries should pay particular attention to surfaces with pits and fissures, which have always been the first to be affected. Occlusal caries are more prevalent in

children as a result of the morphology of surfaces with pits and fissures, which are vulnerable areas where plaque that is formed is anatomically protected from toothbrush filaments due to the size of the cracks. It seems likely that the period most susceptible to caries of a first permanent molar is the long eruptive phase. The immature enamel and the ignorance on the part of the child and the parents of the presence of tooth eruption further hinder good dental hygiene in this area [8].

Two materials which have different properties are currently used to seal pits and fissures: resins and glass ionomer cements. While resin sealants can prevent seepage of nutrients from the oral cavity into the cracks, glass ionomer cements inhibit caries by releasing fluoride ions [9,10]. During the eruptive period of the first permanent molar, absolute isolation of the operative field is impossible, preventing the sealing of pits and fissures with resin sealants.

Fissure sealing with glass ionomer cements was introduced by McLean and Wilson in 1974 [11]. Glass ionomer cements can adhere chemically to the tooth structure, are less hydrophobic than resinous sealants, and release fluoride ions, providing a valid alternative in situations where there is high probability of contamination during application of the sealant [12]. Despite the high loss of ionomeric macroscopic sealants, they have a caries preventive effect because the material remaining at the bottom of the pits and fissures can act as a slow release depot of rechargeable fluoride ions [13-17]. Conventional glass ionomers are difficult to handle and have low resistance to wear and fracture. The high density glass ionomer developed for the atraumatic restorative technique (Fuji IX, GC and Ketac Molar, 3M ESPE) has greatly improved physical properties [18].

The recently introduced Fuji VII glass ionomer (GC, Tokyo, Japan) offers the ideal properties for a pit and fissure sealant [7]. This pink glass ionomer allows quick identification by dentist and patient, and the presence of this pigment absorbs light energy. Setting time can be accelerated and mixing time is reduced by using light bulb radiation of appropriate wavelength and intensity. Moreover, its rate of fluoride release is more than 6 times higher than in Fuji IX [19].

The aim of this study was to evaluate microleakage by the degree of penetration of dye into the pits and fissures of premolars extracted for orthodontic reasons and sealed with Fuji VII and Fuji IX.

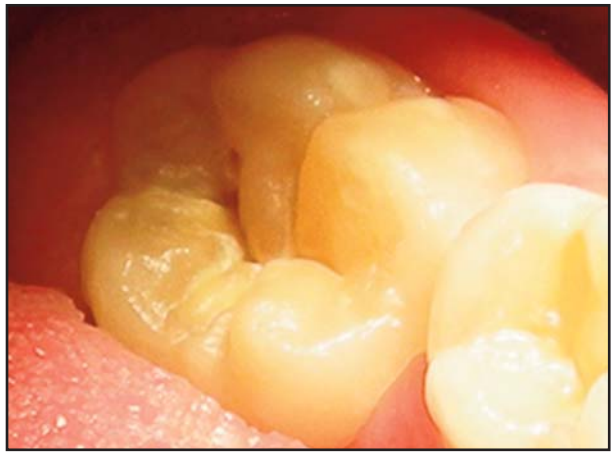

Fig. 1: Clinical Occlusal view of mandibular first permanent molar.

MATERIALS AND METHODS

Thirty first and second, upper and lower healthy premolars extracted for orthodontic reasons were selected to be used as samples.They were divided into two equal groups (n =15) as follows:

Group 1: sealed using the ionomer Fuji VII (GC, Tokyo, Japan).

Group 2: sealed using the ionomer Fuji IX (GC, Tokyo, Japan).

Sample Preparation

The thirty premolars were cleaned with a prophylaxis brush to remove any remaining plaque biofilm or residual stains. Then they were washed with water with a triple air/water syringe from the dental kit and kept in artificial saliva at room temperature for 15 days (Naf Oral Solution, NAF Laboratories, Buenos Aires, Argentina).

Sealing of samples

The material was applied following the manufacturer's instructions. The steps were:

1. Remnants of the occlusal surfaces were removed with water and a prophylaxis brush attached to a low-speed handpiece.
2. Occlusal surfaces were washed with water and dried gently.
3. The occlusal surfaces of the premolars of both groups were treated with 10% polyacrylic acid for 30 seconds and then washed with water and dried with cotton rolls to prevent desiccation of the surfaces.

4. Materials were prepared according to manufacturer's instructions.
5. The capsule material was placed in a syringe provided with the sealant kit.
6. The teeth were sealed with Fuji VII or Fuji IX by keeping the syringe tip at the end of the fissure until the completion of the sealant application to prevent air bubble formation.
7. Fuji VII was polymerized with a light lamp (Coltolux® 75, Coltene, USA) for 20 seconds. For teeth sealed with Fuji IX, the initial setting was indicated by loss of gloss of the material.
8. Sealing surfaces were then protected with petroleum jelly to prevent drying of the material in its initial curing periods.

Thermocycling

Thermocycling was performed to simulate the oral cavity environment at 5 °C, 37 °C and 60 °C for 250 cycles with a period of 30 seconds in between. The low temperature cycle was obtained with ice and water in a glass beaker. Higher temperatures were obtained with water in a container at controlled temperatures. Then teeth were stored in artificial saliva for 15 days, ensuring that they were completely immersed.

Dye penetration and acrylic inclusion of teeth

Samples were removed from the artificial saliva and immersed in a 2% alcohol gentian violet solution for 24 hours [7]. Teeth were then washed in running water to remove excess dye.

Then the root portion of the teeth was removed. The crown portion of each premolar was included in self-curing acrylic to create a contact surface between the tooth and the cutting machine. Premolars were sectioned with a Struers- Minitom electric cutting machine with diamond-coated disks. The cut was performed in a bucco-lingual direction.

Table 1: Dye Penetration in premolars sealed with Fuji VII and IX. *Significant difference between two groups (p=0.006)*

	Fuji VII	Fuji IX
Grade 0 (without penetration)	0	4
Grade 1 (1/3 penetration)	1	3
Grade 2 (2/3 penetration)	0	2
Grade 3 (more than 2/3 penetration)	14	6
Total	15	15

Polishing of samples

Surfaces of interest were prepared for viewing under a microscope using a grinding and polishing protocol. Grinding was performed with a sequence of grain size 500, 1000 and 2000 sandpaper using a metallographic polishing machine Prazis PUL-01 model with running water cooling. Polishing was performed using the same polishing machine with polishing cloths and a series of 3 and 0.25 micrometer diamond pastes (Prazis), respectively.

Microscope observation

Once polished, samples were cleaned with running water and dried with air (dryer at room temperature). The samples were then placed under an optical reflection microscope (Olympus BX- 60M, USA) to evaluate the extent of penetration of the dye in the material-tooth interface.

For evaluation, the Williams and Winter criterion [20] of Grades was applied:

Grade 0: no dye penetration.

Grade 1: penetration extending 1/3 of the total length of the interface between the sealant and the dental structure (depth of groove).

Grade 2: penetration extending between 1/3 and 2/3 of the total length of the interface.

Grade 3: penetration extending beyond 2/3 of the total length of the interface.

RESULTS

Evaluation of Marginal Sealing

Table 1 shows the results obtained. The number of samples with each grade is shown for each group. Two different degrees of dye penetration are shown as examples in Fig. 2, sealed with Fuji VII and Fuji IX respectively. Fig. 3 show details of Fuji IX adhesion to enamel surface.

The complete results were analyzed with Fisher's exact test (p=0.006) and showed statistically significant differences between the two materials, indicating that Group 2 (Fuji IX) provided better marginal sealing that Group 1 (Fuji VII).

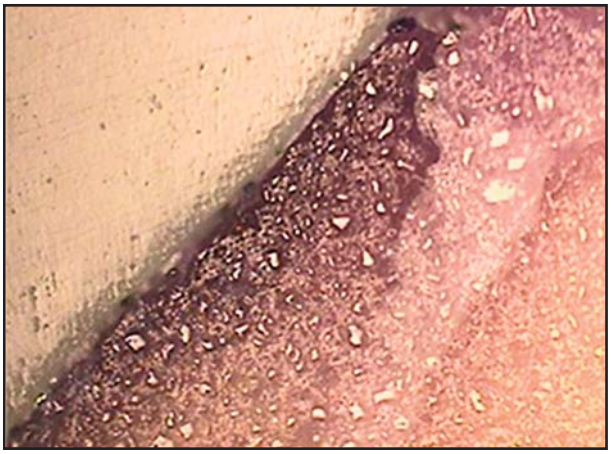

Fig. 2: Microphotography dye penetration: a: Fuji VII: Grade 3. b: Fuji IX: Grade 0. Light microscopy, Magnification 12.5x.

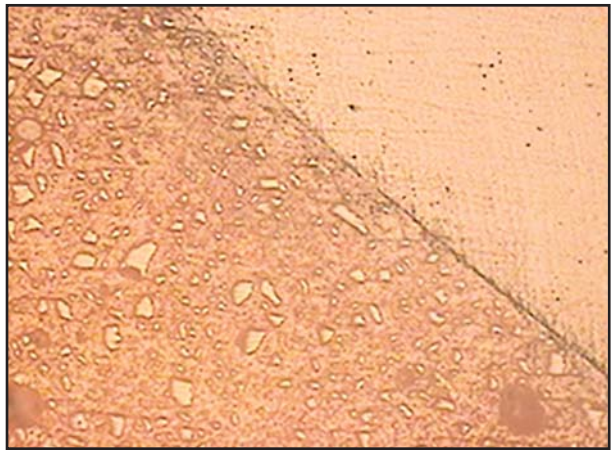

Fig. 3: Microphotography showing good adhesion with Fuji IX to adamantine wall (right side). Arrows shows interface. Light microscopy, Magnification 125x.

DISCUSSION

The prevalence of caries in pits and fissures emphasizes the importance of sealants in preventing caries [21].

The effectiveness of sealants lies in their ability to isolate pits and fissures from bacteria, nutrients and metabolic product acids[22]. Sealants are the best preventive measure against caries, pits and fissures, especially in those patients at high cariogenic risk such as children with incisive molar hypomineralization[23-25].

The clinical effectiveness of pit and fissure sealants is directly related to their retention, which depends on pit and fissure morphology, proper insulation, characteristics of the sealant material and application techniques. Furthermore, sealant retention can be improved by cleaning the occlusal surface before insertion, using prophylaxis pastes, air abrasion and mechanical preparation of fissures, known as invasive techniques. Another factor in the success of the sealer is marginal integrity, which can be evaluated by measuring microleakage. This can be defined by the entry of bacteria and oral fluids into the space between the tooth and the restorative material [26].

The ability of a sealant to prevent microleakage is an important parameter, since a carious process can be initiated and sustained under the sealant [27].

The advent of new directions for the use of glass ionomers such as pit and fissure sealants has made it necessary to re-examine their physical characteristics and application techniques. *In vitro* studies have allowed us to determine marginal leakage and predict the marginal sealing ability of different materials used as pit and fissure sealants [26].

In this study, *in vitro* microleakage was assessed by measuring dye penetration between the sealant and the tooth structure following the criteria of Williams and Winter. The results, analyzed with Fisher's exact test (p=0.006), showed statistically significant differences between the two materials, indicating that Fuji IX I provided better marginal sealing than Fuji VII.

With respect to marginal sealing ability, there are numerous works in the literature studied that analyze the clinical and *in vitro*behavior of Fuji VII compared to different resinous sealants (i.e. Concise) [7], with the conclusion that the latter has more satisfactory behavior. The authors based their findings on the pattern of tooth-sealant bond and on the poor resistance to marginal leakage of ionomers. However, other authors report different results, concluding that there is no difference in microleakage (p> 0.05) when comparing Fuji VII and Concise resin sealant (3M) [27]. Traditionally, glass ionomer cements are not used as pit and fissure sealants because of the risk of microleakage. However, the increased resistance to microleakage of glass ionomer cement Fuji VII can be explained on the basis of enamel conditioning prior to chemical adhesion to enamel structure and absence of polymerization shrinkage [27]. The results of our study agree with those reported by these authors in relation to marginal sealing ability, although they indicate that Fuji IX was better than Fuji VII.

In 2011, Singla[26] conducted an *in vitro* comparison of microleakage between Fuji II LC and Dyract Flow with fluoride release using non-invasive and invasive application techniques. Fuji II LC is a resin-modified glass ionomer and Dyract Flow is a fluid compomer. More microleakage was found in the glass ionomer. This can be attributed to different reasons but the most acceptable one is the solubility of the glass ionomer in the oral environment. It should be noted that the glass ionomer is hydrophilic and tends to absorb dye, thus giving false positive results. The use of the dye in *in vitro* studies simulates bacteria and their products. The leakage of the dye into the ionomer structure would not be important since, when simulating bacterial biofilm, it would be neutralized by the effect of the fluorine contained in the material [26] and in this study we did not consider dye penetration into the structure of the materials employed. However, in this study, potential false positives would cancel each other out since we compare the same materials with similar hydrophilicity values that neutralize this limitation.

CONCLUSIONS

Under the experimental conditions studied Fuji IX showed better marginal sealing than Fuji VII.

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CHANGES IN pH OF IRRIGATING SOLUTIONS AFTER CONTACT WITH HUMAN ROOT DENTIN

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ABSTRACT

The aim of this study was to analyze the in vitro behavior of the pH of different irrigating solutions, used alone or consecutively, after contact with extracted human teeth. Mandibular human premolars were selected. The middle thirds were divided into 6 parts. The specimens obtained were divided into 6 groups and treated with irrigating solutions: 1) distilled water; 2) 1% NaOCl; 3) 1% Citric Acid (CA); 4) 17% EDTA; 5) 1% CA + 1% NaOCl; 6) 17% EDTA + 1% NaOCl. Specimens were immersed in 1 mL of each solution at 37ºC, those of groups 1, 2, 3 and 4, for 5 minutes, and the rest, consecutively for 2.5 minutes in each solution. Initial and final pH of the solutions were determined. Data were analyzed by the T Test, one-way analysis of variance (ANOVA) and Tukey multiple comparison Test. At 2.5 and 5 minutes there were significant differences between the initial and final pH for all solutions. The pH values decreased for distilled water and NaOCl, while they increased for CA and EDTA. In vitro, the pH of all solutions was modified after contact with root dentin at both test times (2.5 and 5 min).

Key words: *pH, irrigating solutions, dentin.*

VARIACIONES DEL pH DE SOLUCIONES DE IRRIGACIÓN ENDODÓNTICAS EN CONTACTO CON DENTINA RADICULAR HUMANA

RESUMEN

El objetivo de este trabajo fue estudiar in vitro el comportamiento del pH de diferentes soluciones de irrigación endodónticas, usadas solas o en forma consecutiva, después del contacto con dientes humanos extraídos. Se seleccionaron premolares inferiores. El tercio medio radicular se dividió en 6 partes. Los especímenes obtenidos se dividieron en 6 grupos, de acuerdo a la solución de irrigación empleada: 1) agua destilada; 2) NaClO 1%; 3) Ácido Cítrico 1% (AC); 4) EDTA 17%; 5) AC 1% + NaClO 1% ; 6) EDTA 17% + NaClO 1%. Los especímenes fueron sumergidos en 1 mL de cada solución a 37°C. Aquellos del grupo 1, 2 y 3 durante 5 minutos, y el resto, consecutivamente 2,5 minutos. Se determinaron pH inicial y final para cada solución. Los datos fueron analizados utilizando Test T, ANOVA y Test de comparaciones múltiples de Tukey. A los 2,5 y 5 minutos de exposición hubo diferencias estadísticamente significativas entre el pH inicial y final en todas las soluciones. El pH disminuyó en el caso de agua destilada e NaClO, mientras que aumentó en AC y EDTA. In vitro, el pH de todas las soluciones se modificó después del contacto con dentina radicular humana en ambos períodos de tiempo (2,5 y 5 minutos).

Palabras clave: *pH - irrigación - dentina.*

INTRODUCTION

Endodontic instrumentation produces a smear layer and plugs of organic and inorganic particles of calcified tissue and organic elements such as pulp tissue debris, odontoblastic processes, microorganism and blood cells in dentinal tubules[1]. Irrigation is considered the best method for removing tissue remnants and dentin debris during instrumentation[2,3]. Irrigating agents also provide lubrication, destruction of microbes and dissolution of tissues.

The efficiency of irrigating agents depends on root canal length, penetration depth of the substance, application time, dentin hardness, and concentration and pH of the solutions[2,4,5]. Because each solution is most effective at a specific pH, changes in pH value could modify its properties.

It has been suggested that chelating agents improve chemical-mechanical debridement in the root canal treatment by removing the smear layer from the root canal and demineralizing and softening dentin. The most commonly used chelating agents are based on different concentrations of ethylenediaminetetraacetic acid (EDTA) and citric acid (CA)[6,7]. Other non-chelating agents, such as sodium hypochlorite (NaOCl), have also widely been recommended as irrigants.

Initially, the use of EDTA solution was proposed by Ostby (1957) to assist with the instrumentation of calcified, narrow or blocked canals because of its ability to foster the chelation of the calcium ions at a pH close to neutral[8]. Its efficiency in removing inorganic dentin particles, preventing the formation of smear layer during instrumentation has been demonstrated[9-12]. It is used at 15-17% and pH 7-8.

CA, a weak organic acid, has a chelating demineralizing effect on calcified dentin components[13]. It has been previously applied on root surfaces altered by periodontal disease and flap surgery in order to increase cementogenesis and to accelerate healing, regeneration and normal periodontal attachment[14]. In operative dentistry, CA has been proposed as a mild etchant for hard dental tissues, particularly for dentinal conditioning, and enhanced smear layer and plug removal[6]. In endodontic treatments it is used at a concentration of 1%-50% and pH 0.8-1.9.

NaOCl has been widely recommended as an irrigant for chemical-mechanical debridement of root canals due to its solvent activity for necrotic and living tissues, in addition to its ability as an effective agent against broad spectrum bacteria[15-17]. It is used at a concentration of 1%-5.25% and at pH 11.9.

For maximum effect during and after instrumentation, chelating agents should be followed by tissue solvents. Alternating the use of EDTA or CA and NaOCl solutions has gained wide acceptance as an effective irrigation regimen[18-20].

The aim of this study was to evaluate *in vitro* the behavior of the pH of different irrigating solutions, used alone or consecutively, after contact with extracted human teeth.

Fig. 1: Root dentin segments. A: coronal third; B, C and D: middle thirds; E: apical third.

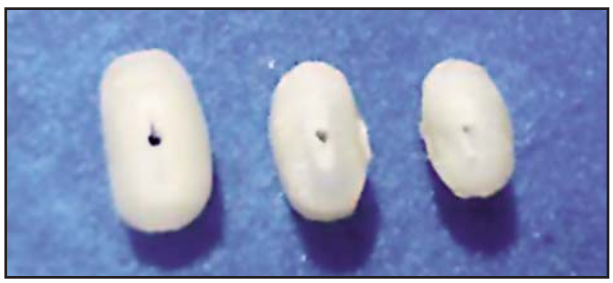

Fig. 2: Root dentin middle third segments.

Fig. 3: Sections of root dentin middle third segments.

MATERIAL AND METHODS

Experimental teeth and solutions

Ten recently extracted single-root human mandibular premolars were selected on the basis of their similarity in morphology and size. They were kept in distilled water at 4ºC until used. Debris, calculus and soft tissue remnants on the root surfaces were cleaned using a Gracey curette (Hu-Friedy, NC, USA). The crowns were sectioned at the cement-enamel junction using a high speed bur # 2200 (KG Sorensen, SP, Brazil) and water-irrigation. Cementum was removed using a Gracey curette. Root canals were enlarged up to a number 50 K-file (Maillefer, East Lansing, MI, USA),), at a working length of 1mm from the apex. They were cleaned and shaped using the step-back technique. After each instrument change, root canals were irrigated with 2 mL of distilled water, using a 25G needle (BD Precision Glide, Curitiba, Brazil). The apical and coronal third of the roots were removed and the remaining parts were cut transversally into three parts using a high speed bur # 2200 (KG Sorensen, SP, Brazil) (Fig. 1 and 2). Each slice was then bisected in buccolingual direction, obtaining a total of six sections of each root (Fig. 3). Sections of the same teeth were used to compare all the solutions. The sections were weighed on a precision scale (Acculab, BA, Argentina) (accuracy ≤0.1 mg) and found to have an average weight of 46.0 mg ± 13 mg. Then they were stored at 4ºC until use. The 60 specimens were divided into six experimental groups

(ten specimens each) and treated with different irrigating solutions: group 1 (Control), distilled water (DW) pH 7; group 2, 1% NaOCl pH 11.6; group 3, 1% CA pH 1.8; group 4, 17% EDTA pH 7.2; group 5, 1% CA pH 1.2 + 1% NaOCl pH 11.6; group 6, 17% EDTA pH 7.2 + 1% NaOCl pH 11.6. The specimens in groups 1, 2, 3 and 4 were immersed in 1 mL of the irrigant at 37ºC for 5 minutes, and those in groups 5 and 6 were left in contact with 1 mL of each solution for 2.5 minutes resulting in a 5-minute immersion. Specimens were not washed between irrigants. All specimens were then removed and the pH of each solution was analyzed.

pH measurement

The pH of each solution was determined before and after contact with the dentin specimens using a digital pH meter (Broadley-Yames Corp. Irvine, Ca, USA) for small volumes (accuracy ≤0.01). The pH was determined by placing the refillable Calomel electrode in a 30 µL sample on a slide for 10 sec. The electrode was washed with distilled water and wiped dry between readings.

Statistical analysis

Data were analyzed using the T Test to compare the initial and final pH of each solution for related samples, and the final pH at different times for independent samples. Finally, one-way analysis of variance (ANOVA) was performed to compare the pH of the NaOCl solution when it was used alone or consecutively to CA or EDTA. Means were compared using the Tukey multiple comparison test.

RESULTS

Table 1 shows the pH values of the experimental solutions after contact with the sections of root dentin. At 5 minutes there were statistically significant differences ($p \leq 0.01$) between the initial and final pH values for all the solutions, including the control solution ($p \leq 0.05$). The pH values decreased for DW and NaOCl and increased for CA and EDTA.

When the irrigating solutions were used consecutively (Table 2), similar results were obtained: the pH values for DW and NaOCl decreased significantly ($p \leq 0.01$), while for CA and EDTA, they increased significantly ($p \leq 0.01$) after remaining in contact with the dentin for 2.5 minutes.

A comparison of the final pH values for DW, CA and EDTA solutions at both exposure times (5 minutes and 2.5 minutes) (Tables 1 and 2) showed no difference ($p \geq 0.05$) between the pH values of the DW and EDTA groups. However, CA showed statistically significant differences ($p \leq 0.05$) resulting in even higher pH values at 2.5 minutes than at 5 minutes contact time.

Regarding NaOCl solutions (Table 3), after the use of CA, and even more so with EDTA, pH was significantly lower ($p \leq 0.01$) at 2.5 minutes contact time compared to the pH value at 5 minutes.

Table 1: Initial and final pH of irrigating solutions after contact with human root dentin.

Solution	Time (min)	Initial pH (x ± SE)	Final pH (x ± SE)
DW	5	7.02 ± 0.00[a]	6.46 ± 0.13[b]
1% NaOCl	5	11.60 ± 0.00[a]	11.40 ± 0.00[b]
1% CA	5	1.80 ± 0.00[a]	2.00 ± 0.01[b]
17% EDTA	5	7.20 ± 0.00[a]	7.32 ± 0.02[b]

DW: Distilled water; NaOCl: Sodium hypochlorite; CA: Citric acid; EDTA: Ethylenediaminetetraacetic acid
*Significant differences are expressed by different letters ($p \leq 0.05$).

Table 2: Initial and final pH of consecutively used irrigating solutions after contact with human root dentin.

Solution	Time (min)	Initial pH (x ± SE)	Final pH (x ± SE)
DW	2.5	7.00 ± 0.00[a]	6.75 ± 0.08[b]
1% CA	2.5	1.80 ± 0.00[a]	2.11 ± 0.03[b]
1% NaOCl	2.5	11.60 ± 0.00[a]	11.30 ± 0.00[b]
17% EDTA	2.5	7.20 ± 0.00[a]	7.36 ± 0.02[b]
1% NaOCl	2.5	11.60 ± 0.00[a]	11.26 ± 0.01[b]

CA: Citric acid; NaOCl: Sodium hypochlorite; EDTA: Ethylenediaminetetraacetic acid
*Significant differences are expressed by different letters ($p \leq 0.05$).

Table 3: Final pH of NaOCl solution alone and consecutively used after contact with human root dentin.

Solution (Time)	Final pH (x ± SE)
1% NaOCl (5 min)	11.40 ± 0.00[a]
1% NaOCl (2.5 min) after 1% CA (2.5 min)	11.30 ± 0.00[b]
1% NaOCl (2.5 min) after 17% EDTA (2.5 min)	11.26 ± 0.01[c]

NaOCl: Sodium hypochlorite; CA: Citric acid; EDTA: Ethylenediaminetetraacetic acid
*Significant differences are expressed by different letters ($p \leq 0.05$)

DISCUSSION

The decalcifying action of CA, which has an acid pH, is greater than its chelating action, as reported in a paper by Machado-Silveiro *et al.* 2004[21] comparing CA to sodium citrate. They considered that sodium citrate may only have the chelating activity of the original acid, which is low and may explain why sodium citrate has lower decalcifying activity than CA.

De-Deus *et al.* 2006[22], reported that 10% CA caused peritubular and intertubular dentin erosion. Machado-Silveiro *et al.* 2004 also found stronger results with 1% and 10% CA than with 17% EDTA, while Spanó *et al.* 2009 contradict these results reporting that, when used for 5 min, 15% EDTA removed more calcium ions than 10% CA. Di Lenarda *et al.* 2000[23] found similar results for 1 $ml.L^{-1}$ CA and 15% EDTA. Haznedaroglu 2003[24] studied the effect of pH variation on the chelating effectiveness of CA, concluding that pH is a more important factor than concentration. These results are in agreement with Hennequin *et al.* 1994[8]. Thus, decalcification was higher with a CA solution at pH 1.1[9]. In addition to the pH variations of CA, EDTA and NaOCl with exposure time, we have demonstrated in other studies that these irrigating solutions did not significantly affect organic and inorganic human dentin composition at 2.5 minutes or 5 minutes exposure time[25].

Renewal of the solution increases the effectiveness of its action compared to a single continuous application over the same period of time[26] because it maintains the pH at natural levels, thereby increasing its moisturizing and decalcifying capacity[27]. Zehnder *et al.* 2005[28] reported that CA and EDTA may interfere with NaOCl action and should therefore be used separately. Both CA and EDTA immediately reduce the available chlorine in solution, rendering the sodium hypochlorite irrigant ineffective on bacteria and necrotic tissue. In our experience, NaOCl, like DW, did have lower pH after contact with the dentin, as if some acidic component of the exposed root tissue could be slightly sensitive to solubilization. On the other hand, CA and EDTA as chelating agents may act on the calcium dentin component, which may be responsible of the rise in the pH of the solution. However, exposure time might not affect the action of EDTA, as was demonstrated for CA, which had lower pH at 5 minutes than at 2.5 minutes, as if the dentin had shown buffering capacity. After the application of CA and EDTA, the solubilization effect of NaOCl may be much greater, since the dentinal tissue would be much more destabilized. However, it should be taken into consideration that cementum was absent from the root specimens in this experiment.

Dentin exposed to NaOCl may be sensitive to solubilization, an effect that may appear after the application of CA and EDTA, which may act on dentinal calcium. The exposure time used may not affect the pH of NaOCl and EDTA as it did for CA, which may provide evidence of dentinal buffering capacity at 5 min.

Further studies are needed to determine the pH behavior of the solutions, used alone and consecutively, in contact with human root dentin at higher exposure times.

This study should be complemented with others to determine biocompatibility of these drugs when used in endodontic treatments.

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A LABORATORY ASSESSMENT OF BACTERIAL LEAKAGE IN MTA APICAL PLUGS EXPOSED TO PHOSPHATE-BUFFERED SALINE

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ABSTRACT

This study evaluated the influence of the exposure of mineral trioxide aggregate (MTA) - with and without calcium chloride ($CaCl_2$) - to phosphate-buffered saline (PBS) on apical microleakage. Sixty root segments were divided into 4 experimental groups (n=15). Apical cavities were filled with MTA with or without $CaCl_2$, and the root canals dressed with a moistened cotton pellet or PBS: 1) MTA/cotton pellet; 2) MTA/PBS; 3) MTA+ 10%$CaCl_2$/cotton pellet; 4) MTA+10%$CaCl_2$/PBS. After 2 months, E. faecalis penetration was analyzed along the apical plugs. Samples were observed weekly for 70 days, and leakage was detected by turbidity of the medium in contact with the root segment. Teeth in the control groups (n=2) were either made completely impermeable or kept without an apical plug. The Kaplan–Meier method was used to analyze survival and the Log-rank test was used to compare the survival curves (p<0.05). All specimens in the positive control group showed evidence of leakage within 24h, while none in the negative control group showed leakage up to 70 days. There was no statistically significant difference among the experimental groups (p=0.102). The use of PBS as intracanal dressing may improve MTA sealing ability, but cannot prevent bacterial leakage. The addition of $CaCl_2$ to the MTA did not improve MTA sealing ability.

Key words: *apexification, dental leakage, endodontics, mineral trioxide aggregate.*

AVALIAÇÃO LABORATORIAL DA INFILTRAÇÃO BACTERIANA EM PLUGS APICAIS DE MTA EXPOSTOS AO TAMPÃO FOSFATO-SALINO

RESUMO

O presente estudo avaliou a influência da exposição do agregado de trióxido mineral (MTA) – com e sem cloreto de cálcio ($CaCl_2$) – ao tampão fosfato-salino (PBS) sobre a microinfiltração apical. Sessenta segmentos radiculares foram divididos em 4 grupos experimentais (n=15). As cavidades apicais foram preenchidas com MTA, com ou sem $CaCl_2$, e os canais radiculares receberam uma bolinha de algodão umedecida ou PBS, como medicação intracanal: 1) MTA/ bolinha de algodão umedecida; 2) MTA/PBS; 3) MTA+10% $CaCl_2$/ bolinha de algodão umedecida; 4) MTA+10%$CaCl_2$/PBS. Após 2 meses, a penetração de E. faecalis ao longo dos plugs apicais foi avaliada. As amostras foram observadas semanalmente durante 70 dias e a infiltração detectada através da turbidez do meio em contato com os segmentos radiculares. Dentes pertencentes aos grupos controle (n=2) foram mantidos completamente impermeáveis ou sem plug apical. A análise de sobrevivência e a comparação das curvas foram realizadas por meio dos testes Kaplan-Meier e Log-rank (p<0.05), respectivamente. Todas as amostras do grupo controle positivo apresentaram evidência de infiltração dentro de 24h, enquanto nenhuma amostra do grupo controle negativo apresentou infiltração ao longo dos 70 dias. Não houve diferença significativa entre os grupos experimentais (p=0.102). O uso do PBS como medicação intracanal pode melhorar a capacidade de selamento do MTA, mas não é capaz de impedir a infiltração bacteriana. A adição de $CaCl_2$ ao MTA não melhora sua capacidade de selamento.

Palavras-chave: *apexificação, infiltração dentária, endodontia, agregado de trióxido mineral.*

INTRODUCTION

Coronal leakage of microorganisms and their byproducts is considered a common reason for the failure of endodontic treatment. Therefore, any material intended to seal communications between the root canal and the periodontium[1-3] should be effective.

Mineral trioxide aggregate (MTA) used as an apical plug or as a retrofilling material rarely provides a totally efficient seal[4,5]. Some studies have shown that the interaction between MTA and phosphate-buffered saline (PBS)[6] or tissue fluid[7] leads to the formation of a layer with tag-like structures at the cement-dentin interface, which may positively

influence its sealing ability[3,8]. Despite the promising results obtained in these studies, a more recent investigation demonstrated that the use of PBS as intracanal dressing provided a slightly, though not significantly improved sealing[9].

It was believed that adding calcium chloride ($CaCl_2$) to MTA might have a positive influence on the biomineralization process[6] and contribute to its sealing ability; however, experiments found that the opposite was true, with the mixture allowing greater glucose leakage[9].

Due to the ongoing controversies regarding the benefits of PBS as an intracanal dressing and regarding the effect of adding $CaCl_2$ to MTA on microleakage, more evidence is needed. The bacterial leakage model, considered to be the most clinically relevant and biologically significant[10], might provide a more reliable result. The purpose of this study was to evaluate the influence of the exposure of MTA - with and without $CaCl_2$ - to PBS on apical microleakage using a bacterial penetration model.

MATERIALS AND METHODS

Sixty-four single-rooted, extracted human teeth were used under a protocol approved by the Ethics Committee for Research with Human Beings of the Federal University of Santa Catarina (protocol number 1861).

The procedures were performed as described by Almeida et al.[9]. The crowns were sectioned, and a 2-mm root tip resection was performed with a high-speed bur under water cooling, so that all root segments were about 12 mm long. The canals were cleaned and shaped using #1-5 Gates-Glidden drills in a crown-down fashion, and 1% sodium hypochlorite (NaOCl) was used for irrigation. A standardized open apex was created by retrograde preparation of the canal with a #6 Gates-Glidden drill (±1.50-mm diameter). The final canal rinse was performed with 17% EDTA followed by 1% NaOCl.

Apexification procedures

The root sections were randomly divided into 4 experimental groups (n = 15). The apical cavities were filled and the root canals dressed as described in Table 1.

MTA cement was mixed following the manufacturer's recommendations, and MTA+$CaCl_2$ was mixed following Bortoluzzi et al.[11]: 1 g MTA with 0.1 g $CaCl_2$ mixed with 0.18 mL H_2O.

The cement mixture was placed into the canal, condensed with moistened paper points, and compacted with pluggers (Dentsply, Tulsa Dental, Tulsa, OK, USA) to create a 4 mm-thick apical plug. Radiographs were taken of all root segments to ensure void-free MTA placement and plug thickness.

In Groups 1 and 3, following the manufacturer's recommendations, a cotton pellet moistened with distilled water was placed in the cervical region of each root segment, and replaced with a dry one after 24 h. In Groups 2 and 4, in order to favor the biomineralization process[6], the remaining canal space was filled with PBS (Dermus Farmácia Dermatológica e Cosmética Ltda, Florianópolis, SC, Brazil; pH = 7.2) as an intracanal dressing (Table 1).

All access openings were covered with cotton pellets and filled with temporary cement (Cimpat, Septodont Brasil Ltda, São Paulo, SP, Brazil). The root segments were placed in plastic vials containing floral foam moistened with 20 mL PBS, and stored for 2 months at 37°C.

After the experimental period, the external surfaces of all specimens were made impermeable with two layers of nail varnish, except for the 1 mm around the apical foramen. An apparatus with the root segment was mounted [similar to the model developed by de Leimburg et al.[12]], sterilized by ethylene oxide gas (ACECIL, Central de Esterilização Com. Ind. Ltda., Campinas, SP, Brazil), and adapted to a sterile 20-mL syringe containing 5 mL of Brain Heart Infusion broth (BHI), so that the most apical portion of each root segment was immersed in the culture medium. The syringe embolus allowed closure of the apparatus, which was kept in an oven at 37°C for 4 days to confirm sterilization.

Table 1: Groups, Materials Used to Form the Apical Plug and Intracanal Dressing.

Group	Apical plug	Intracanal dressing
1	MTA*	Moistened cotton pellet
2	MTA*	PBS
3	MTA*+$CaCl_2$#	Moistened cotton pellet
4	MTA*+$CaCl_2$#	PBS

* MTA Branco - Angelus Soluções Odontológicas, Londrina, PR, Brazil.
# Vetec Química Fina, Rio de Janeiro, RJ, Brazil.

Bacterial leakage test

For the leakage assay, a standard strain of *Enterococcus faecalis* (ATCC 29212) was used. Previously to testing, the *E. faecalis* counts in the BHI were determined by decimal dilutions. Aliquot portions were plated on the surface of trypticase soy agar (TSA) (Difco Laboratories, Becton Dickinson and Company, Franklin Lakes, NJ, USA) and incubated at 37ºC for 24 h. After the incubation period, the number of colony forming units (CFU mL-1) was determined. For the bacterial leakage test, 500 µL aliquots of *E. faecalis* were transferred to the upper portion of the pipette connected to the root segment. Every 7 days during the experimental period, the BHI inoculated with *E. faecalis* was replaced with a new 500 µL aliquot of sterile BHI. The aliquot removed was tested to confirm bacterial viability. The number of leaking samples for each group was observed weekly for 70 days. Leakage was detected by turbidity of the BHI medium in contact with the apical portion of the root segment.

For the positive control group, two root segments without apical plugs were used. For the negative control group two root segments with apical plugs were made completely impermeable by the application of two layers of nail varnish. Additionally, one specimen of each experimental group was used as negative control and inoculated with BHI without *E. faecalis*.

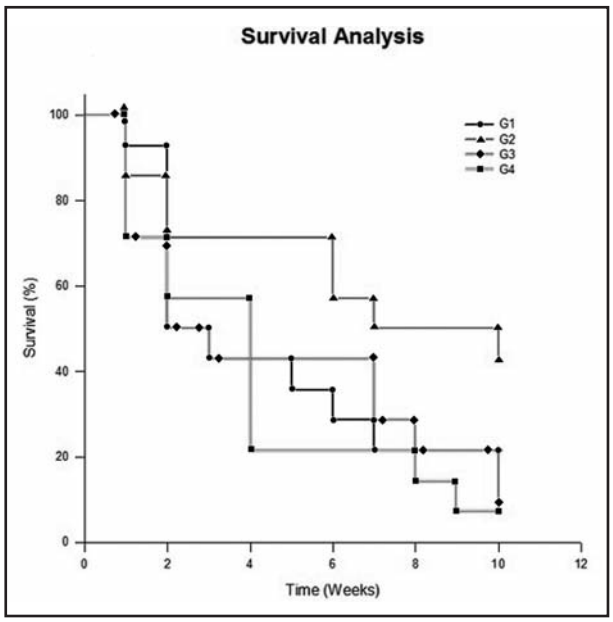

Fig. 1: Plot of Kaplan-Meier survival analysis for bacterial leakage in the experimental groups over the 70-day period.

Statistical analysis

The Kaplan-Meier method was used to estimate survival curves for each experimental group. Log-rank testing was used to compare the survival curves at the 5% significance level.

RESULTS

Control groups

All specimens in the positive control group exhibited leakage within 24 h, and the inoculums were confirmed to contain *E. faecalis,* while none in either of the negative control groups showed leakage up to 70 days.

Experimental groups

Fig. 1 shows the survival curves. Over the 70 days there was no statistically significant difference among groups ($p = 0.102$).

DISCUSSION

Several *in vitro* methodologies are available for evaluating the quality of the apical seal provided by apical plugs, such as glucose penetration[9], fluid filtration techniques[13] and bacterial leakage[14]. Due to the controversial history of the reproducibility and clinical relevance of these methods, the issue of microleakage has been intensively discussed[15,16]. However, among all *in vitro* methods, the bacterial leakage test reflects clinical reality, since it uses bacteria, which are etiologic agents of apical periodontitis, as markers[17]. The 70-day experimental period was carried out based on the methods used in previous bacterial leakage studies[18,19]. The medium in contact with the root segments of the negative control groups showed absence of turbidity up to the end of the experiment, and confirmed the suitability of the apparatus and the absence of contamination. The culture medium in contact with the root segments of the positive control group, without MTA apical plugs, became turbid within the first 24 h, confirming that microorganisms might reach the apical region in the absence of apical plugs.

Monitoring of the experimental groups up to 70 days showed that, regardless of the addition of $CaCl_2$ to MTA or the use of PBS as intracanal dressing, most of the apical plugs did not prevent bacterial leakage. A possible reason for this was contemplated in a similar study using the glucose leakage method[9]. Through-and-through voids in the body of the material or the cement-dentin interface[20]

as well as interconnected pores[21] probably served as a route for bacterial penetration.

It is worth noting that under our experimental conditions, the interaction of PBS as intracanal dressing with MTA apical plugs provided better sealing, although it was not significantly different. Despite using different leakage methodologies, recent investigations have demonstrated similar results when MTA was kept in contact with PBS[3,8,9]. The most acceptable explanation is based on the formation of carbonated apatite at the biomaterial-dentin interface[6]. The association of PBS as intracanal dressing that diffused through the apical barrier encourages the occurrence of the biomineralization process along the MTA apical plugs[22], probably reducing the leakage.

Despite its benefits regarding setting time and pH[11], the addition of $CaCl_2$ to MTA jeopardized its sealing ability, regardless of the use of PBS as intracanal dressing. It was noticed that a high number of MTA+ $CaCl_2$ apical plugs (28.57%) allowed bacterial leakage in the first week and by day 70 almost all the samples had leaked (92.85%). These results agree with a recent leakage study[9]. The addition of $CaCl_2$ to MTA promotes greater absorption of water by the cement, leading to the formation of capillary pores and loss of sealing ability[23].

The findings of this study confirm that the use of PBS as intracanal dressing may improve MTA sealing ability, but cannot totally prevent bacterial leakage. The addition of $CaCl_2$ to the MTA did not improve its sealing ability. Although there are still controversies in microleakage studies, it is very useful to know the behavior and effectiveness of the MTA in different situations.

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EDENTULISM AND DENTAL PROSTHESES IN THE ELDERLY: IMPACT ON QUALITY OF LIFE MEASURED WITH EUROQOL – VISUAL ANALOG SCALE (EQ-VAS)

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ABSTRACT
The objective of this study was to measure the impact of edentulism and dental prostheses on quality of life (QOL) in older adults in Bogotá, Colombia. Edentulism is a frequent condition in older adults and has great impact on their QOL. No epidemiological data are currently available on edentulism among older adults in Colombia. Data were obtained from the SABE-Bogotá study, a cross-sectional study conducted in 2012, and used to analyze the EQ-VAS (Visual Analog Scale) from the EuroQol instrument to measure the perception of quality of life (QOL) in relation to edentulism. The study included 2,000 individuals over 60 years old. The Spearman-Rho correlation was used to analyze the correlation between EQ-VAS and edentulism. Chi-Square, ANOVA and t-test were used to study the differences in EQ-VAS scores between edentulous and healthy subjects. Statistical significance was set at $p<0.05$. Of the 2000 respondents, 98.3% were edentulous, 73.0% reported half or more missing teeth, 76.9% used dental prostheses and 23.7% had related eating problems. Older age, lower social class and lower education were related to edentulism. Individuals with fewer teeth and dental prostheses had lower EQ-VAS scores ($p<0.05$) and dental prosthesis did not improve EQ-VAS scores ($p=0.22$). Edentulism also showed a significant negative correlation with EQ-VAS scores (rho= -0.102, $p<0.01$). In summary, EQ-VAS is a useful tool for measuring the perception of QOL in dental health scenarios. Edentulism significantly affects QOL in older adults and the use of dental prosthesis does not improve the perception of QOL.

Key words: *Mouth, Edentulous, Quality of life, Aging, Dental prosthesis.*

EDENTULISMO Y PRÓTESIS DENTALES EN EL ADULTO MAYOR: IMPACTO SOBRE LA CALIDAD DE VIDA MEDIDO CON EUROQOL – ESCALA VISUAL ANÁLOGA (EQ-VAS)

RESUMEN
El objetivo de este estudio fue medir el impacto del edentulismo y el uso de prótesis dentales en la calidad de vida de los adultos mayores en Bogotá, Colombia. El edentulismo es frecuente en los adultos mayores y afecta profundamente su calidad de vida. Actualmente existen pocos datos epidemiológicos disponibles sobre este tema en nuestro medio. Los datos fueron extraídos del estudio SABE-Bogotá, estudio transversal por conglomerados llevado a cabo en el 2012. Se uso el EQ-VAS (escala visual análoga) como instrumento de medición de la percepción de la calidad de vida. El estudio incluyó 2.000 individuos mayores de 60 años. Se usó el test de Sperman-Rho para analizar la correlación entre el EQ-VAS, el edentulismo y el uso de prótesis. Las pruebas de Chi-cuadrado, ANOVA y t-test se usaron para estudiar las diferencias en los puntajes del EQ-VAS entre los sujetos edéntulos y sanos. La significancia estadística se estableció en <0.05. De los 2000 encuestados, 98.3% fueron edéntulos, 73.0% reportaron perdida de mas de la mitad de sus dientes, 76.9% reportaron usar prótesis dentales y 23.7% problemas relacionados con la alimentación. La edad avanzada, el estrato social bajo y el bajo nivel educativo se relacionaron con mayor pérdida dental. Individuos con pocos dientes y prótesis dentales tuvieron puntajes bajos en el EQ-VAS ($p=0.22$). La presencia de edentulismo también mostró una correlación negativa con los puntajes del EQ-VAS (rho= -0.102, $p<0.01$). Se concluye que EQ-VAS es un instrumento de gran utilidad para la medición de calidad de vida en contextos relacionados a salud oral. El edentulismo afecta significativamente la calidad de vida en los adultos mayores y el uso de prótesis dentales no mejora la percepción de la calidad de vida.

Palabras clave: *Boca, Edentulismo, Calidad de vida, Envejecimiento, Prótesis dentales.*

INTRODUCTION

The elderly population has increased rapidly worldwide in recent decades due to the demographic transition[1] which is accompanied by an increase in longevity and age-dependent chronic diseases[2]. Oral diseases are among the most prevalent conditions in the elderly[3,4] and affect quality of life (QOL) as a result of infections, functional impairment, poor self-esteem, socialization issues, communication and chewing problems[5,6]. Edentulism therefore

represents a difficult challenge for public health given that poor oral health leads to a broad spectrum of comorbidities, such as malnutrition, frailty, and deterioration of preexistent chronic diseases, and decreases overall health status[7,8]. Most importantly, these conditions increase morbidity and mortality rates [9].

The most frequent causes of poor dental health are caries, periodontal disease and edentulism; the latter being a consequence and a common outcome of caries and periodontal disease[10]. Edentulism is the loss of at least one tooth (partial edentulism) or the loss of all the teeth (total edentulism)[4]. In the elderly, high prevalence rates have been reported for caries (47 to 91.9%), periodontal disease (36 to 89%) and edentulism (20 to 65%)[3,6,11-18].

In clinical practice, oral and dental examinations are the basis for identifying early oral diseases, signs of malnourishment and systemic diseases and infections that could be the result or the cause of dental health issues. Recently developed technology has enabled successful treatment of these diseases. Nevertheless, there are still serious problems involving dental health in the elderly, due to the following reasons: access to oral health services is limited, services are expensive and frequently not covered by insurance companies[19], poor hygiene habits and the lack of dental health information provided to older adults[20,21].

Dental prosthesis usage has been shown to increase QOL by improving dental function. However, it has been reported that people are often not aware of the proper use of the prosthesis or simply do not use it despite their need[19,22]. Furthermore, a complete nutritional assessment is a priority in edentulous individuals and dental prosthesis users because they typically eat food with low nutritional value to facilitate chewing. Adequate nutritional status in edentulous patients is maintained by proper usage of the prosthesis and health care of the gingiva and remaining teeth.

Several approaches have been used to measure QOL[23]. One of the most widely used instruments for measuring QOL is the EuroQOL questionnaire[24], developed by "The International European Quality of Life Group'' to be applied internationally and standardized to measure QOL in the context of different diseases[25,26]. {Leeds, 2004 #850} It has also been used and validated for Latin American countries in standard Spanish. This study used the Spanish version to analyze the relationship between QOL and dental health in the elderly [27,28].

This study evaluates the impact of edentulism and the usage of dental prostheses on the self-perceived QOL of older adults in the city of Bogotá, Colombia

MATERIAL AND METHODS

The SABE Bogotá study (Health, Wellbeing and Aging) is a probabilistic cross-sectional study. Data were collected from a sample selected by clusters in a multistage process (Sectors, sub-sections of neighborhoods, blocks, and sets of 10 houses); a correction factor was used for a statistical confidence of 95%. The total response rate was 81.9%. The sample of 2000 older adults aged 60 years and older was statistically representative of this population.

The Pan American Health Organization designed the SABE survey for Latin American countries including 11 main topics[29]: 1) identification of the house and the elderly older adults to be interviewed; 2) house and home characteristics; 3) personal and family data; 4) experiences of violence; 5) cognitive status; 6) health status; 7) characterization and causes of disabilities; 8) functional evaluation; 9) medication usage and access to health services; 10) anthropometrics and physical performance tests; 11) health, disease and a social history framework (biological component).

Data were collected by fieldwork teams including: 1 supervisor, 3 to 4 interviewers and 1 anthropometrics specialist. The teams were trained by the principal investigator, field researchers, a statistician and a field coordinator. The collected data were digitalized and saved in Microsoft Excel, software version 2011. All respondents signed an informed consent in order to participate in the study, which was approved by ethics committee of the Pontificia Universidad Javeriana.

Variables

Dependent Variables

To characterize edentulism, we used related questions: 1. How many missing teeth do you have? (None, 1 to 4, 4 to half, more than half, all the teeth); 2. Do you use dental prosthesis? (Yes/no); 3. During the last 12 months, have you experienced any difficulties in eating related to issues with your teeth or dental prosthesis? (Yes/no).

To measure QOL we used the EuroQol instrument, which has two main components: 1. EQ-5D, which evaluates 5 dimensions (Mobility, self-care, usual

activities, pain/discomfort and anxiety/depression) and 2. EQ-VAS (Visual Analog Scale) which is a scale of 0 to 100 that allows individuals to place themselves according to how they perceive their overall health status (0 is the worst and 100 the best health status). For the purposes of this study we used the EQ-VAS as a tool for measuring health-related QOL in edentulous older adults.

Independent Variables

Socio-demographic variables: Age (age groups); Sex (male or female); Social class [Social class in Colombia is divided into strata (1-6, with 1 being the lowest and 6 the highest). We created three categories using the 6 classes 1-2 (Lower), 3-4 (middle) and 5-6(upper).[30]]; and Educational level [evaluated as years of education (0, 1-5, ≥6)]. The questions related to edentulism were also used as independent variables when we evaluated EQ-VAS scores.

Statistical Analysis

We performed a descriptive analysis of edentulism and the socio-demographic variables with Chi-Square test to find differences between groups. Then we used the EQ-VAS as a continuous dependent variable to evaluate perceived QOL. This variable followed a normal distribution and we performed parametric analyses. Results are presented as mean scores ± SEM (standard error of the mean); One Way-ANOVA or t-test was used to find statistically significant differences between edentulous and healthy subjects. When appropriate, a post-hoc test (LSD-Fisher) was used to determine significant differences between groups. Statistical correlations were estimated with Spearman-Rho correlation test given that the distribution for edentulism categorical variables was not normal. Statistical significance was accepted for p-values less than 0.05. Data analysis was performed using the software IBM SPSS Statistics version 21 for Mac and for figure preparation Sigma Plot by SYSTAT Software Inc. version 12 for Windows.

RESULTS

General Demographics and Prevalence of Edentulism

Of the 2000 respondents, 62.4% were female and 37.6% were male. Age (in years) groups had similar frequencies: 60-64 with 25.3%, 65-69 with 22.7%, 70-74 with 19.9%, and ≥75 had the highest frequency (32.1%). For socioeconomic status, the highest proportion was in the lower social class (51.9%), followed by middle social class (44.9%) and upper social class (3.3%). A total 98.3% were edentulous, 73.0% had lost more than half their teeth and 1.7% had complete natural teeth. 77.0% used dental prosthesis, 23.0% were edentulous and did not use dental prosthesis. Individuals with eating problems related to teeth or dental prosthesis were 23.7% (Table 1).

Table 2 shows the prevalence of edentulism by categories. Individuals who have lost more than half their teeth showed higher prevalence among women (76.6%), older age (82.5% over 75 years), lower

Table 1: Socio-demographic data Demographics about of the elderly population in the SABE Bogotá-study.

Category	n (%)
Sex	
Male	751 (37.6)
Female	1249 (62.5)
Age (years)	
60-64	506 (25.3)
65-69	454 (22.7)
70-74	398 (19.9)
≥75	642 (32.1)
Social Class	
Lower class	1038 (51,.9)
Mild Middle class	897 (44,.9)
Upper class	65 (3,.3)
Educational level (years)	
0	244 (12,.2)
1-6	1111 (55,.6)
≥6	645 (32,.3)
Edentulism (number of missing teeth)	
All teeth present	34 (1.7)
Less than four missing	276 (14)
Four to half missing	222 (11.3)
More than half missing	1441 (73.0)
Edentulism related conditions	
Dental prosthesis	1535 (77.0)
Edentulous with no prosthesis	459 (23.0)
Eating problems related to issues with natural teeth or prosthesis	474 (23.7)

Table 2: Missing teeth by sex, age groups, social classes and educational level.

	All teeth present n (%)	Less than half missing n (%)	More than half missingn (%)	Total n (100%)	p value[a]
Sex					
Male	13 (1,.7%)	231 (31,.0%)	500 (67,.2%)	744	*
Female	21 (1,.7%)	267 (21,.7%)	941 (76,.6%)	1229	*
Age (years)					
60-64	12 (2,.4%)	188 (37,.5%)	301 (60,.1%)	501	*
65-69	9 (2,.0%)	112 (24,.9%)	329 (73,.1%)	450	*
70-74	6 (1,.5%)	95 (24,.2%)	292 (74,.3%)	393	*
≥75	7 (1,.1%)	103 (16,.4%)	519 (82,.5%)	629	*
Social class					
Lower class	8 (0,.8%)	206 (20,.3%)	801 (78,.9%)	1015	*
Mild Middle class	22 (2,.5%)	258 (28,.9%)	613 (68,.6%)	893	*
Upper class	4 (6,.2%)	34 (52,.3%)	27 (41,.5%)	65	*
Education level (years)					
0	2 (0,.8%)	35 (14,.7%)	201 (84,.5%)	238	*
1-6	17 (1,.6%)	231 (21,.2%)	843 (77,.3%)	1091	*
≥6	15 (2,.3%)	232 (36,.0%)	397 (61,.6%)	644	*

[a] Chi-square test * p<0.001.

social-class (78.9%) and lower educational level (84.5%). The frequency of complete teeth decreased from younger to older age groups (2.4% for 60-64 ys., 2.0% for 65-69 ys., 1.5% for. 70-74 ys. and 1.1% for individuals ≥ 75 ys), from higher to lower social class (6.2% for upper class, 2.5% for middle class and 0.8% for lower class) and from higher to lower educational level (2.3% for more than 6 years education, 1.6% for 1-5 years education and 0.8% for 0 years education). Statistically significant values were obtained for all categories.

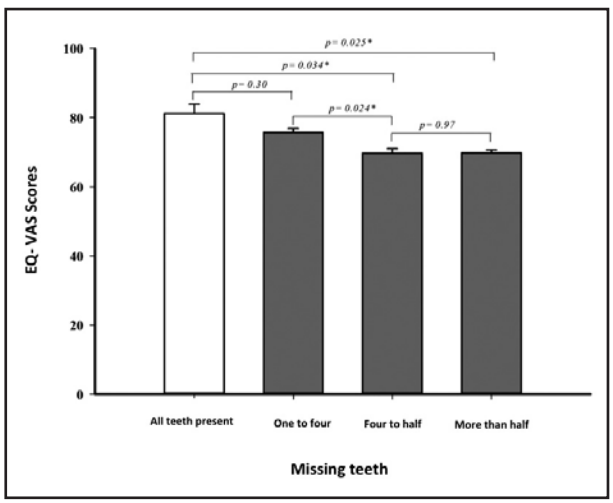

*Fig. 1: Quality of life (EQ-VAS) scores for groups with missing teeth and healthy subjects. One-way ANOVA, (LSD-Fisher). *p<0.05. (n= 2000).*

Quality Of Life in Edentulous Older Adults Measured with EQ-VAS

Fig. 1 shows the differences in EQ-VAS scores between edentulous groups and subjects with complete teeth. EQ -VAS scores for those with complete teeth were 81.2 ±2.7 and significantly lower in edentulous groups. EQ-VAS scores decreased as the number of missing teeth increased with a significant negative correlation for edentulism and EQ-VAS, (rho= -0.102, p<0.01). (Data not shown).

EQ-VAS scores for edentulous groups that used dental prosthesis (70.1±0.5) did not differ significantly (p= 0.22) from those that did not use dental prosthesis (72.9±2.2). However, the presence of eating problems related to issues with the teeth or dental prosthesis was significantly lower 65.3±1.0 than no eating problems 72.2±0.5 (p<0.05, Table 3).

DISCUSSION

Dental health progressively deteriorates in the elderly, leading to a complex scenario for overall health in this population. The most frequent dental health problems include caries, periodontal disease, pathological tooth migration, loss of alveolar supportive tissue and finally, partial and total edentulism[31-33]. The aging process may contribute to these problems, but does not cause them[21,34]. The main etiologies are lack of hygiene and appropriate dental health habits, as has been widely discussed and reported in the literature.[20,33,35] Previous studies of the Colombian population (ENSAB III) reported data on individuals up to 69 years of age and did not characterize edentulism.[36] This is the first study that provides epidemiological data for edentulism and its impact on QOL in the elderly in the city of Bogotá, Colombia.

We found that the prevalence of edentulous individuals is close to 100% and most of them have lost more than half their teeth, representing a relevant public health problem. This prevalence is highest in women, those in lower social class, individuals with lower educational level and those over 75 years of age. Edentulism also showed a deleterious impact on QOL, as shown by significantly lower scores in EQ-VAS in edentulous individuals. As expected, the highest scores corresponded to older adults with complete teeth, and although their perceived QOL was higher, it did not differ significantly from those with up to four missing teeth. However, QOL was considerably affected in individuals with a greater number of missing teeth, who reported significantly lower scores in the edentulous groups that have lost less than half or more than half of their teeth, suggesting that the loss of more than four teeth affects the perception of QOL as much as the loss of more than half of the natural teeth.

On the other hand, an important measure for improving health and QOL in edentulous people is the use of dental prostheses, although users must know how to use and handle them[19]. We found that 23.1% of individuals with edentulism did not use prostheses and those who did use them did not report an improvement in their perceived QOL. This is not surprising, given that dental prosthesis implementation requires a complete oral rehabilitation process, including continuous follow-up, patient education, special care and adaptation or change of the device if necessary, all of which are limited in this population. Furthermore, the presence of eating problems related to issues with the natural teeth or prosthesis has a significant detrimental effect on QOL compared to the absence of eating problems.

Table 3: Visual analogue scale by for eating problems related to issues with natural teeth or prosthesis.

Problems	EQ-VAS Mean ±SEM	p value[a]
Yes	65,.3 ±1	*
No	72,.2 ± 0.5	

aT-test *p<0.0001, (n= 1534).

The fact that QOL does not improve in older adults with the use of dental prostheses suggests that they are missing out on important advantages and benefits. Further studies on this population are needed in order to better understand why these dental health measures do not have the intended impact. It is a priority to increase the coverage of dental health care in older adults and improve their understanding on how to use these devices properly, through prevention and education campaigns focused on the oral health of the elderly.[37] Despite the complexity of measuring QOL, the EuroQol (EQ-VAS) instrument provides a very good tool which is easy to use at a very low cost and can be implemented routinely in these dental health studies.

This study has some limitations. It was a cross-sectional study and it was not possible to evaluate the precise sequence of events and factors leading to edentulism. It was not possible to determine either the causes of or factors related to missing the benefits of dental prosthesis. Despite these limitations there are also several strengths. It is the first study to examine dental health, the prevalence of edentulism and prostheses and their impact on QOL of the elderly in Colombia.

Finally, there is high prevalence of edentulous individuals among the older adults of Bogotá, many of whom do not use dental prostheses. Most of these individuals had lost more than half their teeth and we found a clear increased association with sex (females), older age groups, lower social-classes and lower educational levels. These vulnerable groups in the older adult population are at high risk of having poor dental health, which in turn is widely

reported to be associated with a wide range of illnesses such as malnourishment, chronic diseases and disability[4,38]. These findings highlight the importance of creating public policies to improve dental health in populations that are less able to afford dental healthcare or do not have enough knowledge about dental hygiene and health, as is the case in lower social classes and lower educational levels. Studies are also needed to identify the reasons why women are more edentulous than men. Most importantly, such public policies should focus on a continuous educational oral rehabilitation process, especially in elderly people who require dental prostheses.

EQ-VAS is a useful tool for measuring the perception of QOL in dental health scenarios. Herein we report that edentulism significantly affects QOL in older adults and that strikingly, dental prosthesis usage did not improve the perception of QOL, possibly due to the lack of knowledge on their appropriate use. It is imperative to collect new epidemiological data about the current status of oral health in the elderly population.

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INFLUENCE OF ALGINATE IMPRESSION MATERIALS AND STORAGE TIME ON SURFACE DETAIL REPRODUCTION AND DIMENSIONAL ACCURACY OF STONE MODELS

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ABSTRACT

This study compared the surface detail reproduction and dimensional accuracy of stone models obtained from molds prepared using different alginate impression materials (Cavex ColorChange, Hydrogum 5, or Jeltrate Plus) and with different storage times (1, 3, and 5 days) to models from molds that were filled immediately with no storage time. The molds were prepared over a matrix containing 50-μm line, (ISO 1563 standard) under pressure with a perforated metal tray. The molds were removed 2 minutes after loss of sticky consistency and either filled immediately or stored in closed jars at 100% relative humidity and 37°C for 1, 3, or 5 days. The molds were filled with dental plaster (Durone IV). Surface detail reproduction and dimensional accuracy were evaluated using optical microscopy on the 50-μm wide line, which was 25 mm in length, according to ISO 1563 standard. The dimensional accuracy results (%) were subjected to analysis of variance. The 50-μm wide line (ISO 1563 standard) was completely reproduced by all alginate impression materials regardless of the storage time. There was no statistically significant difference in the mean dimensional accuracy values of stone models made from molds composed of different alginate impression materials and with different storage times (p = 0.989). In conclusion, storing the mold for five days prior to filling did not change the surface detail reproduction or dimensional accuracy of the alginates examined in this study.

Key words: *dental impression materials, alginates, dimensional measurement accuracy.*

INFLUÊNCIA DOS ALGINATOS E TEMPO DE ARMAZENAMENTO NA REPRODUÇÃO DE DETALHES DA SUPERFÍCIE E ESTABILIDADE DIMENSIONAL DE MODELOS DE GESSO

RESUMO

Este estudo comparou a reprodução de detalhes da superfície e estabilidade dimensional de modelos de gesso obtidos a partir de diferentes alginatos (Cavex ColorChange, Hydrogum 5, Jeltrate Plus) e com diferentes tempos de armazenagem (1, 3, e 5 dias) para modelos obtidos de moldes que foram preenchidos imediatamente sem tempo de armazenagem. Os moldes foram preparados sobre matriz contendo linha de 50 μm (norma ISO 1563) realizado sob pressão com moldeira de metal perfurada. Os moldes foram removidos 2 minutos após a perda de consistência pegajosa e preenchidos imediatamente ou armazenados em frascos fechados com temperatura (37°C) e umidade relativa (100%) controladas por 1, 3 ou 5 dias. Os moldes foram preenchidos com gesso dental (Durone IV). A reprodução de detalhes da superfície e a estabilidade dimensional foram avaliadas usando microscopia óptica na linha 50 μm com 25 mm de comprimento, de acordo com a norma ISO 1563. Os resultados de estabilidade dimensional (%) foram submetidos á análise de variância. A linha de 50 μm (norma ISO 1563) foi completamente reproduzida por todos os alginatos, independentemente do tempo de armazenagem. Não houve diferença estatisticamente significativa nos valores médios de estabilidade de modelos de gesso obtidos de moldes de diferentes alginatos com diferentes tempos de armazenagem (p = 0.989). Em conclusão, o armazenamento do molde durante cinco dias antes do preenchimento não alterou a reprodução de detalhes da superfície ou estabilidade dimensional dos alginatos examinadas neste estudo.

Palavras-chave: *materiais de moldagem dentais, alginatos, exatidão de mensuração dimensional.*

INTRODUCTION

Impression materials are used in dentistry to make accurate casts of oral tissues[1]. They must be capable of recording the anatomic topography of the desired area and remain dimensionally stable[1]. Alginate impression materials have been used in dentistry since 1947[2]. Alginates are commonly used as a two-component system of powder and water. The powder contains sodium or potassium alginates (soluble alginates), diatomaceous earth acting as

filler particles, calcium sulfate as a reactor, a fluoride as an accelerator, and sodium phosphate as a retarder[3].

In the alginate structure, gel fibrils are held together by primary bonds occurring due to the substitution of sodium ions by calcium ions on two neighboring molecules[4]. The gel forms as a complex, entangled structure, which traps sodium alginate that has not reacted with the calcium salt, excess water, charged particles, and reaction byproducts[4]. Under these conditions, the final alginate structure is very sensitive to conditions that can change the amount of water trapped in the fibrillar assembly. Consequently, the dimensional stability of an alginate mold is highly vulnerable the weather and moisture conditions during storage, before it is used to make the plaster model.

Surface detail reproduction and dimensional accuracy are necessary to make a true copy of the molded anatomical structures. Thus, these properties are used to analyze the quality of impression materials[5]. A previous study reported that the dimensional changes of alginate impressions in 100% relative humidity varied with the brand of the impression material[6]. However, molds are generally filled with plaster as quickly as possible, avoiding long exposure to air and the resulting syneresis and evaporation. If immediate casting is not possible, it is recommended that the mold be kept in an environment with 100% relative humidity to preserve the water balance within the material. Many alginate manufacturers recommend that models be made within 12 h of casting because increased dimensional changes occur after 12-24 h[7]. This study evaluated the surface detail reproduction and dimensional accuracy of stone models obtained from molds prepared using different alginate impression materials and with different storage times (1, 3, and 5 days) compared to stone models produced from molds that were filled immediately with no storage time. The null hypotheses tested were that the surface detail reproduction and dimensional accuracy of stone models are not affected by the alginate impression material or the storage time.

MATERIALS AND METHODS

The following alginate impression materials were used in this study: Cavex ColorChange (batch number 120817, Cavex Holland BV, Haarlem, The Netherlands), Hydrogum 5 (batch number 161477, Zhermack, Badia Polesine, RO, Italy), and Jeltrate Plus (batch number 757944E, Dentsply Caulk, Milford, DE, USA). The dimensional accuracy and surface detail reproduction were evaluated in accordance with the ISO 1563 standard[8]. The molds were prepared over a matrix (38 mm outer diameter and 29.97 mm internal diameter) containing three parallel lines that were 20, 50, and 75 μm wide and 25 mm in length and spaced 2.5 mm apart. Two additional lines marked X and X' were used to determine the dimensional accuracy and surface detail reproduction on the 50-μm wide line (Fig. 1). Before performing the impression procedure, the matrix was ultrasonically cleaned and dried with compressed air. The alginate impression materials were prepared in accordance with the manufacturer's instructions. A perforated metal tray (31 mm internal diameter, 5 mm high) was placed on a glass plate and filled with the molding material (Fig. 1). The tray was joined to the matrix, and a pressure of 2 kgf was applied using a pneumatic press to simulate the impression process and allow for leakage of excess material[1] (Fig. 2).

The molds were removed 2 minutes after loss of sticky consistency. Then the molds were rinsed with 150 mL of distilled water and dried. For the control groups, the molds were immediately filled with gypsum plaster (Durone IV, batch number 821320F; Dentsply Caulk). In the other groups, the molds were sealed in closed jars at 100% relative humidity (humidifier) and stored at 37°C (greenhouse) for 1, 3, or 5 days and then cast.

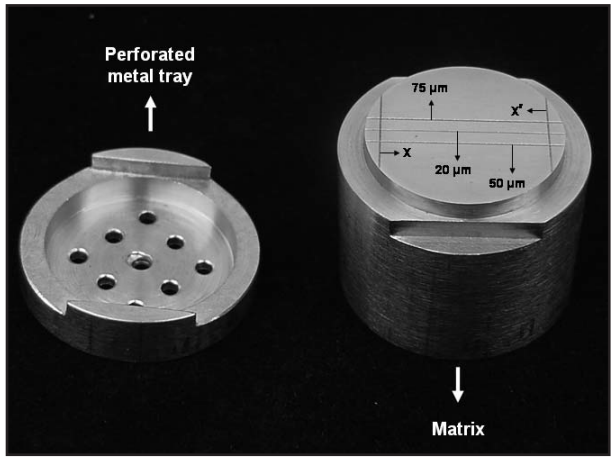

Fig. 1: Matrix in accordance with the ISO 1563 standard and tray.

Fig. 2: Method of positioning of the tray and matrix with a pressure of 2 kgf using a pneumatic press.

Thus, the samples were divided into 12 groups (n = 5) according to storage time and alginate impression material: Group 1: no storage time (control group) + Jeltrate Plus; Group 2: no storage time (control group) + Cavex ColorChange; Group 3: no storage time (control group) + Hydrogum 5; Group 4: stored for 1 day + Jeltrate Plus; Group 5: stored for 1 day + Cavex ColorChange; Group 6: stored for 1 day + Hydrogum 5; Group 7: stored for 3 days + Jeltrate Plus; Group 8: stored for 3 days + Cavex ColorChange; Group 9: stored for 3 days + Hydrogum 5; Group 10: stored for 5 days + Jeltrate Plus; Group 11: stored for 5 days + Cavex ColorChange; and Group 12: stored for 5 days + Hydrogum 5. The stone models were separated from the tray containing the alginate 1 h after the start of stone mixing.

Measurements of surface detail reproduction were performed using an optical microscope (SZM; Bel Engineering SRL, MI, Italy). The stone models were examined under low-angle illumination at magnifications of ×4 to ×12 to determine whether the 50-μm wide line was completely reproduced over the full 25 mm length between the intersecting reference lines (X and X'), in accordance with the ISO 1563 standard (13). Dimensional accuracy measurements were performed on the stone models using an optical microscope (STM; Olympus Optical, Co., Ltd., Japan) with an accuracy of 0.0005 mm. The dimensional accuracy was expressed as a percentage (L) and was calculated in accordance with ISO 1563[8] standard using the equation: $L = [(L2 - L1) / L1] \times 100$, in which L1 is the distance between the lines on the matrix and L2 is the distance between the lines on the stone model. Then, 100% was added to the results of the equation and the dimensional accuracy results were subjected to the Kolmogorov-Smirnov test for normality, and then to two-way ANOVA (material x storage time).

RESULTS

The surface details of all alginate impression materials were fully replicated regardless of storage time (100% of the 5 samples in the 12 groups). There was no statistically significant difference in the mean values of dimensional accuracy in combinations among the storage times and alginate impression materials ($p = 0.989$) or independent factors (material and storage time) (Table 1).

DISCUSSION

Irreversible hydrocolloids are hydrophilic materials that can capture the details of hard and soft tissues in the presence of moisture[9]. These water-based materials

Table 1: Mean values of dimensional accuracy (%) for combinations among the storage times and alginate impression materials.

Alginate Impression Material	Dimensional Accuracy (%)			
	Immediate	1 day	3 days	5 days
Jeltrate Plus	99.96 (0.14)	100.13 (0.06)	100.13 (0.13)	100.10 (0.08)
Cavex Color Change	99.98 (0.21)	100.16 (0.07)	100.14 (0.14)	100.12 (0.13)
Hydrogum 5	99.96 (0.20)	100.07 (0.12)	100.06 (0.11)	100.10 (0.11)

Standard deviations are provided in parentheses.

are inexpensive and can easily be manipulated by following the manufacturer's instructions[9]. Concerns regarding their performance include their low tear strength, dimensional instability when pouring is delayed, and inability to produce accurate casts upon repouring[10]. Thus, it is not surprising that the dimensional stability of various brands of irreversible hydrocolloids decreases with increased storage time[9]. This decrease in dimensional stability is caused by the gain or loss of water from the impression after setting. Imbibition (absorption of fluid by a colloid that results in swelling), evaporation, and syneresis (expulsion of a liquid from a gel) result in dimensional changes[9]. The effects of water evaporation and imbibition can be minimized by pouring the impression as soon as possible[9]. Moreover, irreversible hydrocolloid impressions may be wrapped in a damp paper towel for shipment to the dental laboratory, rather than pouring the casts immediately in the dental office[11]. Thus, it would be interesting to compare the dimensional accuracy of casts made from irreversible hydrocolloids (i.e., Cavex Color Change and Hydrogum 5), which manufacturers say work better if the cast is poured within 5 days, with a conventional hydrocolloid (Jeltrate Plus). To examine this, we delayed pouring the gypsum to simulate routine clinical procedures by storing the molds for 1, 3, or 5 days.

Studies have supported pouring casts immediately or within 10 minutes[12,13], without wrapping them in a wet towel to avoid any absorption of water by the material[14]. Thus, impressions made using irreversible hydrocolloid materials may undergo dimensional changes if not poured immediately because of water exchange with the surrounding environment or by syneresis, which is inherent to the material. In studies of different brands of hydrocolloid materials with different study conditions and storage intervals, researchers have reported that pouring casts from irreversible hydrocolloid impressions immediately or within 10 minutes to 1 hour after making the impression helps to decrease errors and avoid the discrepancies that may occur with prolonged storage[12,15]. Other studies have shown that the impressions made using certain brands of hydrocolloid materials may be stored for up to 3 hours before being poured[6,16]. In the present study, regardless of the brand of alginate or storage time, there was no statistical difference in surface detail reproduction or dimensional accuracy measured in stone models made from molds within 5 days. Similar results were observed in another study which showed that two irreversible hydrocolloid substitutes (Alginot FS and Position PentaQuick) were dimensionally stable for up to 7 days[17].

Moreover, a previous study, in which impressions were rinsed with water and stored in a sealed plastic container that was maintained in an environment of 100% humidity, showed that the cast surfaces poured after storage were better than those poured immediately after rinsing[18]. Tan et al.[18] reported that this was because the exudates from syneresis, which retard the setting of stone and affect the cast surface, decreased during storage. The decrease in exudates during impression storage was reported to decrease the scratch depth of stone models[19]. This was not observed in the present study. However, the syneresis phenomenon did not have a negative effect on the surfaces of the plaster models. The setting expansion of gypsum plaster might have compensated the contraction in the alginate caused by syneresis.

Torassian et al.[17] compared the dimensional stability of typodont and plaster models cast from molds made from two alginates (Identic and imprEssix) at 72 h, 120 h, and 168 h. Measurements were made in several directions, including the anterior-posterior (measured from the central pit of the first molar to the midline face of the respective central incisor), transverse (measured from the central pit of the first molar to the central pit of the contralateral first molar), and vertical (measured from the incisal edge at the midline of the maxillary right and left central incisors to the gingival margin) dimensions[17]. The Identic alginate exhibited shrinkage in all dimensions, and the intercanine width and vertical measurements of the imprEssix alginate decreased over time. In the present study, there was no statistical difference in dimensions when different methodologies and alginate materials were used. In this study, the ISO 1563 standard was used because dimensional changes could clinically affect dental work involving alginate molds with different storage times. Thus, before carrying out clinical procedures, it is necessary to conduct additional tests using different methodologies, such as those described in the aforementioned study,[17] with the materials used in the present study.

Acceptable methods of measuring the dimensional accuracy of casts include measuring calipers[5,20], micrometers[21], dial gauges[22], and measuring

microscopes[1,23]. The latter device was used in the present study due to its high accuracy (0.5 µm). The largest dimensional deviation between the matrix and stone models was 0.16% in Group 5 (stored for 1 day + Cavex ColorChange), which did not differ statistically from the other material/storage combinations. Alginate impression materials are typically recommended for prosthetics and orthodontic purposes where the level of accuracy is perceived as less critical[5]. However, our results suggest that they have sufficient dimensional accuracy for other uses as well. Furthermore, it should be noted that the study was conducted in the laboratory using a strict protocol for all sample preparation steps. To extrapolate these results to the clinical reality, this strict protocol should be performed and other properties, which were not examined in this study, should be tested in future. Thus, based on the results of this study, the null hypothesis was accepted: there was no difference in the dimensional accuracy of models cast from different alginate molds and stored for different lengths of time.

CONCLUSION

In conclusion, the results indicate there is no difference in surface detail reproduction and dimensional accuracy in plaster models made from alginate molds, regardless of differences in storage time or alginate used. Thus, storing the mold for five days prior to filling did not change the surface detail replication or the dimensional accuracy in this study. However, further studies are needed to confirm these findings clinically.

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CORRELATION BETWEEN GINGIVAL THICKNESS AND GINGIVAL RECESSION IN HUMANS

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ABSTRACT

Gingival recession is characterized by the apical migration of the gingival margin, exposing the root surface. Studies have demonstrated several etiological factors for gingival recession such as periodontitis, traumatic toothbrushing, use of oral piercing, and past orthodontic therapy, among others. It might not be possible to identify and quantify the influence of each factor, and gingival recession at some sites may be the result of the combination of these factors. Gingival recession affects individuals at all ages, with prevalence increasing as time passes. The aim of this study was to observe whether there is correlation between gingival thickness and gingival recession. Fifty-five subjects of both genders aged 18-35 years participated in the study. The volunteers were under treatment at the School of Dentistry of the Federal University of Rio Grande do Sul. Buccal gingival thickness was measured on incisors, canines and bicuspids, under anesthesia, following inclusion and exclusion criteria. Statistical analysis was performed with STATA version 10.1. The results had a Pearson Correlation Coefficient of -0.216. Linear regression had a statistically significant p-value of 0.025. It may be concluded that there is weak negative correlation between gingival thickness and gingival recession.

Key words: *gingival recession, gingival thickness.*

CORRELAÇÃO ENTRE ESPESSURA DO TECIDO GENGIVAL E RECESSÃO GENGIVAL

RESUMO

A recessão gengival é caracterizada pelo deslocamento apical da margem gengival, expondo a superfície radicular. Pesquisas têm apresentado vários fatores relacionados à etiologia da recessão gengival como: periodontite, escovação traumática, uso de piercing oral, tratamento ortodôntico passado, entre outros. Pode não ser possível identificar e quantificar a influência de cada fator, e a recessão gengival, em determinados sítios, pode ser o resultado da confluência de várias causas. A prevalência da recessão gengival atinge indivíduos de todas as idades, aumentando significativamente com o passar dos anos. O objetivo do presente estudo foi observar se existe correlação entre a espessura do tecido gengival e a recessão gengival. Participaram da pesquisa pacientes de ambos os gêneros, com idade entre 18 e 35 anos, que estavam em tratamento nas clínicas odontológicas da Faculdade de Odontologia da Universidade Federal do Rio Grande do Sul. Foram medidas a espessura e recessão gengival das faces vestibulares dos dentes incisivos, caninos e pré-molares, previamente anestesiados, seguindo os critérios estabelecidos para inclusão e exclusão no estudo. Para avaliação e comparação dos resultados foi utilizado o programa estatístico STATA versão 10.1. Os resultados mostraram que a correlação de Pearson entre a espessura gengival e a recessão foi de -0.216. A regressão linear apresentou um p= 0.025, estatisticamente significativo. Pode-se concluir que existe uma correlação fraca e inversa entre espessura gengival e recessão gengival.

Palavras-chave: *recessão gengival, espessura gengival.*

INTRODUCTION

Gingival recession is characterized by the apical relocation of the gingival margin, exposing the root surface. Multiple factors are involved in its etiology, including anatomical, physiological, pathological and traumatic factors which probably do not act simultaneously or to the same degree,[1] and it is probably impossible to identify which the most important factor is. The development of gingival recession seems to be associated to inflammatory processes of different origins[2].

The occurrence of gingival recession varies widely, from 3 to 100%, depending of the population, diagnostic criteria and methods of analysis[3]. A study on a representative sample of subjects in a city in Brazil showed that the prevalence of gingival recession increases with age. The prevalence of at least one site with gingival recession ≥ 1 mm is 29.5% in youths aged 14-19 years and 99% in adults older than 40 years[4]. In France, it was demonstrated that 84.6% of individuals present at least one site with gingival recession[5]. Gingival thickness seems

to be an important risk and prognostic factor for the occurrence of future gingival recession[6]. Studies have shown that the gingival biotype is also a determinant of esthetic results in different therapies[7]. A thick gingival unit is associated with better results, especially concerning the stability of the gingival margin over time. However, most studies do not look at gingival thickness, particularly in healthy individuals. The aim of this study was thus to correlate gingival thickness and gingival recession in adults without history of periodontitis.

MATERIALS AND METHODS

A convenience sample of fifty-five adults (24 male and 31 female) aged 18-35 years participated in this cross-sectional study. Mean age (±SD) was 24.82 ± 5.17. The study included non-smokers, without history of periodontitis, under treatment at the School of Dentistry of the Federal University of Rio Grande do Sul, Brazil. Diabetic patients, pregnant and lactating women, individuals under orthodontic therapy, with history of periodontal surgery, presenting cervical restorations or under medication affecting the periodontium such as cyclosporin A, calcium channel blockers and phenytoin were not included. The research protocol was approved by the Institutional Review Board and all participants signed an informed consent form. The study followed the guidelines of the Declaration of Helsinki.

Sample size estimation

In order to estimate the sample size, data from the prevalence of gingival recession in the metropolitan area of Porto Alegre in the same age range were used[4]. Considering a prevalence of gingival recession higher than 70%, alpha and beta errors of 5 and 10%, it was determined that 43 individuals were necessary for the study. Twenty percent oversampling was used.

Clinical examination

Clinical periodontal parameters and gingival thickness were evaluated in upper or lower teeth (15-25 or 35-45) previously anesthetized for dental treatment. A previously trained examiner performed all clinical measurements. The following parameters were evaluated:

a) Visible Plaque Index (VPI)[8]: absence or presence of visible plaque after drying was scored.
b) Gingival Bleeding Index (GBI)[8]: absence or presence of gingival bleeding was scored after gentle probing of the gingival margin.
c) Gingival Recession (GR): the distance from the cemento-enamel junction (CEJ) to the gingival margin was measured in millimeters and rounded to the nearest millimeter. When the CEJ was not clinically visible, the measurement was given a negative sign.
d) Probing Depth (PD): the distance between the gingival margin and the most apical probeable part of the crevice was measured in millimeters and rounded to the nearest millimeter. A Williams periodontal probe was used.
e) Bleeding on Probing (BoP): absence or presence of bleeding after probing the bottom of the crevice up to 30 seconds after probing was recorded.
f) Clinical Attachment Level (CAL): obtained by adding PD and GR.

Measurement of gingival thickness

Gingival thickness (GT) was evaluated by piercing with a needle with a rubber stent perpendicular to the root surface at the mid-point between the gingival margin and the muco-gingival junction. The stent was pressed until it touched the gingival surface. After removing the needle, the distance from the end of the needle to the stent was measured with a digital caliper. All measurements were performed by the same examiner[9].

Reliability

Before the study began, the examiner was trained and calibrated for the GW measurements by measuring 5 individuals twice, with a 40-minute interval. The Intraclass Correlation Coefficient (ICC) was 0.98. During the study, trans-experimental reliability was tested in 5 patients, with the same methodology, and the ICC was 0.99.

Statistical analysis

Data analysis comprised descriptive and analytical approaches. Outcome values of all continuous parameters are shown as mean and standard deviation (SD). Correlations between gingival thickness and gingival recession were calculated using the Pearson correlation coefficient with the corresponding 95% confidence intervalthickness. After analysis of data distribution, linear regression models were analyzed (STATA 10.1 for Macintosh).

RESULTS

Full mouth periodontal clinical conditions of the individuals included in the study are shown in Table 1. Participants had good standards of oral health, with an average of 29.18 existing teeth. Plaque control showed values of visible plaque, gingival bleeding and bleeding on probing of approximately 15-20%. It was observed that 11.5% of existing teeth exhibited some degree of loss of attachment.

Table 1: Full mouth periodontal clinical variables of the subjects.

Variable	Mean Value	Standard Deviation
Existing Teeth (n)	29.18	1.85
Teeth with Loss of Attachment (%)	11.49	9.90
Visible Plaque Index (%)	18.86	19.60
Gingival Bleeding Index (%)	21.16	18.38
Bleeding on Probing (%)	16.25	17.37

Table 2: Clinical periodontal parameters at sites where gingival thickness was measured.

Variable	Mean Value	Standard Deviation
Probing Depth (mm)	1.28	0.49
Bleeding on Probing (%)	0.12	0.32
Gingival Recession (mm)	1.01	0.65
Gingival Thickness (mm)	1.40	0.28

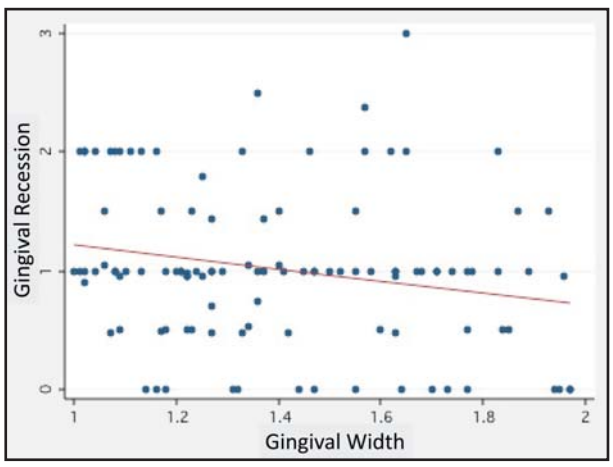

Fig. 1: Correlation between gingival thickness and gingival recession.

Table 2 shows periodontal clinical parameters at sites where gingival thickness was measured. At these sites, mean probing depth was 1.2 mm (range 0.95 – 3.00 mm). Gingival recession had a mean value of 1.01mm (range 0 – 3.5mm). Mean gingival thickness varied from 1 to 1.97mm, with a mean of 1.40mm.

Fig. 1 is the main outcome of the present study and demonstrates the correlation between gingival thickness and gingival recession: the smaller the gingival thickness, the greater the gingival recession. The Pearson correlation coefficient was -0.22. The simple linear regression model demonstrates a statistically significant relationship ($p= 0.02$).

DISCUSSION

This study evaluated possible association between gingival thickness and gingival recession, finding a statistically significant correlation between them. These results should be interpreted in the light of the literature and taking into account the strengths and limitations of the study.

This is a cross-sectional study, therefore causality cannot be claimed. However, a series of methodological principles were taken into consideration in order to increase the validity of the results. The study comprises a sample of young adults aged 18-35 years. The restriction to a specific age range relates to the fact that it has been demonstrated that the prevalence, extent and severity of gingival recession increase with age.[10,11] Susin et al.[4] demonstrated, in the same area where our study was performed, that in individuals 20-29 years old, 18% of teeth had gingival recession ≥ 1 mm. In our study, the mean extension of gingival recession was 13.6 teeth (range 6-26).

Individuals with previous exposure to periodontitis were excluded in order to focus attention on recession unrelated to periodontal disease. However, this potential confounder cannot be ruled out. On the other hand, even in individuals with loss of attachment due to periodontal disease, whether or not gingival recession occurred could have the gingival biotype as a predisposing factor.[9]

Studies demonstrate that the presence of oral biofilms and periodontal breakdown are associated with gingival recession. In this study, approximately 18% of the examined surfaces without recession presented visible plaque, sites with gingival recession did not present visible plaque. Moreover,

it should be noted that examined sites presented less bleeding on probing than full mouth scores (16.25 vs. 0.12%). Virtually no inflammation was observed at examined sites, suggesting that traumatic brushing could be part of the causal chain.

Additionally, this study did not include smokers, in order to reduce the potential confounder, since smoking has been strongly associated with higher degrees of loss of periodontal attachment[4].

Severity of gingival recession in this study was relatively low, with a range of 0-3.5mm and mean value 1.01mm. This finding is in accordance with the study by Susin et al.[4], where higher degrees of recession affected a very small proportion of individuals younger than 40 years of age.

This study restricted the examination to non-molars because anterior teeth tend to present higher degrees of gingival recession[2,3,4]. In addition, it is easier to measure gingival thicknessthickness on them, increasing the chances of higher reliability. Moreover, the sample size was estimated in order to ensure that the number of individuals included would be sufficient for drawing conclusions.

It should be noted that gingival recession is not an unavoidable physiological process due to aging, but may be explained by the cumulative effects of trauma and/or inflammation of the periodontium[3,4]. In this context, gingival thickness could play a role that should not be ruled out.

Vandana and Savitha[12] demonstrated that gingival thickness varies according to age, gender and dental arch. Younger individuals, men and upper jaws tend to present thicker gingiva. This would suggest a separate analysis for these factors. However, in this age range it was not possible to demonstrate differences in these aspects, therefore a combined analysis was performed[4]. Mean gingival thickness in our study was 1.40 mm, similar to that in the literature.[12] In the study by Vandana and Savitha[12], for example, mean gingival thickness was 1.63 - 1.73mm (1.59-1.78mm in 16- to 24-year-olds and 0.93-1.07mm in 25- to 38-year olds). thicknessThey suggest that alterations in the oral epithelium caused by aging may thin the epithelium and diminish keratinization.

Gingival thickness may be measured by invasive and non-invasive methods, but there are few studies comparing them. Savitha and Vandana[13] demonstrated that trans-gingival probing and ultrasound are both reliable. In our study, reliability was ensured by double measurements with an excellent intra-class correlation coefficient both prior to and during the experiment.

Gingival recession as a multifactorial entity has been extensively studied. Among possible etiological/ predisposing factors, the amount of keratinized gingiva has been suggested. However, studies failed to demonstrate causality in this respect.[14] Evidence suggests that even in the absence of keratinized gingiva, gingival recession is not a natural consequence.[14]

On the other hand, studies that associate gingival recession with gingival thickness are few and controversial. Our study found that the lower the gingival thickness, the higher the degree of gingival recession. However, due to data dispersion, a low Pearson Correlation Coefficient was observed among these variables. On the other hand, the statistically significant correlation suggests a consistent association. The regression line demonstrated a statistically significant p-value (0.025). This means that even though the correlation could be interpreted as low, a negative linear relationship exists among gingival recession and gingival thickness.

The limitations of our study should be taken into consideration in the conclusion process. These limitations include the fact that the study was cross-sectional and restricted to young adults. However, it is within the age range of our subjects that preventive strategies could be implemented. Follow-ups of cohorts starting early in adolescence could be an interesting way of overcoming these limitations. In conclusion, gingival thickness is inversely correlated to gingival recession in young adults with lower degrees of gingival inflammation.

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THE CONCENTRATION OF IL-1β IN SALIVA OF CHILDREN WITH ORAL LESIONS ASSOCIATED TO HISTIOCYTOSIS

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ABSTRACT

Langerhans Cell Histiocytosis (LCH) is a disease whose etiology and pathogenesis are still unknown. It affects several organs and tissues, producing lesions of different severity. Its histopathology and clinical picture suggest the participation of cytokines in its pathogenesis. IL-1β might have an important role in its development.

The purpose of this study was to determine the concentrations of IL-1β in saliva of pediatric patients diagnosed with LCH, with and without oral manifestations (Groups 1 and 2 respectively) compared to a Control Group (Group 3) of pediatric patients without medical antecedents or oral lesions.

The saliva of twenty patients with LCH was studied and compared to a Control Group consisting of eleven pediatric patients without medical antecedents. The children with histiocytosis, aged four months to sixteen years, were referred by the Oncohaematology Service at Garrahan Hospital and Hospital de Clínicas, to the Department of Comprehensive Children's Dentistry, School of Dentistry, University of Buenos Aires (UBA).

The concentrations of IL-1β in the different groups were determined using the Enzyme Immune Assay Kit (Cayman MI, USA) and expressed in pg/ml.

Results were analyzed by the Kruskall Wallis test. Significant differences between the three cohorts were found, ($H = 20.36$, $P < 0.001$). Dunn´s multiple comparison analysis was performed, which showed significant differences between Groups 1 and 2, and between Groups 1 and 3 ($P < 0.05$). Higher values of IL-1β were found in the patients with histiocytosis with oral manifestations (Group 1) than in patients without manifestations (Group 2) and patients in the Control Group (Group 3).

Key words: *Saliva, Histiocytosis, Langerhans cells, Interleukin, 1beta, mouth disease, child.*

CONCENTRACION DE IL-1β EN SALIVA DE NIÑOS CON LESIONES BUCALES ASOCIADAS A HISTIOCITOSIS

RESUMEN

La Histiocitosis de células de Langerhans (HCL) es una enfermedad de etiología y patogenia aún desconocidas. Afecta diferentes órganos y tejidos en los que produce lesiones de distinta gravedad. La histopatología de las lesiones y la clínica sugieren la participación de citoquinas en su patogenia. La IL-1β podría tener un rol importante en el desarrollo de la enfermedad.

El objetivo de este estudio fue determinar las concentraciones de IL-1β de las salivas de pacientes pediátricos con diagnóstico de Histiocitosis de Célula de Langerhans con y sin manifestaciones bucales (grupos 1 y 2 respectivamente), en relación a un grupo control (grupo 3), de pacientes pediátricos que no presentaron antecedentes médicos ni lesiones bucales.

Fueron estudiadas las salivas de 20 pacientes con la enfermedad de HCL, en relación a un grupo control de 11 pacientes pediátricos que no presentaron antecedentes médicos. Los niños con Histiocitosis cuyas edades oscilaban entre 4 meses y 16 años fueron derivados del servicio de Oncohematología del Hospital Garrahan y Hospital de Clínicas, a la Cátedra de Odontología Integral Niños de la Facultad de Odontología de la Universidad de Buenos Aires.

Se determinaron las concentraciones de IL-1β en los diferentes grupos, y se utilizó el Enzyme Inmune Assay Kit (Cayman, MI, USA), se expresó en pg/ml.

El análisis de los resultados se realizó según el test de Kruskall Wallis, se obtuvieron diferencias significativas entre los tres grupos ($H = 20,36$; $P<0,001$). Luego se realizó el análisis de comparaciones múltiples de Dunn que mostró diferencias estadísticamente significativas entre los grupos 1 y 2 y entre los grupos 1 y 3 ($p < 0,05$). Se observaron valores más elevados de IL-1β en los pacientes con Histiocitosis con manifestaciones bucales (grupo 1), en relación con el grupo sin manifestaciones bucales (grupo 2) y con el grupo control (grupo 3).

Palabras clave: *saliva, Histiocitosis, células de Langerhans, interleuquina 1beta, lesiones bucales-niños.*

INTRODUCTION

Langerhans Cell Histiocytosis (LCH) is a disease whose etiology and pathogenesis are still unknown. It is characterized by a proliferation of histiocytes, lymphocytes and eosinophilic cells that form clusters producing several types of lesions[1,2].

Differents organs and tissues are affected, mainly skin, bones, lungs and liver. Bone tissue is affected

in 72% of pediatric patients[3]. The most compromised parts are cranium (27%), femur (13%), jaws (11%), pelvis (10%), vertebral bodies (8%), ribs (8%), humerus (5%), and tibia (3%)[4,5]. Its presentation may vary from a single bone lytic lesion with possible self-resolution without therapeutic intervention, to a disseminated form requiring medical treatment, corticoid administration, and/or chemotherapy[6-8].

In addition, lesions may appear within the oral cavity. The most frequent are early dental eruption, dental loss, gingivitis, periodontitis, gingival enlargements and gingival bleeding [9-11].

The anatomical-pathological study of histiocytic lesions shows presence of Langerhans cells, which are gigantic multinucleated histiocytes similar to osteoclasts. Electron microscope images show intra-cytoplasmic inclusions: tennis racket shaped granules, called Birbeck granules[12-14], which are specific to pathological Langerhans cells. Their presence confirms diagnosis of LCH[15,16].

The diagnosis is completed through histochemical analysis to mark proteins CD1a and S 100 for these cells[4].

The etiology and pathogenesis of this disease are still unknown; however, there are numerous hypotheses about its origin, including infectious[17,18], immunological[3,19], genetic[20] and oncologic[13,17,21].

The immunological theory is one of the most convincing, because histologically the disease shows similarities to diseases of immune-reactive nature, and patients have diminished suppressing lymphocytes (CD8). In addition, the immunological alteration may be regulated by lymphokines and other growth factors that might modify the migration and maturation of Langerhans cells[22].

The pathogenesis of LCH is unknown and there is controversy regarding whether it is a reactive or a neoplastic process[23].

LCH is not recognized as a malignancy. Despite the existence of cell cloning, the disease does not spread through the lymphatic system, and no metastasis occurs[5,24-26].

After an indeterminate period of time, reactivations (new isolated lesions) appear in most patients. The reactivations may or may not differ from the initial lesion, and may even involve different tissues[25].

Despite the unknown origin of LCH, what is known is that many immunological mechanisms are involved in its pathogenesis, with the clinical picture and lesion histopathology suggesting that cytokines may participate in it[10,27].

Classification

Lichtenstein (1853) proposed the term *Histiocytosis X* to group three clinical entities:

Eosinophilic granuloma, Hand – Schüller – Christian´s disease, and Letterer –Siwe´s disease, adding the letter X to them because he did not know their etiology.[28]

In 1973, Neselof et al. proposed Langerhans cells (LC) as responsible for the disease, which they called Langerhans Cell Histiocytosis (LCH)[29].

To systematize the disease according to the organs involved and further treatment, in 1985 the *Histiocyte Society,* an international scientific entity, proposed the present classification[9,12,30,31], as follows:

Unifocal single-system histiocytosis: involves a single organ or tissue, usually bone or skin, and has a single location (focus). Corresponds to eosinophilic granuloma in the former classification.

Multifocal single-system histiocytosis: involves a single organ or tissue, with multiple foci. Corresponds to Hand – Schüller – Christian´s disease in the former classification.

Multisystem histiocytosis: involves multiple organs or tissues, with multiple foci. It is acute and usually occurs in infants from birth. Corresponds to Letterer Siwe´s disease in the former classification.

The aim of this study was to determine the concentrations of IL-1β in saliva of pediatric patients diagnosed with Langerhans Cell Histiocytosis, with and without associated oral histiocytosis lesions.

MATERIALS AND METHODS

The sample consisted of a total 31 pediatric patients, who were divided into three groups:

- Group 1: 10 patients with Langerhans Cell Histiocytosis, with lesions in the oral cavity.
- Group 2: 10 patients with Langerhans Cell Histiocytosis without oral lesions.
- Group 3: 11 patients with neither medical antecedents nor oral lesions.

Patients in Groups 1 and 2 were referred by the Oncohaematologic Service, Garrahan Hospital, diagnosed with Langerhans Cell Histiocytosis. Group 3 consisted of healthy pediatric patients who visited the Department of Comprehensive Children's Dentistry, School of Dentistry, University of Buenos Aires (UBA), for dental assistance.

The inclusion criteria for Groups 1 and 2 were confirmed diagnosis of Histiocytosis, having signs –

symptoms compatible with LCH seen by light microscopy, plus confirmation by electron microscopy of presence of Birbeck´s granules and positive inmuno- tracing with CD1a in the lesion cells (Table 1).
Patients taking any medication were excluded from all three Groups (Table1).

Methodology

1. Parent´s informed consent for performing this study was obtained. The study was accepted by The Ethics Committee of the School of Dentistry.
2. Dental files and history of the disease in each child were made.
3. Lesions of the oral mucosa were located and diagnosed through visual examination and palpation. Biopsies were sent to Department of Pathological Anatomy, School of Dentistry, Buenos Aires University.
4. Periapical and panoramic radiographs were taken of each child in order to detect osteolytic lesions.
5. Samples of non-stimulated saliva from the three groups were collected in sterile tubes, covered, and sent to the Department of Pharmacology at the same School in order to establish the concentration of IL-1β. The samples were stored in a freezer at -80º until they were analyzed.

All the procedures used are specified in the protocol for diagnosis of IL-1β.

RESULTS

Values for Group 1 (with lesions in the mouth) ranged from 2,625.26 to 749 pg/ml, most of them higher than 1,000 pg/ml.
Values for Group 2 (without oral lesions) ranged from 53.84 to 503.44 pg/ml.
Values for Group 3 (control) ranged from 10 to 496.78 pg/ml.
Medians were 1,309.11 for Group 1; 243.54 for Group 2, and 139.00 for Group 3 (Control) (Table 2).

Table 1: Inclusion criteria.

Groups	Inclusion	Exclusion
1	Signs-symptoms compatible with Langerhans Cells Hystiocitosis through Light microscopy. Confirmation of Birbeck's granules through Electronic microscopy	Ingestion of medicines
2	Positive inmuno tracing with monoclonal CD1a in the lesional cells	
3	With neither medical antecedents nor oral lesions	

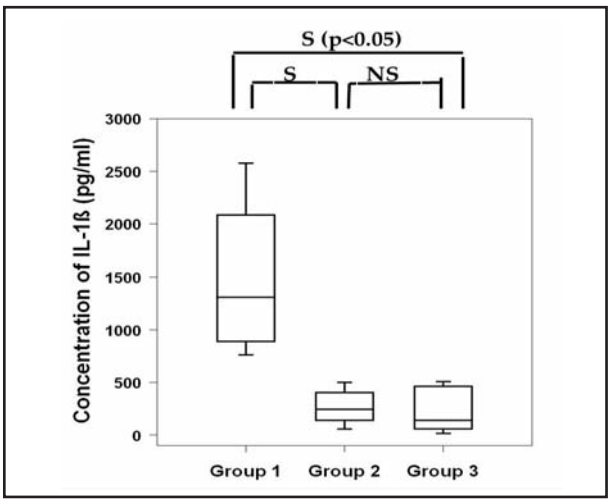


Fig. 1: Dunn´s multiple comparisons analysis (graphic). s: significant differences ns: non-significant differences.

Table 2: Concentrations of IL-1β in saliva collected from the study patients.

Group 1	Group 2	Group 3
2625,26	70,11	493,44
2146,92	503,44	10,00
1866,08	153,44	115,67
749,00	237,89	210,11
869,00	225,67	139,00
2075,67	53,84	496,75
885,67	249,19	32,33
1037,67	391,45	66,78
1168,61	467,19	470,02
1449,60	324,92	171,33
		50,30

The Kruskall Wallis test applied to the results showed significant differences among the three groups ($H = 20.36; P < 0.001$).

Dunn´s multiple comparison analysis showed statistically significant differences between Groups 1 and 2, and between Groups 1 and 3 ($p < 0.05$) (Fig. 1). Values of IL-1β were significantly lower in patients presenting lesions outside the mouth, and with no significant difference compared to Group 3 (Control).

DISCUSSION

For over 40 years, saliva has been considered an auxiliary tool in the diagnosis of oral diseases, because organic molecules of proteinic nature have been detected in its composition, mainly specific antigens, proteinic cell particles – receivers, glycoproteins, cytokines, (interleukines and derivatives), which may be linked to oral lesions[32,33].

Serum components can also reach saliva through the gingival crevicular fluid, providing potential application in the diagnosis of certain disorders [34].

The etiology of Langerhans Cell Histiocytosis has not yet been established; nevertheless, it is accepted that it is the expression of an immunological disorder[35].

Similarly, the pathogenesis of LCH is enigmatic, although the altered expression of cytokines and cellular adhesion molecules, which are important for migration and homing of the normal Langerhans cells (LC), may play an important role.[36-37]

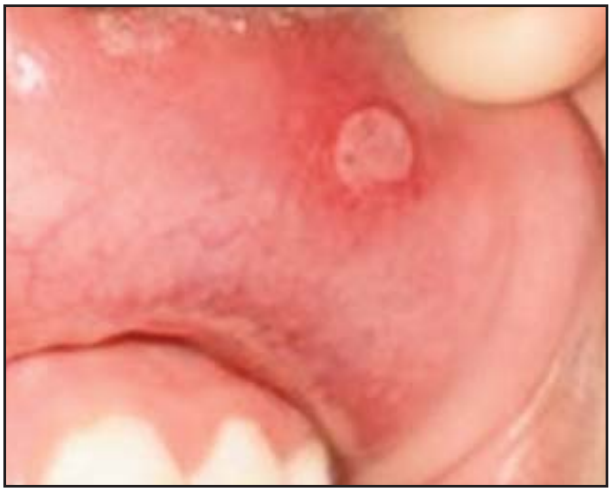


Fig. 2: Child, eleven years old, with multi-system LCH. Reactivation two years later: an erosive lesion on mucosa, compatible with an aphtha with more than 30 days of evolution is observed. Concentration of IL-1β in saliva: 1037.67 pg/ml.

Kannourakis et al. (1994) and Egeler (1999) suggested that cytokines participate in the histopathology of the lesions. It has been observed that the production of cytokines plays an essential role in reactivations of the disease, as well as in the inflammatory and immunological processes[27,38] Specifically, the IL-1β are important in the development and evolution of Langerhans cells; and the Langerhans cells also produce them. Interleukines act on osteoclasts linked to bone resorption[27,38].

IL – 1 was found in bone granulomas diagnosed as histiocytosis [10].

However, the regulation of cytokine production is still unknown [10].

In other oral diseases, high cytokine values were found in saliva. Kaushik et al. (2011) found high levels of IL-1β in saliva of patients with untreated chronic periodontal disease (comparable to those found in this study in chidren with oral lesions), and those levels decreased significantly after basic periodontal therapy[39]. Katakura et al. (2007) reported a significant increase in cytokines IL-1β and IL 16 in serum and saliva of patients with oral cancer compared to the healthy Control Group[40].

However, no previous study analyzing saliva of patients with histiocytosis was found.

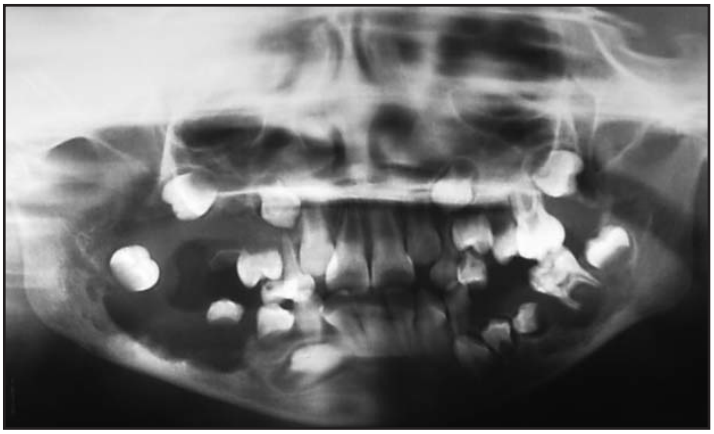
In the present study, among the patients with Langerhans Cell Histiocytosis who showed oral lesions, eight were primary and two were reactivations.

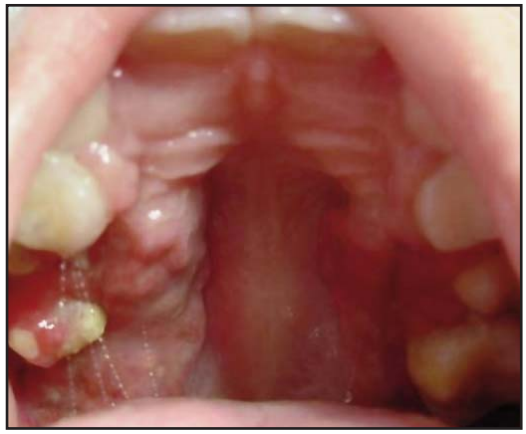
Among the oral manifestations, Hernandez and Juyol et al., found mandibular osteolysis, which can lead to a reduction in mandible height and bone loss linked to inflamed gingiva, looking like "floating teeth"[41] . The "floating tooth" is a pathognomonic sign of the disease[42].

In this study, the lesions in mucosa in Group 1 clinically look like erosions (Fig. 2), and osteolytic lesions of support bone associated to atypical enlargements of oral mucosa were observed (Figs. 3 and 4).

Solitary osteolytic lesions in maxillary bone (Fig. 5), bone reactivations with localized bone loss, similar to periodontal disease (Fig. 6), and bone lesions located in deciduous teeth ("floating tooth") were observed (Fig. 7).

In this study, open lesions were diagnosed in the oral cavity with inflammatory exudates and closed jaw injuries without any exudation. In all cases, high IL-1β values were found in saliva.





Figs. 3 and 4: Girl, nine years old, with multi-system LCH. Radiolucent areas compatible with large osteolytic lesions and loss of teeth are observed. Clinically, gingival enlargements are seen. Some teeth presented severe mobility. Concentration of IL-1β is 2075.67 pg/ml.

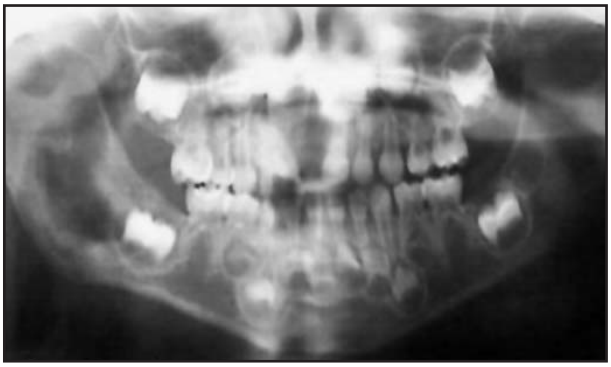


Fig. 5: Panoramic radiograph of a child, four years of age, with single-system unifocal LCH. A single extensive radiolucent area, compatible with an osteolytic lesion, in mandibular ramus. Concentration of IL-1β : 1866.08 pg/ml.

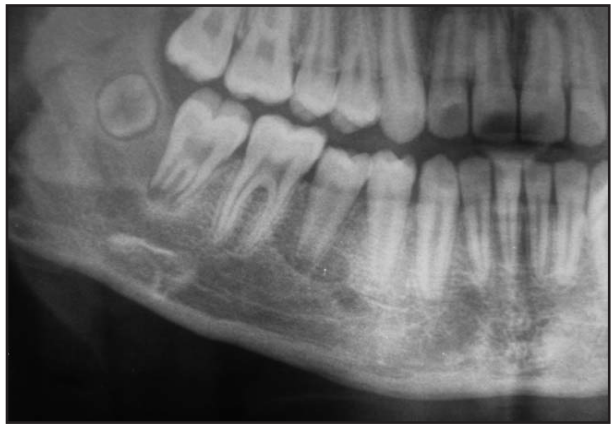


Fig. 6: Radiograph of child, 13 years old, with multi-system LCH. Tooth 4.5 submerged and a radiolucent area at the apex of the same tooth, compatible with a periodontal lesion are observed. Clinically, it presented a periodontal pocket, 11 mm depth located in 4.5. Concentration of IL-1β: 1449.60 pg/ml.

Figs. 2 to 7 are clinical examples showing the diversity and aggressiveness of oral lesions associated with LCH.

The panoramic radiograph (orthopantomograph) is the method of choice to study the jaws. It allows observation of the integrity of the cortex, presence of osteolytic lesions in maxilla and mandible that may compromise tooth buds, and comparisons with later radiographs during the follow-up of each patient[9,10].

The active bony lesions present indefinite borders, and are rounded or elliptic. In contrast, when the lesion is healing, the size and density of the trabecular bone within the lytic areas diminishes, and the thickness of the lesion margin increases, appearing as a radiopaque halo similar to cortex bone[43]. The high levels of IL-1β found in saliva of children with histiocytosis lesions in the oral cavity

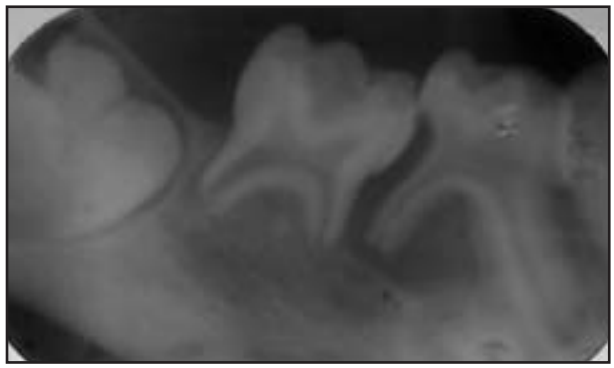


Fig. 7: Radiograph of child, 2 years of age, with multi-system LCH. A radiolucent area compatible with a localizad osteolytic lesion, at support bone of 8.5 "floating tooth" is observed. Concentration of IL-1β: 2146.92 pg/ml.

allow us to assess or raise the value of saliva as a fluid that could contribute to the diagnosis of LCH. It is concluded that:

- Pediatric patients with Langerhans Cell Histiocytosis with oral manifestations show high values of IL-1β in saliva, which are higher than those in patients who suffered the disease without oral manifestation, and higher than those in the group of healthy children.
- A saliva sample, which is a simple, non-invasive procedure, may allow detection of high concentrations of IL-1β associated to primary or reactivation lesions in the mouths of children with Langerhans Cell Histiocytosis.
- Further studies should be conducted on a larger sample to confirm the association between the oral lesions and the interleukines in saliva from patients with LCH.

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AN EXPERIMENTAL MODEL OF DISUSE IN THE ALVEOLAR RAT BONE. A HISTOMORPHOMETRICAL STUDY

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ABSTRACT

Lack of mechanical stimulation is known to cause disuse osteopenia in bones. However, experimental models for disuse osteopenia on bones other than jawbones are not applicable to jawbones. The little available information in this field has been applied to the study of overeruption of teeth lacking antagonists. However, the absence of an antagonist in the opposite jaw means that there is no stimulation by occlusion. Our hypothesis is that the lack of stimulation due to the absence of teeth causes disuse osteopenia in the interradicular bone of the antagonist teeth. Our aim was to develop a model of disuse osteopenia due to the absence of occlusal forces. We used male Wistar rats with 215-230 g body weight, divided into 2 groups: one absolute control group (C) and one experimental group in which the three right lower molars were extracted (E). The left side of the jaw in the experimental group was used as a paired control (PC). The animals were euthanized 7 days after extraction. The jaws were placed in occlusion, fastened and fixed in 10% formalin. The heads were cut in half and radiographs made of both jaws. The upper jaws were processed histologically. After decalcification, bucco-palatine oriented sections were cut at the level of the mesial root and distal roots of the first upper molars. On the radiographs, the distance from the tip of the cusp on the first upper molar to the antagonist edentulous ridge (DA) was measured. On the microphotographs, the following parameters were measured: passive eruption degree (PED), height of periodontal ligament at the level of the furcation (HPL) and interradicular bone volume (BV_I). The data were compared statistically using ANOVA and Bonferroni's post-hoc test, considering $p< 0.05$ as statistically significant. DA in experimental animals was 0.34±0.048 mm. PED in experimental animals was significantly greater than in the control groups, both for the buccal plate and for the palatal plate. HPL showed no significant difference between groups. BV_I was significantly lower in the experimental group than in the control group. The results showed that the model used produces a condition of disuse osteopenia, shown by the statistically significant reduction in interradicular bone volume. The use of this model at different experimental times will enable the evaluation of cell responses in periodontal tissues, particularly bone tissue, e.g. to compare them to known responses such as the application of orthodontic forces.

Key words*: disuse, experimental model, alveolar bone.*

MODELO EXPERIMENTAL PARA EL ESTUDIO DEL EFECTO DEL DESUSO EN EL HUESO MAXILAR SUPERIOR DE RATA

RESUMEN

Se sabe que la falta de estímulo mecánico produce un cuadro de osteopenia por desuso en huesos de la economía. Los modelos experimentales de osteopenia por desuso utilizados en otros huesos no son aplicables a los huesos maxilares. La escasa información que existe en este campo se aplicó al estudio de la sobreerupción de dientes sin antagonista. Sin embargo, la ausencia de antagonista en el maxilar opuesto hace que falte el estímulo de la oclusión. Por tal razón, nuestra hipótesis es que la falta de estímulo por ausencia de piezas dentarias provoca un cuadro de osteopenia por desuso en el hueso interradicular de los dientes antagonistas. Nuestro objetivo fue poner a punto un modelo de osteopenia por desuso debido a la ausencia de fuerzas oclusales. Se emplearon ratas Wistar machos de entre 215-230 g de peso corporal divididas en 2 grupos, un grupo control absoluto (C) y un grupo experimental al que se le extrajeron los tres molares inferiores derechos (E). El lado izquierdo del maxilar del grupo experimental, fue utilizado como control apareado (CA). A los 7 días de efectuadas las extracciones se realizó la eutanasia de los animales. Los maxilares se colocaron en oclusión, se precintaron y así ubicados se fijaron en formol 10%. Se dividieron las hemicabezas y tomaron radiografías de ambos maxilares siendo procesados histológicamente los maxilares superiores. Luego de la descalcificación se obtuvieron cortes orientados en sentido buco-palatino a nivel de la raíz mesial y de las raíces distales de los 1°molares superiores. Sobre las radiografías se midió la distancia desde el vértice cuspídeo del 1° molar superior al reborde antagonista desdentado (DA), y sobre microfotografías se midieron los siguientes parámetros: grado de erupción pasiva (GE), altura del ligamento periodontal a nivel de la furcación (ALP) y volumen óseo interradicular (VO_I). Los datos se compararon estadísticamente mediante el test ANOVA y prueba post hoc de Bonferroni, considerando una $p< 0,05$ como estadísticamente significativa. La DA en los animales experimentales fue de 0,34±0,048 mm. La GE en los animales experimentales fue significativamente mayor que en los grupos control, tanto a nivel de la tabla vestibular como de la tabla palatina. La ALP no mostró diferencias significativas entre los grupos. El VOi fue significativamente menor en el grupo experimental con respecto a los controles. Los resultados mostraron que el modelo empleado logra una condición de osteopenia por desuso manifestada por la disminución del volumen óseo interradicular. La utilización de este modelo de desuso a diferentes tiempos experimentales permitirá evaluar las respuestas celulares de los tejidos periodontales, especialmente del tejido óseo, permitiendo por ejemplo, compararlas con respuestas conocidas como la aplicación de fuerzas ortodóncicas.

Key words: *desuso, modelo experimental, hueso alveolar.*

INTRODUCTION

Disuse osteoporosis is characterized by bone loss due to the absence of skeletal mechanical loads[1]. It occurs in situations of immobility such as paralysis associated to lesions of motor nerves or muscular dystrophy, or to changes in the mechanical environment (e.g. space flight and long-term bed rest), some of which have been taken as human models of disuse osteoporosis[2]. The most frequently cited experimental models in animals that replicate these conditions are the spinal cord injury model[3] and the hind limb unloading model[4]. Non-surgical models which have become relevant in recent years include intramuscular injection with botulinum toxin A (BTX)[5], which leads to transient muscle paralysis, resulting in a rapid loss of muscle mass and function[6,7]. All these disuse models lead to bone loss in long bones and vertebral column[5,8].

With regard to the jawbones, the model of the unopposed rodent molar has been used to study the mechanisms of tooth eruption (axial movement of teeth)[9-11]. In this model, unloading of the right-side mandibular teeth is accomplished by extraction of the right-side maxillary molars. Functional occlusion of the molars on the left side is maintained, and the time between the extractions and euthanasia has varied widely, from hours to days, weeks and even a month[12]. These studies showed that unloading-induced biological tooth movement in mice is a result of osteoclastic bone resorption on the distal aspect of the alveolar socket combined with alveolar bone and cementum formation on the mesial and apical aspects of the alveolar socket[9,10]. However, the unloading capacity in this model has not been used to evaluate potential disuse-related bone loss, which is of interest given recent use of orthodontic treatment in adults who may be suffering osteoporosis. Thus, our aim was to develop an experimental model in order to evaluate the effect of disuse on the upper jawbone in rats based on the hypothesis that the absence of teeth will lead to disuse-related bone loss in the interradicular bone of antagonist molars due to the absence of occlusal forces.

MATERIALS AND METHODS

Animal procedures

Ten male Wistar rats weighing 215-230 grams b.w. were used. All procedures were reviewed and approved by the Ethics Committee of the School of Dentistry of the University of Buenos Aires (UBACYT 2011-2014-3), which follows the Guide for the Care and Use of Laboratory Animals (NRC 1996). The animals were divided into two groups, an absolute control group (C) with n=5, and an experimental group with n=4, in which the three lower right molars were extracted, so that the zone of the upper right molars was the experimental side (E) and the zone of the upper left molars were the paired control (PC). The animals were housed in cages and fed a soft diet *ad libitum* for the duration of the experiment. Seven days after the extractions, they were euthanized by barbiturate overdose under general anesthesia.

Dental extractions

Extraction of lower molars of right hemimandible was performed under general anesthesia by intraperitoneal (IP) injection of ketamine 40 mg/kg (Ketamina 50, Holliday-Scott S.A., Beccar, Buenos Aires, Argentina) and xylazine 5 mg/kg (kensol König, Laboratorios König S.A., Avellaneda, Buenos Aires, Argentina) following the technique described by Guglielmotti and Cabrini[13]. The contralateral maxilla (left) was considered to be its paired control, and the maxillae of another five animals with all their teeth were used as absolute controls.

Sampling

The animals were sedated with 0.5 mg/kg b.w. of acepromacine and then euthanized by sodium thiopental (Pentotet®, Richmond Vet, Buenos Aires, Argentina) overdose. The jaws were positioned in occlusion, and fastened by wrapping with elastic bands to ensure contact between continuously growing incisors and molars and prevent the dental arches from separating (Fig. 1). The heads prepared in this way were resected and fixed in 10% buffered formalin.

Radiographic Techniques

After fixing, the two halves of the head were separated, while keeping the jaws closed, and standardized radiographs were taken of the jaws in occlusion (Fig. 2). Radiographs were taken using periapical dental radiographic film and MTX 70 mV 8mA dental X-ray equipment (Dental San Justo, Buenos Aires, Argentina) at 70 Kv and 0.8 sec exposure time; the focus-to-film distance

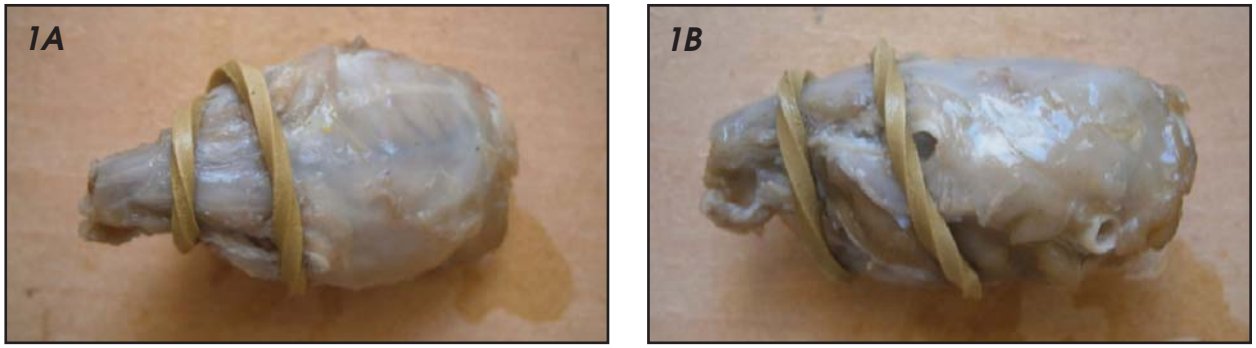

Fig. 1: Heads of animals after euthanasia, fastened in occlusion with elastic bands to ensure contact between continuously growing incisors and molars, and to prevent the separation of dental arches. A) Top view; B) lateral view.

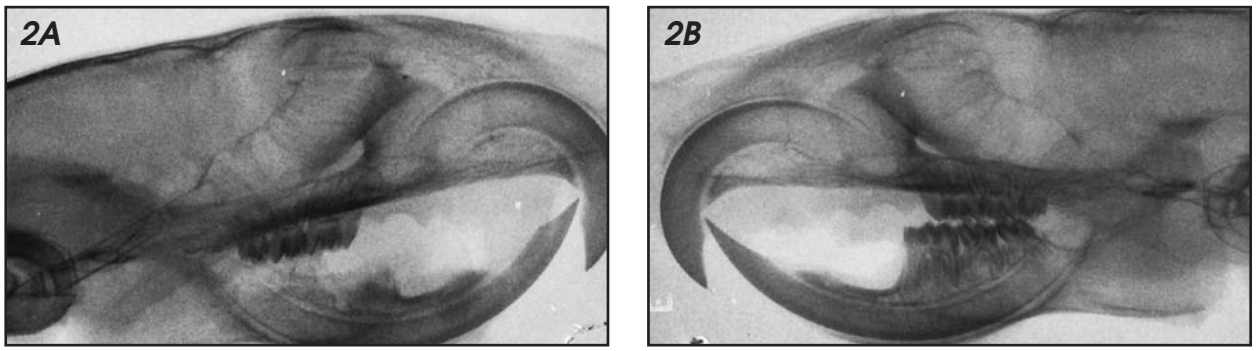

Fig. 2: Standardized radiographs of the heads with jaws in occlusion showing contact between continuously growing incisor and palatal mucosa. A) Right half of the head from the experimental group showing absence of lower molars and absence of contact between upper molars and antagonist ridge. B) Left half of the head showing molars in occlusion in an animal from which teeth were not extracted.

was 40 cm. Radiographs were processed following standard protocols. Checking contact between the continuously growing incisor and the palatal mucosa in the radiograph confirms the position in occlusion of the edentulous hemimandibles.

Sample processing

The hemimandibles were decalcified in EDTA (ethylenediaminetetraacetic acid) for four months at pH 7 and room temperature, and embedded in paraffin. Six-micron thickness bucco-palatal oriented sections at the level of the mesial root and at the level of the distal roots of the first upper molar were obtained and stained with hematoxylin-eosin (H-E) following standard protocols.

Morphometry and Histomorphometry

The radiographs were scanned with an Hp Scanjet G2710 scanner, on TMA negative scanning mode, grayscale and micrographs were taken of the histological preparations using a Canon Powershot A640 10.0 megapixel camera with 4x optical zoom (Canon Inc, Tokyo, Japan) mounted on a Carl Zeiss Axioscop 2 optical microscope (Carl Zeiss mikroscopie, Jena, Germany). The Image Pro-Plus 5.1 software was used to measure the distance from the tips of the cusps of the first upper molar to the ridge of the edentulous antagonist (DA) on the radiographs (Fig. 3A) and the following parameters on the micrographs: passive eruption degree (PED) (Fig. 3B), height of periodontal ligament (HPL) and interradicular bone volume (BV_I) (Fig. 3C). The data were compared statistically with ANOVA and Bonferroni's *post hoc* test, taking $p<0.05$ as significant.

RESULTS

Distance from the tip of the cusp of the first upper molar to the ridge of the edentulous antagonist (DA)

In the experimental group, DA showed positive values with an average of 0.34±0.048 mm, indicating that in no case was there contact between the first right upper molar and the antagonist ridge.

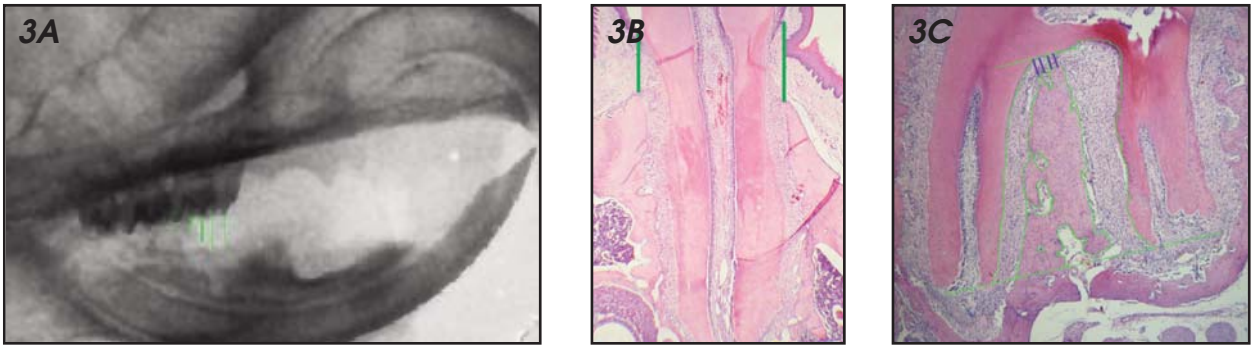

Fig. 3: Morphometry and histomorphometry measurements. A) Radiograph of one half of the head of an experimental animal showing the measurement of the distances (green lines) from the tips of the cusps of the first upper molar to the ridge of the edentulous antagonist (DA); B) Microphotograph of the mesial root of the first upper molar showing the measurement of the passive eruption degree (PED) determined as the distance from the vestibular and palatal alveolar crests to the cement-enamel junction (green lines); C) Microphotograph of distal roots of the first upper molar showing the measurement of the height of the periodontal ligament at the level of the furcation (HPL) (blue lines) and measurement of the interradicular bone volume (BVI) (green line).

Passive eruption degree (PED)

The distance from the crest of the buccal and palatal plates to the cement-enamel junction of the first upper molar was significantly greater in the experimental group than in either of the control groups and on both plates, with no difference between the paired control group (PC) and the absolute control group (C) (Table 1).

Height of the periodontal ligament (HPL)

There was no significant difference in the height of the periodontal ligament among the 3 groups (Table 2).

Interradicular bone volume (BV/TV)

Interradicular bone volume was significantly lower in the experimental group than in the control groups, and there was no difference between control groups (Table 2).

DISCUSSION

Although the literature includes studies in which occlusal forces are eliminated, this is the first study to conduct a histomorphometric evaluation of the antagonist alveolar bone to the edentulous ridge[9-11]. It is important to note that most experimental orthodontic models used universally are applied on the first upper molar. Thus, having a model that replicates a disuse condition in the upper jaw allows us to compare conditions with and without forces on the same bone.

Disuse osteoporosis corresponds to bone loss due to skeletal mechanical unloading. The condition has been observed in various situations, and can be described as models with immobilizations linked to changes in mechanical environment, such as space flight or long term bed-rest, and pathological immobilizations, such as those observed during neurological or muscular diseases, spinal cord injury being the most frequently quoted model[1,2]. The notion of disuse osteoporosis appeared in the literature in the seventies with the reports of histomorphometry analysis of iliac crest bone biopsies performed at various times after the spinal cord injury[14]. Trabecular bone volume exhibits rapid bone loss in the first three

Table 1: Degree of passive eruption (DE). Distance from buccal bone crests (BP) and palatal bone crest (PP) to the cementoenamel junction.

	BP	PP
E	523.55 ± 5.68 µm*	949.83 ± 136.85 µm*
C	251.73 ± 53.61 µm	427.51 ± 142.53 µm
PC	295.54 ± 82.85 µm	509.30 ± 58.96 µm

E: experimental side; C: absolute control;
PC: paired control side; *p<0.05

Table 2: Height of the periodontal ligament (HPL) measured at the level of the furcation and interradicular bone vloume (BV/TV).

	E	PC	C
HPL	124.5±11.99µm	163.76 ±91.96µm	156.11±78.26µm
BV/TV	0.78±0.12*	0.88±0.03	0.82±0.06

E: experimental side; C: absolute control
PC: paired control side; *p<0.05

months of disuse, followed by slower loss. In parallel, bone remodeling is found uncoupled with an increase in bone resorption and a decrease in static and dynamic bone formation parameters[15].

The use of this model with different experimental times will enable an evaluation of cell responses in periodontal tissues, particularly bone tissue, which can be compared to the known response of the bone to the application of forces such as orthodontic forces, and in various systemic conditions. In addition, it will subsequently allow us to compare the results with other models of absence of force in other parts of the body (tibia, femur, spine), and to compare them to the external application of forces or of different systemic conditions. The fact that there was no significant difference in the results between the Control group and the Paired Control group means that fewer experimental animals will be needed in further studies.

Potential results of further experiments replicating the disuse condition in the upper maxillary bone due to absence of occlusal forces caused by missing antagonist teeth will be transferred to the clinical understanding of the biological mechanisms governing periodontal tissues and alveolar bone of partially edentulous patients who do not use dental prostheses to replace their missing teeth.

The frequency of aged patients receiving orthodontic treatment has increased in recent years. Osteoporosis is often associated with aging. Recent studies[16,17] report that ovariectomy (OVX) accelerated orthodontic tooth movement (OTM) in rats. Acceleration of OTM is expedient for orthodontists because it can reduce treatment duration; however, it also involves the risk of side effects. These studies showed that OVX accelerates OTM but also induces severe root resorption. With the increasing demand for orthodontic treatment in adult patients, orthodontists need up-to-date knowledge regarding age-related metabolic changes and the effects of medications.

The reduction in bone volume measured after applying the proposed model shows that it is effective for inducing disuse oestopenia in the alveolar bone in the jaws of rats.

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ANTISEPTIC MOUTHWASHES: *IN VITRO* ANTIBACTERIAL ACTIVITY

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ABSTRACT

Mouthwashes are used as an adjunct to tooth brushing for improving breath and preventing oral diseases. The aim of this study was to compare the in vitro Maximum Inhibitory Dilution (MID) of 3 mouthwashes with different active ingredients against mutans streptococci (MS). The products analyzed were Periogard®, Cepacol® and Plax® Fresh Mint. Their antibacterial activity was assessed in duplicate in 96-well microtiter plates against 36 clinical isolates of MS. Each mouthwash was submitted to a serial two-fold dilution (1/2.5 to 1/5120) using double concentration of Tryptose Soy Broth with 1.0% yeast extract. The final volume in each well was 100 mL plus 5 mL of a bacterial suspension, equivalent to 10^7 CFU/mL. They were incubated microaerobically at 37ºC for 48 hours and the MIDs determined. MID was 1/320 for Periogard® and Cepacol®, and 1/20 for Plax®. Statistical analysis revealed that the MID of Periogard® MID did not differ from that of Cepacol® ($p>0.05$), and was higher than that of Plax® ($p<0.05$). In conclusion, the antiseptic mouthwashes containing chlorhexidine (Periogard®) and cetylpyridinium chloride (Cepacol®) had higher in vitroantibacterial activity (MID) against MS than the antiseptic mouthwash containing triclosan (Plax®), according to microbiological method employed.

Keywords: *Microbial Sensitivity Tests, Mouthwashes, Streptococcus.*

ANTISSÉPTICOS BUCAIS: ATIVIDADE ANTIBACTERIANA

RESUMO

Os antissépticos bucais são utilizados mundialmente como adjuvantes da escovação para melhoria do hálito e prevenção de doenças bucais infeciosas. O objetivo deste estudo foi comparar in vitro a Diluição Inibitória Máxima (DIM) de 3 antissépticos bucais com diferentes princípios ativos contra estreptococos do grupo mutans (EGM). Os produtos analisados foram Periogard®, Cepacol® e Plax® FreshMint. A atividade antibacteriana foi avaliada em duplicata em placas de microtilulação de 96 poços contra 36 isolados clínicos de EGM. Cada antisséptico bucal foi submetido a diluição dupla seriada (1/2,5 a 1/5120) com o emprego de concentração dupla de TryptoseSoyBrothwith adicionado de 1,0% de extrato de levedura. O volume final em cada poço foi de 100 mL mais 5 mL da suspensão bacteriana equivalente a 10^7 UFC/mL. A incubação foi realizada em microaerofilia a 37ºC por 48 horas e a DIM deteminada. Periogard® e Cepacol® apresentaram DIM de 1/320, e Plax® de 1/20. Os resultados submetidos asanálises estatísticas revelaram que a DIM do Periogard® não foi diferente do Cepacol® ($p>0,05$) sendo maior que do Plax® ($p<0,05$). Em conclusão, os antissépticos bucais contendo clorexidina (Periogard®) e cloreto de cetilpiridínio (Cepacol®) demonstraram maior atividade antibacteriana in vitro (DIM) contra os EGM do que o antisséptico bucal contendo triclosan (Plax®) de acordo com o método microbiológico utilizado.

Palavras-chave: *Testes de Sensibilidade Microbiana, Antissépticos Bucais, Streptococcus.*

INTRODUCTION

Mouthwashes have been widely employed in the fields of Preventive Dentistry and Periodontics[1-3]. The use of antimicrobial mouthwashes has been proposed as a means for reducing the levels of oral bacteria, specifically *Streptococcus mutans*. A wide range of mouthwashes containing different active ingredients is available in the market.

Periogard® is a mouthwash which contains 0.12% chlorhexidine gluconate and other ingredients (Table 1). Chlorhexidine is a cationic biguanide with broad-spectrum antimicrobial action, effective against dental biofilm and gingivitis[3-6]. Chlorhexidine has an important feature, substantivity, due to its ability to bind to oral tissues and act for extended periods after application[4].

Cepacol® is a cetylpyridinium chloride-based (CPC) mouthwash (Table 1). CPC is a quaternary ammonium compound included in the group of the cationic surface-active agents[7]. It acts primarily by penetrating the cell membrane, causing leakage of cell components, disruption of the bacterial metabolism, inhibition of cell growth, and finally, cell death[8].

Plax® contains 0.03% triclosan (2,4,4'-trichloro-2'-hydroxydiphenyl ether), 0.20% polyvinyl-methyl ether/maleic acid (PVM/MA) copolymer which is used jointly with triclosan to increase its antimicrobial activity,[9] and other ingredients (Table 1).

Due to their ability to form biofilms on teeth, mutans streptococci (MS) are considered major etiological agents of human dental caries[10]. Thus, mouthwashes containing active ingredients against them may help prevent caries. Mouthwashes are expected to maintain their antimicrobial activity even after dilution in oral fluids, and their antimicrobial activity against different strains of *S. mutans* can be evaluated *in vitro*,

The aim of this study was to compare *in vitro* Maximum Inhibitory Dilution (MID) of 3 mouthwashes containing different active ingredients against 36 clinical isolates of MS.

MATERIAL AND METHODS

The following products were evaluated: Periogard® (Colgate-Palmolive, São Bernardo do Campo, SP, BR), Cepacol® (Aventis Pharma, Suzano, SP, BR) and Plax® Fresh Mint (Colgate-Palmolive, São Bernardo do Campo, SP, BR) as shown in Table 1.Antibacterial activity was assessed in duplicate in 96-well microtiter plates against 36 clinical isolates of mutans streptococci (MS).

Each mouthwash was submitted to a serial two-fold dilution (1/2.5 to 1/5120) using double concentration of Tryptose Soy Broth with 1.0% yeast extract. The final volume in each well was 100 mL plus 5 mL of a bacterial suspension equivalent to 10^7 CFU/mL. They were incubated microaerobically at 37ºC for 48 hours and the MIDs determined (the highest dilution of each product that inhibited the bacterial growth).

Statistical analysis

Results were expressed as scores determined from MID. Groups were compared using the Kruskal-Wallis nonparametric test. When this test showed significant difference between groups, Dunn's multiple comparison test, which allows two-by-two comparison between groups, was applied. The significance level in the statistical testing was 5% ($p<0.05$).

Table 1: Chemical composition of the mouthwashes.

Mouthwash	Composition	
Periogard® (Colgate-Palmolive, São Bernardo do Campo, SP, BR)	Chlorhexidinegluconate (0.12%) Water Glycerin Ethanol Polysorbate 20 Flavoring agents Sodium saccharin FD&C Blue nº1	
Cepacol® (Aventis Pharma, Suzano, SP, BR)	Cetylpyridinium chloride (0.05%) Disodium EDTA Sodium saccharin Polysorbate 80 Glycerin Water Sodium phosphate monobasic anhydrous	Disodium phosphate anhydrous Eucalyptol Menthol Methyl salicylate Mint oil Chinese cinnamon flavor Yellow tartrazine Ethyl alcohol 96GL
Plax® Fresh Mint (Colgate-Palmolive, São Bernardo do Campo, SP, BR)	Triclosan (0.03%) Sodium fluoride (227ppm fluoride) PVM/MA copolymer(0.20%) Ethanol Disodiumphosphate Glycerin Sodium hydroxide	Sodium lauryl sulphate Sodium methyl taurate Sodium saccharin Sorbitol Blue CI42090 Yellow CI47005 Flavoring agents Water

RESULTS

The mouthwashes had different MIDs. Statistical analysis revealed that the MID of Periogard® did not differ from that of Cepacol® ($p>0.05$) but was higher than that of Plax® ($p<0.05$) against 36 clinical isolates of MS (Table 2).

DISCUSSION

A wide range of mouthwashes containing different active ingredients is available on the market. It is important to know their antimicrobial activity because they are mainly employed to control microorganisms. This study conducted a comparative analysis among three different mouthwashes - chlorhexidine gluconate, cetylpyridinium chloride and triclosan-based solutions – which were effective against MS from the initial dilution of 1/2.5.

Chlorhexidine gluconate mouthwashes have been available on the market for a long time with concentrations ranging from 0.12% to 0.2%. The chlorhexidine-based mouthwash evaluated in this study (Periogard®) contains 0.12% chlorhexidine gluconate.

In this study, Periogard® had the best *in vitro* antibacterial activity against MS, because it inhibited all the clinical isolates of MS at the 1/320 dilution. However, our results disagree with MIDs found in other studies, of 1/16[11], 1/80[12] and 1/160[13]. Other studies found that Cepacol® was able to inhibit MS at 1/20[12] and 1/40[13] dilutions. In contrast, our study found that all clinical isolates of MS were inhibited by Cepacol® at 1/320 dilution.

In our study, Plax® had lower MID than Periogard® and Cepacol® ($p<0.05$) and inhibited the growth of all MS at the 1/20 dilution, in agreement with André et al. [13]. Thus, triclosan used with PVM/MA copolymer had *in vitro* antibacterial activity against the different clinical isolates of MS.

McMurry, Oethinger and Levy[14] demonstrated in a study with *Escherichia coli* that the antibacterial activity of triclosan is due to its ability to block the synthesis of fatty acids by inhibiting the enoyl-acyl carrier protein reductase enzyme. This specific action affects several lipid synthesis-dependent processes, resulting in secondary effects on the cytoplasmic membrane[15].

Giertsen[16] analyzed mouthwashes containing triclosan (3.5mM) associated with sodium lauryl sulphate (17.4mM), which were found to have a synergic effect against *S. mutans*(NTCC 10449). The MIC of triclosan and sodium lauryl sulphate against *S. mutans*was 13.49µM. Moreover, sodium lauryl sulphate is an anionic surfactant that has often been used in mouthwashes and toothpastes and it is included in the Plax® formula (Table 1). In addition, Plax® contains a sodium fluoride

Table 2: Data of 36 clinical isolates of mutans streptococci inhibited by each dilution of the mouthwashes.

	Periogard®				Cepacol®				Plax®			
	Inhibited strains		Cumulative data		Inhibited strains		Cumulative data		Inhibited strains		Cumulative data	
Dilution	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
1/5120	1	2.8	1	2.8	0	0	0	0	0	0	0	0
1/2560	1	2.8	2	5.6	2	5.6	2	5.6	0	0	0	0
1/1280	25	69.4	27	75.0	21	58.3	23	63.9	0	0	0	0
1/640	8	22.2	35	97.2	12	33.3	35	97.2	2	5.6	2	5.6
1/320*	1	2.8	36[a]	100.0	1	2.8	36[a]	100.0	25	69.4	27	75.0
1/160	-	-	-	-	-	-	-	-	1	2.8	28	77.8
1/80	-	-	-	-	-	-	-	-	0	0	28	77.8
1/40	-	-	-	-	-	-	-	-	0	0	28	77.8
1/20*	-	-	-	-	-	-	-	-	8	22.2	36[b]	100.0
1/10	-	-	-	-	-	-	-	-	-	-	-	-
1/5	-	-	-	-	-	-	-	-	-	-	-	-
1/2.5	-	-	-	-	-	-	-	-	-	-	-	-

*Kruskal-Wallis's nonparametric test and Dunn's multiple comparison test ($p<0.05$). Different letters indicate statistically significant difference.

concentration of 270 ppm (270 µg/mL), which is less than that required for the MIC of 600 µg/mL against *S. mutans*[17].

Herrera et al. [18] evaluated the antimicrobial activity of four commercially available mouthwashes, three containing 0.12% chlorhexidine (one containing alcohol and two alcohol-free) and one containing 0.12% chlorhexidine and 0.05% CPC (alcohol-free). They observed that the product containing alcohol was more effective than the alcohol-free rinses, except for the formulation including chlorhexidine and CPC, which had greater antimicrobial activity.

The difference in the MID results of these studies may be related to the different sources of the MS (ATCC strain[11], saliva[13] and dentures[12]).

According to Carlinet al. [19] Periogard® and Plax Whitening® can induce genetic damage. DNA damage is considered to be the prime mechanism during chemical carcinogenesis, and these data may be relevant in risk assessment for protecting human health and preventing carcinogenesis. Moreover, the ethanol concentration present in Cepacol induced mitotic recombination between homologous chromosomes in the Drosophila SMART assay involved in the genesis of numerous diseases, including cancer[20].

In conclusion, the mouthwashes containing chlorhexidine (Periogard®) and cetylpyridinium chloride (Cepacol®) had higher *in vitro* antibacterial activity (MID) against MS than the mouthwash containing triclosan (Plax®), according to microbiological method employed. Further studies should be conducted to evaluate the *in vitro* and *in vivo* antimicrobial activities of Periogard®, Cepacol®, Plax® and other mouthwashes against MS and a wide range of oral microorganisms, as well as their cytotoxicity.

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ASSOCIATION AMONG SALIVARY FLOW RATE, CARIES RISK AND NUTRITIONAL STATUS IN PRE-SCHOOLERS

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ABSTRACT

Modéer T. et al.(2011) claim that there is association between decreased salivary flow rate and caries in obese adolescents. The aim of this study was to determine the association among nutritional status, salivary flow rate and caries risk in preschoolers. The study comprised 60 children aged 3 to 6 years attending kindergartens in areas immediately adjacent to Buenos Aires City, Argentina. Body weight and height of the children were determined. Body mass index was calculated and the population was classified anthropometrically according to the WHO 2007 (WHO Anthro. Program). Caries risk was determined. Saliva was collected in sterile graduated wide-mouth containers, without stimulation and without food restrictions. Salivary flow rate (SFR) was determined. Statistical analysis was performed using Pearson's test. It was found that 56.7% (IC95%: 37.7-74.0) of anthropometrically adequate children (Ad) and 37.0% (IC95%: 20.1-57.5) of overweight and obese children (OW/Ob) had caries. The odds ratio for caries (OR=3.78; IC95%: 1.2–11.8, p=0.02) was almost 4 times higher in adequate children than in the others. SFR was 0.534 ± 0.318 ml/min in Ad and 0.439 ± 0.234 ml/min in OW/Ob. Pearson's test showed no correlation between SFR and nutritional status (r= 0.004592, p= 0.5977). Although the presence of caries was lower in overweight and obese children, no correlation was found between nutritional status and salivary flow rate.

Key words: *nutritional status; saliva; dental caries, child.*

RELACIÓN ENTRE LA TASA DE FLUJO SALIVAL, RIESGO DE CARIES Y ESTADO NUTRICIONAL EN NIÑOS PRE-ESCOLARES

RESUMEN

Modéer T. et al. (2011) afirman que en las poblaciones de adolescentes obesos existe asociación entre reducción de tasa de flujo salival y caries. El objetivo del presente estudio fue determinar la asociación entre el estado nutricional, la tasa de flujo salival y el riesgo de caries en preescolares. Se estudiaron 60 niños de 3 a 6 años de edad, que concurrían a Jardines de Infantes del conurbano de la ciudad de Buenos Aires, Argentina. En este grupo de niños se midió el peso corporal y la talla. Se calculó el índice de masa corporal y se categorizó antropométricamente a la población según OMS 2007. (Programa WHO Anthro). Se determinó el riesgo de caries. La saliva se recolectó en frascos estériles, graduados, de boca ancha sin estimulación y sin restricciones alimentarias. Se determinó la tasa de flujo salival (TFS). El análisis estadístico se realizó con el Test de Pearson. Presentaron caries el 56.7% (IC95%: 37.7-74.0) de los niños adecuados (Ad) antropométricamente y el 37.0% (IC95%: 20.1-57.5) de los niños con sobrepeso y obesidad (SP/O). El odds ratio para caries (OR=3.78; IC95%: 1.2–11.8, p=0.02) fue casi 4 veces mayor en los niños Ad, comparados con los SP/O. La TFS fue 0.534 ± 0.318 ml/min en Ad y 0.439 ± 0.234 ml/min en SP/O. El test de Pearson no evidenció correlación entre la TFS y el estado nutricional (r=0.004592, p=0.5977). A pesar que los niños con sobrepeso y obesidad tienen menor presencia de caries no se encontró correlación entre el estado nutricional y tasa de flujo salival.

Palabras Clave: *Estado nutricional; Saliva; Caries Dental; Preescolares.*

INTRODUCTION

Saliva, modulated by the ecosystem, plays a critical role in the homeostasis of the oral cavity[1]. Its functions include lubricating the food bolus; protecting against virus, bacteria and fungi; repairing oral mucosa; buffer capacity and remineralizing teeth. It has buffer and neutralizing capacity against acids produced by microorganisms or in the diet, enabling a relatively constant pH to be maintained in bacterial plaque and the oral cavity. It constantly

provides calcium and phosphates, which are needed for remineralization processes. Salivary buffer capacity depends on bicarbonate concentration and its remineralization capacity depends on calcium and phosphate concentrations. Both are correlated to the saliva flow rate. Reduced salivary flow rate is a risk factor for caries. Salivary secretion rate, which varies according to the type, strength and duration of the stimulus, is the main factor affecting its composition. Some metabolic disorders, such as those found in obesity, may affect saliva synthesis, composition and secretion[2,3]. Quantitative and/or qualitative alterations in salivary secretion may lead to the development of infections (candidiasis), functional alterations of masticatory or digestive processes, or infections of the digestive system (halitosis). Whole unstimulated saliva reflects basal salivary flow and is present in the oral cavity for long periods of time (about 14 hours), and is thus the main factor responsible for protective properties, while whole stimulated saliva is present after eating (physiological stimulation), and thus only acts for about two hours[4].

During the last quarter of the twentieth century, obesity increased worldwide in all age groups, a situation which has become more visible over the past two decades, with little or no distinction among gender, ethnicity or social condition[5-8]. Children are not exempt from this problem, and child obesity has increased dramatically in recent decades, becoming a Public Health issue[9,10]. In Argentina, a study including anthropometric data collected from 2007 to 2012 on a population of 120,000 2- to 18-year-olds, found that 14.5% of preschoolers, 19% of schoolchildren and 17.4% of adolescents were overweight, while obesity affected 9.8% of preschoolers, 17.7% of schoolchildren and 9.9% of adolescents[11]. These data agree with the latest survey on nutrition and health, conducted in 2007, which showed high prevalence of obesity, which affects 10.5% of children of preschool age[12].

The association between diseases caused by plaque biofilm and nutritional status has been studied by different authors, with contradictory results[13-16].

Adipocytes secrete various hormones and cytokines which contribute to obesity. Leptin acts on hypothalamus receptors to suppress food intake and increase energy consumption. A reduction in the sensitivity of the receptors of this hormone can induce the beginning of obesity and affect salivary secretion. This led to obesity being linked to hiposalivation and related pathologies such as dental caries[3]. Overweight or obese children have alterations in the concentration of phosphates, sialic acid, proteins and peroxidase activity, leading to conditions which are favorable to the development of caries[2].

Obesity, in particular abdominal obesity, usually accompanied by metabolic syndrome, is linked to chronic inflammation and therefore to higher risk of diseases such as type 2 diabetes, atherosclerosis, respiratory disorders and periodontal disease[14-16].

Being socially at risk plays a critical part in the etiopathology of obesity and caries because it facilitates synergic interaction among risk factors. Children growing up in urban settings adopt behavior traits that facilitate the progression of the disease. This explains why urbanization and modernization rates are strongly linked to the predominance of obesity[17]. In addition, dental caries and obesity both have multifactorial etiology and are associated to eating habits. The prevalence of caries and obesity is a growing challenge for Public Health authorities. Many studies on these pathologies have produced controversial results, possibly due to the lack of standardized criteria for diagnosis, and the different age groups and designs used in the different papers.

The aim of this study was to determine association among nutritional status, saliva flow rate and caries risk in preschoolers.

MATERIALS AND METHODS

This was a descriptive, correlational study, performed at government-run kindergartens in areas immediately adjacent to Buenos Aires city. The protocol was approved by the Ethics Committee at the School of Dentistry at Buenos Aires University (11/05/2011- nº 07).

Phase 1: Preliminary activities

Parents or guardians received information on the study, following current ethical standards. They provided authorization by signing informed consent for children to be included in the study and information about the children's medical and dental history. Then they attended a meeting during which information was provided about frequent diseases of the oral cavity and advice on oral hygiene and *ad hoc* dietary recommendations.

Phase 2: Sampling

Exclusion criteria were:

a) Children with systemic diseases,
b) Children having received radiation, or
c) Children taking any medication at the time of sampling which would modify the saliva flow rate.

All authorized children were evaluated, diagnosed included in a preventive care program, regardless of the exclusion criteria. The purposive sample included 60 children (30 female and 30 male), aged 3 to 6 years old (mean ± SD= 58.8 ± 10.7 months).

Phase 3: Collecting anthropometric clinical data

Anthropometric data were taken using the measuring techniques suggested by the Growth and Development Committee of the Argentine Pediatric Association[18], by a single professional, at the same time, and after the children had had breakfast and emptied their bladder.

Data were collected and processed as follows:

- Body weight recorded on a CAM brand scale to the nearest ± 100 g, wearing light clothes;
- Height measured with a CAM brand stadiometer to the nearest ± 1 mm.
- Body mass index (BMI) calculated as weight (kg)/ height^2 (m^2).
- Anthropometric classification of the children in the sample using the BMI Z scores corresponding to the WHO standards, by applying the WHO software AnthroPlus[19,20]. The following zBMI cutoff points were used: underweight ≤ -1 SD, adequate -1.0 to +1.0 SD, overweight ≥ +1.0 y < +2.0 SD, obesity ≥ +2.0 SD[21].

Phase 3: Saliva collection and analysis

Unstimulated saliva without food restriction was collected in wide-mouthed, graduated, sterile jars. Spontaneous salivary flow rate was determined (SFR) [22].

Phase 4: Determining history of dental caries and cariogenic risk factors

Clinical examinations were performed by a single professional following validated criteria. The following were calculated:

- dmft index[23]
- dental plaque biofilm index[24]
- diet history

Phase 5: Statistical analysis

The data were analyzed using the following statistical analyses:

- Distribution and frequency of study variables (salivary flow rate, presence of caries and nutritional status) and/or percentages in relation to total cases and their respective 95% confidence intervals, using Fisher's exact probability test for significance, with p<0,005 considered statistically significant difference.
- We expressed SFR as mean ± standard deviation (SD), and analyzed normality assumptions (Kolmogorov & Smirnov test) and homoscedasticity (Bartlett's test). The results were considered significant at a 5% probability level.
- Mann-Whitney's test was applied for comparisons between sexes.
- Pearson's test was used to determine the correlation between SFR and nutritional status and age.

We used the software GraphPad Prism, version 5.03 (GraphPad Software, Inc., San Diego, CA, USA).

RESULTS

Results of studies on anthropometric data

Fifty percent of the children in the study had adequate or normal weight, 23.3% were overweight and 21.7% were obese. Because there were very few underweight children (5%) according to zBMI, they were excluded from the sample. Fig. 1 shows the distribution of the sample according to nutritional status evaluated through the Z score of Body Mass Index (BMI).

Results of studies on teeth

Results for association between anthropometric data and dental status

56.7% (CI 95%: 37.7-74.0) of anthropomorphically adequate children and 37.0% (CI 95%: 20.1-57.5) of overweight and obese children had caries. The odds of having caries (Odds Ratio= 3.78; CI 95%: 1.2– 11.8, p=0.02) was nearly 4 times higher in anthropomorphically adequate children (Fig. 2).

Results for association between SFR and anthropometric data

SFR was 0.534 ± 0.318 ml/min for anthropometrically adequate children and 0.439 ± 0.234 ml/min for overweight and obese children, with no statistically significant difference. Pearson's test showed no correlation between SFR and nutritional status (r= 0.004592, p= 0.5977) (Fig. 3).

Results for association between SFR and gender
When salivary flow rate was analyzed with relation to gender in the study population, girls were found to have higher SFR than boys (mean ± SD= 0.57± 0.29 vs. 0.40±0.27) (Fig. 4).

Results for association among SFR, gender and anthropometric data
When the population was analyzed according to salivary flow rate, gender and anthropometric data, overweight and obese girls were found to have higher salivary flow rate than boys (Table 1).

Results for association between SFR and caries
Both adequate and obese children with caries have higher SFR, although it is only statistically significant in those with adequate weight (0.59 ± 0.30 ml/min vs. 0.37 ± 0.20 ml/min, mean and SD), p=0.0017 (Table 2).

DISCUSSION

There are multiple factors involved in the etiology of dental caries, so the risk analysis should include factors involved in its development, including lifestyle[25].

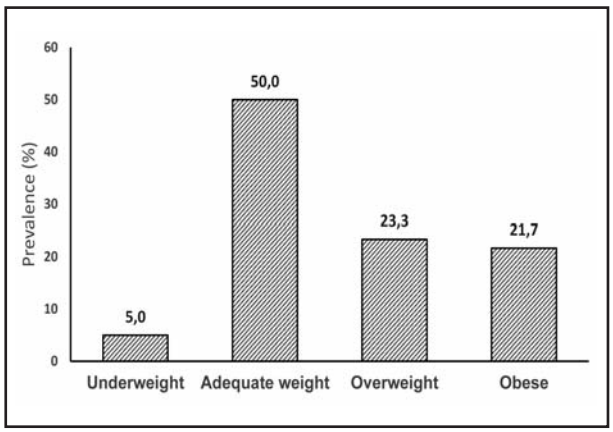

Fig. 1: Distribution of the population according to nutritional status.

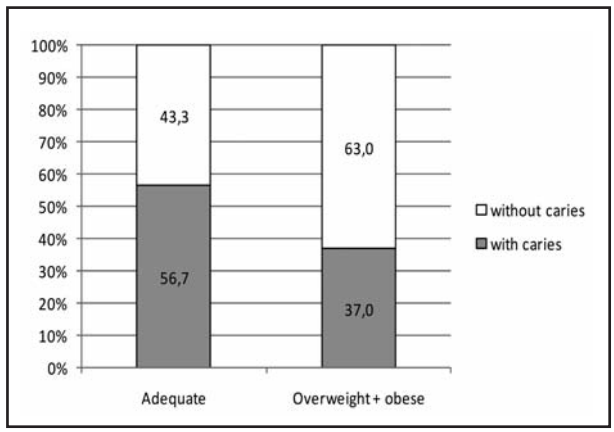

Fig. 2: Presence of caries according to nutritional status.

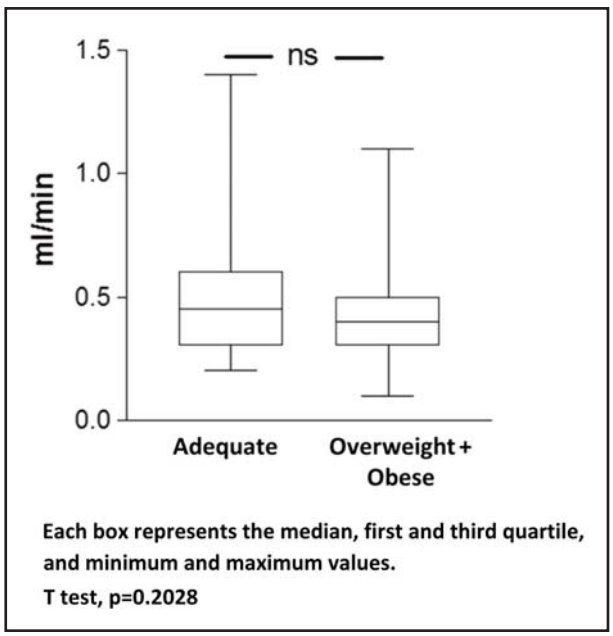

Fig. 3: Salivary flow according to nutritional status.

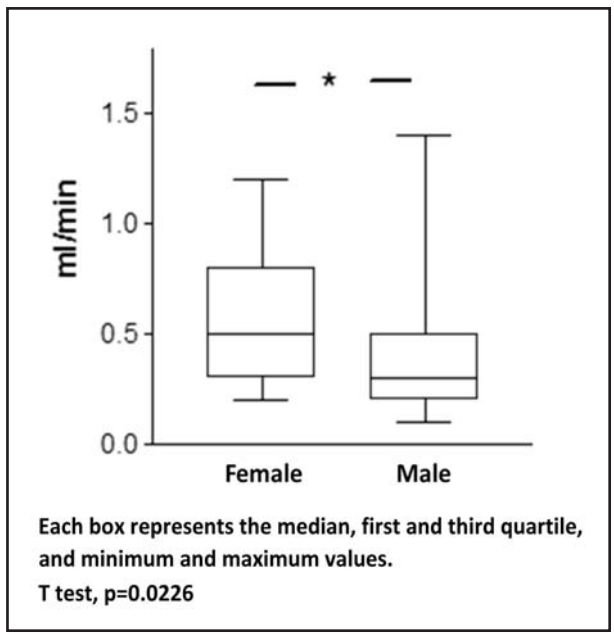

Fig. 4: Salivary flow rate according to sex.

Weight gain is the result of a positive energy balance, with intake exceeding expenditure[26] due to the intake of food with high caloric density[27] and a low level of physical activity[28]. In the "obesogenic environment" of society today, eating habits and sedentary behavior are the main components influencing obesity. Diet quality as a cause of obesity has been the object of many studies in adults, but few in children. The Bogalusa Heart Study found positive association between the prevalence of obesity and the consumption of beverages and salty snacks but not the consumption of desserts and candies[29].

Al-Zahrani et al.[15] suggest that obesity in childhood and youth predisposes to oral disease. Other authors found contradictory results (Table 3): some found significant association between obesity and dental caries[30], while others found that obese children had higher risk of erosion but not necessarily of caries, compared to children of normal weight[31]. Alm et al.[32] found that overweight and obese adolescents had a higher number of proximal caries than those of normal weight, and this finding was associated to higher consumption of snacks since childhood. These findings do not agree with reports by Yen et al.[33].

Table 1: Salivary flow rate according to gender and nutritional status.

	Adequate	OW + Ob	p
Female	0.60 ± 0.30	0.51 ± 0.25	ns
Male	0.45 ± 0.29	0.33 ± 0.14	ns
p	ns	p=0.04	

Table 2: Association among salivary flow rate, nutritional status and caries.

	Adequate	OW + Ob	p
With caries	0.62±0.32	0.51±0.26	ns
Without caries	0.40±0.24	0.36±0.17	ns
p	* p=0.0446	ns	

Table 3: Summary of literature found about the association between obesity and caries and salivary flow rate.

First author (Ref.)	Study design	Year	Nº of subjects	Age range (yr)	Obesity and caries	Obesity and salivary flow rate
Bailleul-Forestier I [30]	prospective	2007	82	12-18	Positive, p=0.01	
Tong HJ [31]	prospective	2014	64	7-15	ns	ns
Alm A [32]	retrospective	2008	402	15	Positive, =0.014	
Yen CE [33]	prospective	2013	329	preschool	ns	
Hayden C [34]	meta-analysis	2013		children	Positive, p=0.0479 only in industrialized countries	
Gupta P [35]	prospective	2014	100	12	ns	
Kopycka-Kedzierawski D. T [36]	retrospective	2008	17748	2-18	ns in 2-5 y group Negative, p=0.03 in 6-18y group	
Fadel HT [37]	prospective	2014	55	13-18	Positive, p<0.05	Negative, p<0.05
Powers PS [38]	Prospective	1982	23	Adults		ns
Flink H [39]	Prospective	2008	1427	20-69		Negative. Hyposalivation is prevalent in younger adults, associated with high BMI
Modéer T [40]	prospective	2010	130	10-18	Positive,	Negative, p<0.001
Aspen VA [44]	prospective	2012	57	9-12	p= 0.002	Positive. Non obese did not become habituated to food cues

Meta-analysis of the association between obesity and dental caries in populations of recently industrialized countries vs. industrialized countries found a significant association between the two conditions in the latter[34].

Gupta et al.[35] found no significant association between presence of caries and body mass index in 12-year-old children, but did find a strong correlation with oral hygiene. Kopycka-Kedzierawski et al.[36] found in a population of 6- to 11-year-old children that obese children were less likely to have caries, in agreement with the findings in our study.

Fadel[37] found that obese adolescents had lower stimulated saliva secretion, higher concentration of secretory IgA ($p<0.001$), and greater presence of dental caries and gingivitis ($p<0.01$), with no significant difference in snack consumption and bacterial plaque index.

Powers et al.[38] found no difference in salivation patterns between obese and non-obese adults. In a cohort of obese adult patients, Flink[39] has shown that there is an association between reduction in stimulated salivary flow rate and increased body mass index. This observation does not agree with the findings of our study, where saliva was not stimulated for collection.

Modéer et al.[40] conducted a cross-sectional study and found lower stimulated salivary flow rate in obese adolescents. This may be attributed to the fact that salivary secretion neuro-endocrinal regulation may be affected due to the altered ratio in abdominal obesity due to the modulation of the immune system, via cytokines, exercised by the central nervous system[41].

Fenoll[42] suggests that salivary flow is lower in females because they may have smaller salivary glands. However, this does not agree with the findings in our study, where salivary flow was significantly higher in females.

Johansson[43] found that stimulated salivary flow declined with malnutrition, while unstimulated saliva did not change. It could be inferred that nutritional status has an influence on changes in salivary flow and thus on oral ecology. In the presence of food, the body's first reaction corresponds to the cephalic phase, which includes increased heart rate, body temperature, gastric activity and salivary flow. Faced with repeated food cues, the individual becomes habituated and learns to ignore them. It has been observed that this behavior is much slower in obese persons, i.e., they continue to have an increased salivary response in the presence of food[44], with a higher rate of salivary flow. It would be worth conducting studies on food consumption to identify what variables might condition these results.

CONCLUSIONS

1) There was no correlation between overweight/obesity and salivary flow rate.
2) Obese children had fewer teeth affected by dental caries.

Both diseases – caries and obesity/overweight – have common causes which require integrated management by multidisciplinary teams promoting healthy diets.

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ULTRASTRUCTURE OF THE SURFACE OF DENTAL ENAMEL WITH MOLAR INCISOR HYPOMINERALIZATION (MIH) WITH AND WITHOUT ACID ETCHING

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ABSTRACT

The aim of the present work was to analyze the ultrastructure and mineral composition of the surface of the enamel on a molar with MIH, with and without acid etching. A permanent tooth without clinical MIH lesions (control) and a tooth with clinical diagnosis of mild and moderate MIH, with indication for extraction, were processed with and without acid etching (H_3PO_4 37%, 20") for observation with scanning electron microscope (SEM) ZEISS (Supra 40) and mineral composition analysis with an EDS detector (Oxford Instruments). The control enamel showed normal prismatic surface and etching pattern. The clinically healthy enamel on the tooth with MIH revealed partial loss of prismatic pattern. The mild lesion was porous with occasional cracks. The moderate lesion was more porous, with larger cracks and many scales. The mineral composition of the affected surfaces had lower Ca and P content and higher O and C. On the tooth with MIH, even on normal looking enamel, the demineralization does not correspond to an etching pattern, and exhibits exposure of crystals with rods with rounded ends and less demineralization in the inter-prismatic spaces. Acid etching increased the presence of cracks and deep pores in the adamantine structure of the enamel with lesion. In moderate lesions, the mineral composition had higher content of Ca, P and Cl.

Enamel with MIH, even on clinically intact adamantine surfaces, shows severe alterations in the ultrastructure and changes in ionic composition, which affect the acid etching pattern and may interfere with adhesion.

Key words: *Dental enamel- hypomineralization- acid etching .*

ULTRAESTRUCTURA DE LA SUPERFICIE DEL ESMALTE DENTAL CON HIPOMINERALIZACIÓN MOLAR INCISIVA (MIH) CON Y SIN GRABADO ÁCIDO

Resumen

El objetivo del presente trabajo fue analizar la ultraestructura y composición mineral de la superficie del esmalte de un molar con MIH, con y sin tratamiento de grabado ácido. Se analizaron una pieza dentaria permanente sin lesiones clínicas de MIH (controles) y una pieza con diagnóstico clínico de MIH leve y moderada, con indicación de extracción con y sin grabado acido (H_3PO_4 37%, 20"). Fueron procesadas para su observación con microscopio electrónico de barrido (SEM) ZEISS (Supra 40) y análisis de la composición mineral con detector EDS (Oxford Instruments). El esmalte del control mostró superficie prismática y patrón de grabado normales. El esmalte clínicamente sano en la pieza con MIH reveló una pérdida parcial del patrón prismático. La lesión leve se presentó porosa con ocasionales grietas. La moderada presentó mayor porosidad, con grietas de mayor tamaño y presencia de gran cantidad de escamas. La composición mineral de las superficies afectadas mostró menor contenido de Ca y P y aumento de O y C. En la muestra con MIH, inclusive con aspecto normal, las desmineralizaciones no responden a un patrón de grabado, mostrando exposición de cristales con redondeamiento en los extremos de las varillas y menor desmineralización en los espacios interprismáticos. El grabado ácido incrementó la aparición de grietas y profundos poros en la estructura adamantina del esmalte con lesión. La composición mineral mostró en las lesiones moderadas una mayor disminución del contenido de Ca, P y Cl.

El esmalte con MIH, inclusive en la superficie adamantina clínicamente intacta, presenta severas alteraciones ultraestructurales y cambios en la composición iónica afectando el patrón de grabado ácido, que podría interferir con los mecanismos de adhesión.

Palabras clave: *Esmalte dental, hipomineralización, grabado ácido.*

INTRODUCTION

Molar incisor hypomineralization (MIH) presents a new challenge in Pediatric Dentistry and Restorative Dentistry. Typical MIH lesions are caused by disturbance during the early mineralization stage[1], affecting teeth at that chronological stage by producing enamel which is deficient in minerals, with normal content of residual amelogenin and rich in albumin[2]. The etiology of MIH is as yet unknown[1-3]. It has been suggested that ameloblasts

are vulnerable during their transition from the secretion stage to maturation. Differences in the susceptibility of ameloblasts at different stages of their development cycle might explain the random distribution of lesions, since not all teeth formed during the same period are affected to the same extent[3]. Clinically, the defects appear as alterations in translucency and color of the affected enamel, showing as demarcated brown-yellow and/or cream-white asymmetrical opacities, mainly on permanent incisors and molars. They can lead to loss of enamel, causing high impact on the need for treatment in children and adolescents. The cream-white colored lesions are classified as mild, following the criteria of Mathu-Muju K and Wright JT, and the brown-yellow/brown lesions are classified as moderate[4, 5]. Moderate lesions have higher porosity, lower mineral density throughout the entire enamel thickness, and lower mechanical strength than mild lesions[6]. Histologically, the microstructure is preserved, indicating normal function of the ameloblasts during the secretion stage. However, in the affected areas, the crystals are disorganized and there are enlarged inter-prismatic spaces[7], suggesting that the problem may occur during the early years of life[2].

Clinical studies conducted in Buenos Aires City reveal that out of 1109 children born between 1993 and 2003, with and without demand for dental healthcare and different social risk levels, the prevalence of MIH was 21.73%, increasing significantly in younger children. Patients with MIH require significantly more frequent restorative interventions and more re-treatment than those without MIH[8]. The results of a study conducted in 2010 on therapeutic alternatives at the Department of Comprehensive Pediatric Dentistry at the School of Dentistry of Buenos Aires University (FOUBA) revealed that 17.8% of molars with MIH required restorations with steel crowns[9]. Moderate and severe lesions at early ages have become a focus of attention in Pediatric Dentistry over the past decade. Clinical complications deriving from MIH include problems related to aesthetics, increased susceptibility to caries, hypersensitivity, anesthetic failure and difficulty in adhesion in techniques employed for restoration of the lesions[10,11]. The latter calls for a study on the microstructural features of affected tissues, in particular the enamel surface, in order to find adequate restorative alternatives. Studies reported in the literature analyze the ultrastructural and biochemical characteristics of the interior of the enamel in permanent teeth diagnosed with MIH. They have found that the affected enamel has lower mineral concentration, less organized crystalline structure, greater porosity, greater carbonate content and lower Ca/P ratio[3, 12,13]. The mechanical properties, hardness and modulus of elasticity of hypomineralized enamel are lower than those of normal enamel[14]. The ultrastructural features and mineral composition of the surface of the enamel on MIH lesions and on the clinically normal enamel on teeth affected by MIH are unknown to date.

In 1955, Buonocore proposed a strategy for improving adhesion to enamel by acid etching to create micro-roughness in the adamantine structure[15]. Still in use today, acid etching produces morphological patterns in the enamel which are classified as types I, II and III. In type I etching, the cores of the adamantine prisms are dissolved; in type II, the periphery of the prisms is dissolved, affecting the inter-prismatic substance. Type III does not produce deep etching and only partially removes the enamel crystals around the prisms. Effective acid etching requires a specific prismatic ultrastructure, which is not available when there is hypomineralization. Research into adhesion to hypomineralized enamel is limited; it has been reported that the affected enamel has a porous interface with fissures and fails in cohesion[16]. The literature does not describe the ultrastructural characteristics of the acid-etched surface of enamel with MIH lesion or the properties of the etching pattern on clinically normal enamel present in teeth affected by MIH lesions.

Thus, the aims of this study were to analyze the ultrastructure and mineral composition of the surface of enamel with and without clinical lesions on a molar with MIH, with and without acid etching.

MATERIALS AND METHODS

Teeth

This study used 2 permanent human teeth: a tooth with a clinical diagnosis of MIH and a healthy tooth (control). The tooth with clinical diagnosis of MIH was a first molar with both mild and moderate hypomineralization lesions, without loss of substance. The control sample was a healthy premolar without lesions on the enamel (hypoplasia, amelogenesis imperfecta, fluorosis, caries). Both teeth were

extracted by orthodontic indication, for reasons unrelated to this study. The experimental design was approved by the Ethics Committee of the School of Dentistry, University of Buenos Aires (UBACYT 208-2110/2 and UBACYT 2011-2014/3).

Sample processing

The teeth were cut in half across their free surfaces (vestibular to lingual). Each half of the tooth with clinical diagnosis of MIH had 3 clear clinically distinguishable zones: mild lesion (cream-white), moderate lesion (brown-yellow) and clinically healthy enamel, which were demarcated on the surface of the enamel as shown in Fig. 1. One half of each tooth was processed with acid etching (H_3PO_4 37%, time: 20") and the other half without. The halves were thoroughly rinsed with distilled water for 60 s and gently air dried, after which they were mounted and sputter-coated for observation under scanning electron microscope (SEM) ZEISS (Supra 40), and mineral composition was analyzed with an EDS detector (Oxford Instruments).

The SEM has a field emission electron gun and a 3rd generation Gemini column. Magnifications of 1.0 KX, 20.0 KX and 70.0 KX were used. The mineral composition of the surface was analyzed using energy-dispersive spectroscopy with an EDS detector Oxford Instrument-INCA, which detects elements as from Boron. Both pieces of equipment belong to the Advanced Microscopy Center at the School of Exact and Natural Sciences, Buenos Aires University.

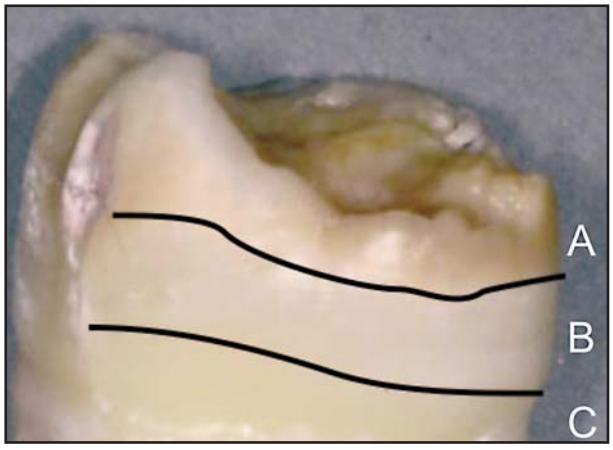

Fig. 1: Molar with MIH on which the zones of enamel with lesions have been marked: A) Moderate lesion, ochre stain; B) Mild lesion, white stain and C) Clinically intact/healthy enamel.

RESULTS

SEM examination of the adamantine surface without acid etching (Fig. 2)

The enamel on the control tooth (without hypomineralization) had a normal prismatic surface without structural alterations. The clinically healthy enamel on the tooth with MIH showed partial loss of the prismatic pattern, with no structural alteration. Higher magnification shows that the crystals forming the prism have more rounded ends than in the healthy control. The enamel with hypomineralization lesions did not have the typical features of prismatic enamel. The surface of the enamel from the cream-white colored (mild) hypomineralized lesion was porous, with occasional cracks and presence of different planes, giving the surface a staggered/ layered appearance. In the zone of the brown-yellow (moderate) hypomineralization lesion, there was more porosity, larger cracks and a large quantity of scale-like lesions.

Mineral composition by EDS of the enamel surface without acid etching (Fig. 3)

The mineral composition of the adamantine surfaces of the tooth with mild MIH lesion had similar Ca and P content as the control, and higher Ca content in the enamel zone that was clinically intact on the tooth with MIH diagnosis. The enamel zone with moderate lesion had lower Ca and P content than the control. All the enamel zones of the tooth with MIH diagnosis had much higher C content with lower O content than the control tooth, even in the clinically healthy enamel zone. The enamel with moderate lesion contained Mg, Zn and Rb, which were absent from the mild lesion and from clinically healthy enamel.

SEM examination of the adamantine surface with acid etching (Fig. 4)

After acid etching, the tooth with MIH diagnosis did not show the typical patterns of demineralization (type I and type II) found in normal enamel. The enamel on the tooth with MIH lost surface matter, partially exposing an underlying prismatic adamantine structure, matching a type III etching pattern. None of these demineralization match deep etching patterns (types I and II), showing exposed crystals with rounded rod ends and less demineralization of inter-prismatic spaces. Acid etching increased the amount of cracks and deep pores in the adamantine structure of the enamel with lesion.

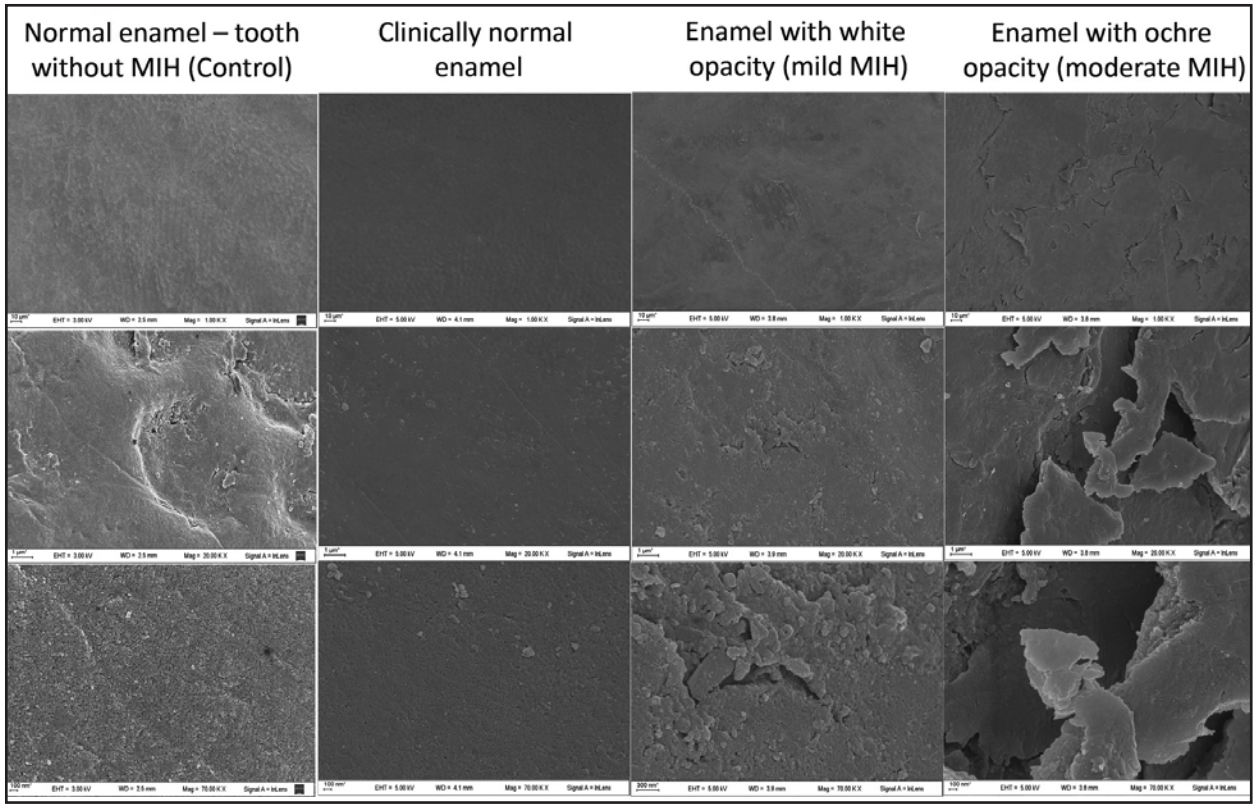

Fig. 2: SEM microphotographs of the adamantine surface without acid etching:a) Normal enamel on tooth without MIH (control): 1. Prismatic enamel surface without structural alterations. Magnification 1.0 KV; 2. Head of an enamel prism. Magnification 20.0 KV; 3. Surface of crystals forming the prism. Magnification 70.0 KV. b) Clinically normal enamel on tooth with MIH: 1. Prismatic enamel surface without structural alterations. Magnification 1.0 KV; 2. Loss of prismatic pattern image, without structural alterations. Magnification 20.0 KV. 3. Crystals forming the prism have a more rounded end than the control. Magnification 70.0 KV. c) Enamel with cream-white opacity (mild MIH): 1. Surface with slight structural alterations compared to the control. Magnification 1.0 KV; 2. Presence of different planes on the surface ("staggering") and cracks. Magnification 20.0 KV; 3. Globulous surface with small cracks. Magnification 70.0 KV. d) Enamel with ochre-brown opacity (moderate MIH): 1. Irregular surface with erosions, circular-edged hollows with visible bottom and scale-shaped enamel plaques. Magnification 1.0 KV; 2. Scaling adamantine surface. Magnification 20.0 KV; 3. Very large, deep "Y"-shaped crack, without visible bottom. Magnification 70.0 KV.

	Control	Normal enamel in MIH	Mild MIH lesion	Moderate MIH lesion
Ca	15.83	23.71	15.08	10.23
P	11.35	12.37	10.28	7.09
C	20.06	30.81	31.16	47.99
O	52.45	32.69	42.91	34.28
Cl	0.31	0.42	0.16	
Na			0.41	
Mg				0.32
Zn				0.01
Rb				0.08

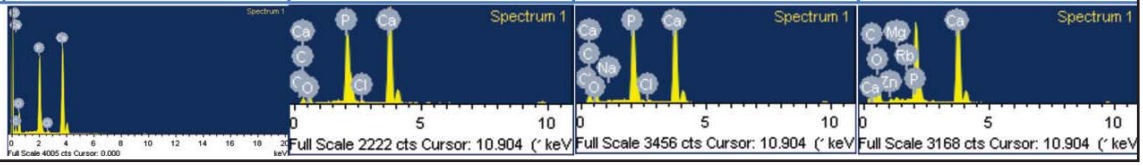

Fig. 3: Graphs and tables for EDS of the adamantine surface without acid etching (a%).

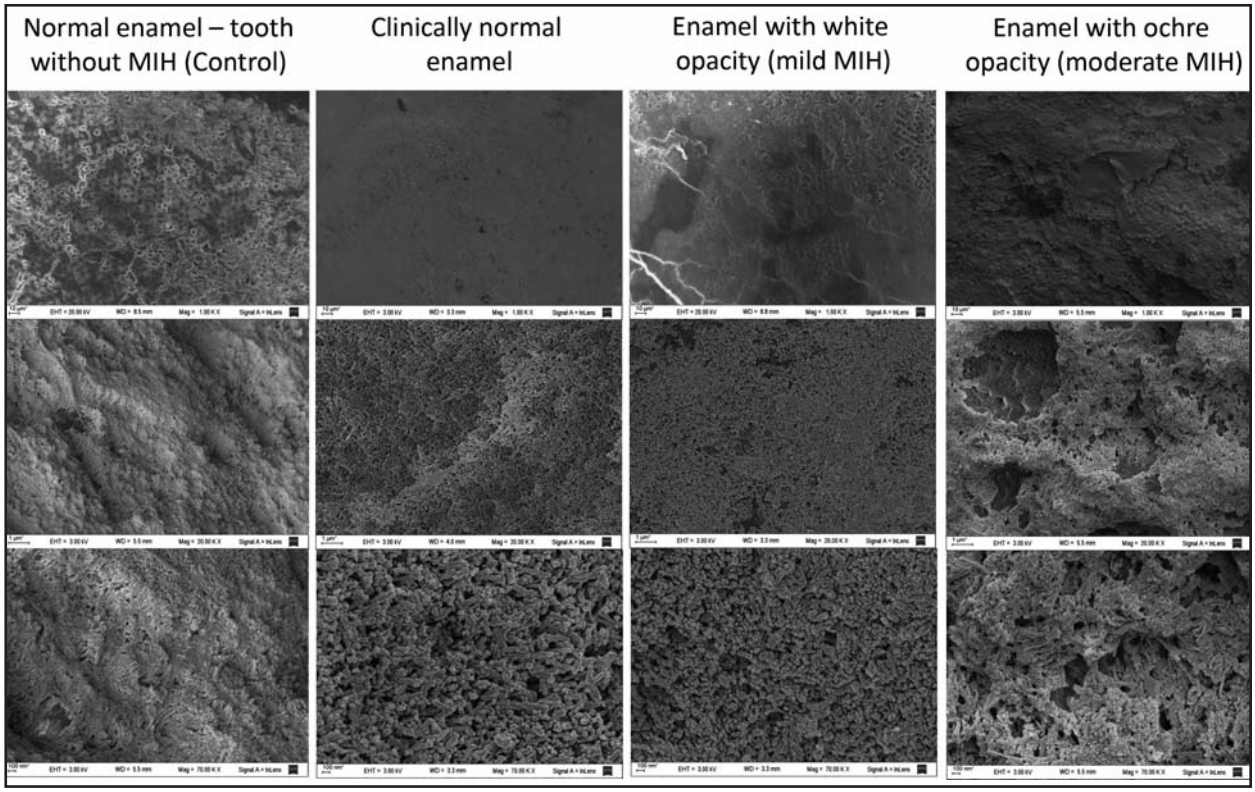

Fig. 4: SEM microphotographs of the adamantine surface with acid etching: a) Normal enamel on tooth without MIH (control): 1. Loss of surface substance with typical type I and II acid etching patterns. Magnification 1.0 KV; 2. The demineralization pattern maintains prismatic structure. Magnification 20.0 KV; 3. Prismatic pattern is maintained. Magnification 70.0 KV. b) Clinically normal enamel on tooth with MIH: 1. No demineralization pattern observed. Magnification 1.0 KV; 2. Irregular loss of substance without pattern. Magnification 20.0 KV; 3. Loss of substance with exposure of crystals. Magnification 70.0 KV. c) Enamel with cream-white opacity (mild MIH): 1. No demineralization pattern observed. Magnification 1.0 KV; 2. Irregular loss of substance without pattern. Magnification 20.0 KV; 3. Loss of substance with exposure of crystals. Magnification 70.0 KV. d) Enamel with ochre-brown opacity (moderate MIH): 1. No demineralization pattern observed. Magnification 1.0 KV; 2. Loss of deep substance with no pattern. Magnification 20.0 KV; 3. Exposure of crystals and deeper surface cracks. Magnification 70.0 KV.

Mineral composition of the acid etched enamel surface, determined by EDS (Fig. 5)

The analysis of mineral composition by EDS showed that after acid etching, the percentages of Ca and P increase while the percentage of C decreases on the surface of the enamel with mild lesion and the zone of clinically intact enamel. The ionic composition of the enamel from the moderate lesion zone was similar to that of normal enamel. It is worth noting Na and Mg were only present in the enamel of the tooth with MIH diagnosis.

DISCUSSION

Enamel with MIH, including clinically intact adamantine surfaces, has ultrastructural alterations and changes in ionic composition which affect the acid etching pattern and may interfere with adhesion.

The reduction in mineral concentration in the enamel of the tooth affected by MIH observed in this study matches the results published by Fearne et al. in 2004[3], reporting that the reduction occurs from the dentin-enamel junction towards the sub-surface zone of the enamel, which is the opposite of what happens in an incipient caries lesion in normal enamel.

The surface of hypomineralized enamel contains increased proportions of C and O, as other authors have reported for the interior of hypomineralized enamel [12]. This increase in C content may indicate persistence of remains of organic matter, probably due to faults in the enamel maturation period, during which proteins in the organic matrix are reabsorbed and the mineral content of the crystal increases. The substitution or loss of carbonate in HA crystals is known to increase their solubility in

	Control	Normal enamel in MIH	Mild MIH lesion	Moderate MIH lesion
Ca	11.62	20.51	15.66	11.04
P	8.09	13.09	10.85	7.09
C	26.42	13.92	15.67	21.03
O	53.58	51.57	56.86	58.66
Cl	0.28	0.36	0.28	0.18
Na		0.56	0.69	0.57
Mg				0.60

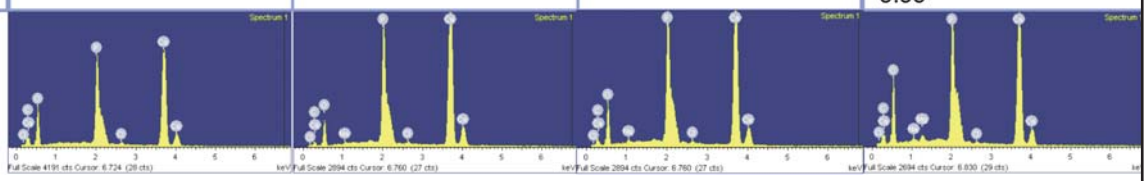

Fig. 5: Graphs and tables for EDS of adamantine surface with acid etching (a%).

acid media. The persistence of carbonate could be expected to have the opposite effect[16]. Another possibility is that the greater porosity of this enamel enables the adsorption of foods and drinks, with the carbonate in the lesion thus coming from the breakdown of organic compounds. It should be noted that the clinically healthy enamel on the tooth with MIH diagnosis had higher Ca and P content than normal enamel. However, considered together with surface morphology, this shows that these ions would not form prismatic structures and would be more closely associated to an amorphous deposit of crystals covering the heads of the prisms, reminiscent of the aprismatic enamel which is more often found in primary teeth.

The analysis of the adamantine microstructure after acid etching of the tooth with MIH does not show type I and II demineralization patterns, which are the ones that really provide retention and clinical certainty of adhesion and marginal sealing. Nevertheless, the pattern found (type III) produces a slight superficial decalcification clinically described as "undesirable" for achieving adhesion of restorative materials to the enamel. In a systematic review, Zhu et al. (2014)[17] propose that there is selective dissolution of enamel prisms or their periphery to enable the penetration of resins to facilitate micromechanical grip which will provide better adhesion. They also report that laboratory studies show a reduction in the quantity of typical patterns in the aprismatic enamel. The same concept could be applied to the findings herein, since the adamantine structure observed differs substantially from the normal, and thus a more deficient adhesion of reinforced resins could be expected. It would be interesting to test this inference with the relevant adhesive strength tests, since there is no information in the literature. The lack of available teeth may make it difficult to carry out a reliable study, since a significant sample size is necessary for obtaining data with the lowest dispersion possible. However, in the same review, Zhu et al. report that maximizing the ideal etching pattern as proposed by laboratory studies might not be clinically relevant.

The analysis of the acid etching results with relation to the mineral composition of the enamel shows that the higher C content on the hypomineralized enamel surface may interfere with the demineralization sought through the acid etching technique, which would thus be unable to create characteristic demineralization patterns. It is very likely that these atypical features of etched enamel are one of the causes of poor adhesion of restorative materials to enamel on teeth diagnosed with MIH, even in enamel zones which are clinically intact. After acid etching, the percentages of Ca and P increase, while the proportion of C decreases on the surface of enamel with mild lesions and zones of clinically intact enamel. This may indicate a deficiency in demineralization caused by phosphoric acid on the adamantine surface. The higher C content observed on the surface of hypomineralized enamel before acid etching may interfere with the demineralization sought at the expense of Ca and P. Phosphoric acid would thus be acting differently on this

enamel, creating random demineralization without a defined pattern.

It would be interesting to complement this study with an evaluation of enamel microhardness in each of the zones described and for each treatment, in order to test whether there is correspondence between their chemical and physical properties. This would be very helpful for establishing appropriate treatment protocols.

In the understanding that to date, the ultrastructure features and mineral composition of the surface of the enamel affected by MIH were unknown, and in particular, that it was unknown whether there was any alteration in the ultrastructure of clinically normal enamel on teeth with MIH, we believe that the preliminary results presented herein are clinically important to pediatric dentistry, which more and more often faces the challenge of restoring teeth with MIH.

CONCLUSIONS

The variations in mineral composition observed on surfaces to which restoration materials should adhere suggest that acid etching with phosphoric acid acts differently on enamel on molars with MIH, and may interfere with adhesion mechanisms. The results hereof reveal that the enamel on molars with MIH, including any clinically intact adamantine surfaces, produce an acid etching pattern which is incompatible with the requirements for an effective adhesive restoration technique.

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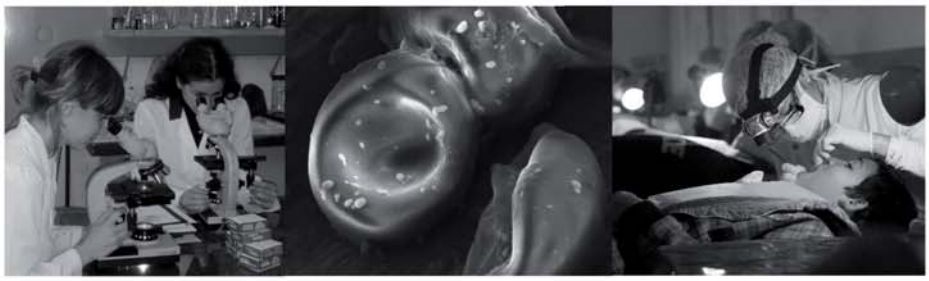

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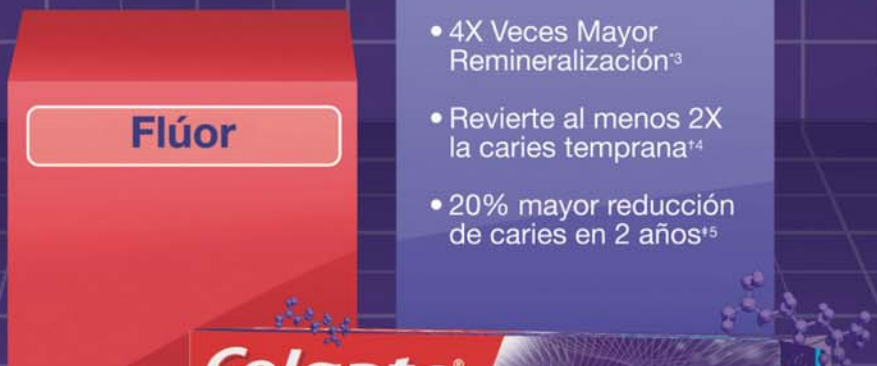

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