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

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

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

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

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

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

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

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Spatial distribution of dental caries among preschool children in Canoas, Southern Brazil

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ABSTRACT

The aims of this study were to analyze the spatial distribution of dental caries among preschool children and create equiprobable scenarios of its occurrence in the city of Canoas, Southern Brazil. Trained, calibrated dentists examined 1,100 children enrolled at public preschools to determine dental caries experience following World Health Organization criteria. The ArcGis 10.0 Geographic Information System was used to analyze spatial and non-spatial data. Geostatistical Modeling Software was used in geostatistical analyses to detect spatial continuity and create maps using stochastic simulation. Overall prevalence of dental caries was 25% with intra-urban differentials in distribution. The findings enabled the generation of 100 equiprobable scenarios and maps with the best and worst scenarios. The highest concentration of dental caries occurrence was found in the western portion of the city, while the lowest probability of occurrence was found in the northern and southern portions. Identifying spatial inequalities in health conditions and visualizing them through the creation of maps can help to qualify and organize public health interventions and provide information to gain better understanding of the influence of the surrounding environment on adverse health conditions.

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Keywords: *Dental Caries; Child, preschool; Spatial Analysis.*

Distribuição espacial de cárie dentária em crianças pré-escolares de Canoas, sul do Brasil

RESUMO

O objetivo do estudo foi analisar a distribuição espacial de cárie dentária entre crianças pré-escolares e criar cenários equiprováveis da ocorrência deste agravo na cidade de Canoas, sul do Brasil. Exame clínico para detecção da experiência de cárie dentária de acordo com o critério da Organização Mundial da Saúde foi realizado por cirurgiões-dentistas treinados e calibrados em uma amostra de 1.100 crianças matriculadas em escolas de educação infantil. Utilizou-se o Sistema de Informação Geográfica ArcGis 10.0 para a inserção de dados espaciais e não espaciais. O programa GeoMS foi utilizado nas análises geoestatísticas para a detecção da continuidade espacial e construção de mapas através da simulação estocástica. A prevalência de cárie dentária foi 25%, com diferenciais intra-urbanos na sua distribuição. Os resultados permitiram a construção de 100 cenários equiprováveis e de mapas com os melhores e piores cenários no município. Uma maior concentração de ocorrências foi encontrada na região oeste da cidade, enquanto que as regiões norte e sul tiveram a menor probabilidade de ocorrência de cárie dentária. A identificação de desigualdades espaciais em condições de saúde e a sua visualização por meio de mapas pode auxiliar na qualificação e organização de intervenções de saúde pública, assim como fornecer subsídios que ajudem no entendimento da influência do meio ambiente sobre as condições adversas de saúde.

Palavras chave: *Cárie dentária; Pré-escolar; Análise espacial.*

INTRODUCTION

Dental caries is the most prevalent adverse oral health condition in childhood and is considered a public health problem[1]. This condition is associated with pain as well as functional and esthetic problems, and has negative impacts on quality of life, social interactions and psychological well-being[2,3].

Although the oral health status of preschoolers has improved considerably over the past decade, oral health problems and treatment needs are distributed unevenly, with greater concentration in a small portion of the population, reflecting a "polarization process"[4,5]. In addition, social inequalities in oral health have been reported as widespread around the world, with people at the lower end of the

socioeconomic scale bearing a greater burden of adverse health conditions than those who are socioeconomically privileged[6,7]. The apparent tendency toward the concentration of adverse health conditions in poor urban areas has been denominated "intra-urban differentials in health" in the literature[8].

Inequalities in oral health have been described as a major challenge for public health authorities, and knowledge of the contextual factors involved is the new paradigm of epidemiology[1,9,10]. Thus, understanding the geographic distribution of adverse health conditions is fundamental to decision-making in epidemiological surveillance systems[11].

The Geographic Information System is a central tool in studies that evaluate the geographic distribution of adverse health conditions, enabling the collection, storage, visualization and analysis of spatial data[11-13]. In turn, geostatistical modeling enables the quantification of the spatial continuity of a given disease and the creation of probability maps for its occurrence through interpolation models and stochastic simulations[14]. In the last decade, health studies have used geostatistics to characterize the spatial distribution of malaria[15], cancer[16,17] and the association between air pollution during pregnancy and low birth weight[18].

In dentistry, few studies have used spatial analysis of information due to a lack of methodological knowledge, deficiencies in cartographic representations and the expense of equipment and software. Thus, only a few studies have used spatial analysis of dental caries[8,10,11,19-21]. Moreover, no study was found in the literature employing this method on a large sample of preschool children in a developing country.

The aim of the present study was thus to identify the spatial distribution of dental caries among preschool children and create equiprobable scenarios of the occurrence of this condition in the city of Canoas in southern Brazil.

MATERIAL AND METHODS

Subjects and study design

This cross-sectional study is part of a larger project of which the aim is to evaluate oral health status among preschool children in the city of Canoas, southern Brazil[2,5]. A total 1316 male and female children aged 0 to five years, enrolled at all public preschools, were examined. According to data provided by the Canoas Municipal Secretary of Education, the source population consisted of 1732 children.

Data collection

Six dentists who had undergone training and calibration exercises collected the data by means of a questionnaire on demographics (age and sex) and socioeconomic characteristics (household income and mother's schooling) administered to parents/guardians at the preschool, and a clinical examination of the preschoolers to determine dental caries experience. Children were examined at the preschool while lying on desks under natural light. Teeth were brushed and dried with gauze, after which a visual clinical examination was performed, following the criteria of the World Health Organization for the diagnosis of decayed (including only cavitated lesions), missing and filled teeth (dmft)[22]. Intra- and inter-examiner reliability for dental caries were assessed using weighted Kappa statistics in two dental examinations performed 10 days apart on 40 children aged 2-5 years. Inter-examiner reliability ranged from 0.83 (95% CI 0.71-0.95) to 1.00 and intra-examiner reliability ranged from 0.93 (95% CI 0.86-1.00) to 1.00.

Data analysis

The ArcGIS 10.0 Geographic Information System was used to integrate the tabulated and spatial data and perform the geoprocessing procedures. Each participant's address was manually georeferenced on a map of the municipality (Fig. 1). The reference datum was the South American Datum of 1969, with the Universal Transverse Mercator projection system on Zone 22 South. The shapes with street names, addresses, neighborhood limits and municipal limits were provided by the Canoas Geoprocessing Institute. The exploratory statistical analysis of the data and the study of the spatial distribution of dental caries based on the creation of variograms were performed using the Geostatistical Modeling Software on the individual level as well as with aggregated data. Dental caries was defined as an indicator variable: I(X) = 1: with dental caries; and 0: without dental caries.

Neighborhood was the spatial aggregation unit and the geographic coordinate for the aggregated data by neighborhood was determined by calculating the centroid of the spatial location of all children residing in the same neighborhood. The probability

of the occurrence of the outcome per neighborhood was adjusted using the direct adjustment rate, which enabled comparison of populations with different structures (age and number of individuals)[23]. After determining the spatial pattern of dental caries, interpolation was performed using Ordinary Kriging for the estimation of dental caries experience in non-sampled locations. Data simulation was performed using Direct Sequential Simulation for the identification of the best and worst equiprobable scenarios for the occurrence of dental caries. The Mann-Whitney test was used to compare the socio-economic level of the families (with and without location) based on household income and mother's schooling, with the level of significance set at 5% ($p < 0.05$).

Ethical aspects

This study was approved by the Human Research Ethics Committee of the Lutheran University of Brazil under process number 2010-056H. All participants' legal guardians signed a statement of informed consent prior to the data collection process.

RESULTS

The final sample comprised 1110 children aged zero to five years, 566 boys (51%) and 544 girls (49%), who were residents of 16 neighborhoods in the city of Canoas. Incomplete information provided by the parents/guardians during interviews and areas not officially registered with the City Hall determined the non-identification of 206 (15%) addresses. No significant difference in household income ($p = 0.383$) or mother's schooling ($p = 0.683$) was found between the located and non-located families.

The prevalence of dental caries (dmft ≥ 1) was 25%, with a range of 6.6 to 68.4% among the different neighborhoods. Fig. 2 shows the distribution of the children based on place of residence and dental caries experience.

The variograms in the individualized analysis revealed no spatial continuity in dental caries. However, the omnidirectional variogram in the analysis of aggregated data per neighborhood demonstrated a spatial pattern in the occurrence of this condition. Thus, a spherical model was adjusted with a range of 4534 meters.

Based on the variogram modeling, data were interpolated using Ordinary Kriging, which allowed the estimation of mean outcome for each location. The Direct Sequential Simulation generated 100 equiprobable scenarios which were equally representative of the outcome. Each simulation provided a single value for each location, representing a possible exposure measure and reproducing the histogram and variogram of the experimental data.

The variance in the set of values from the simulation represents the uncertainty associated with each simulation. In Fig. 3, regions with warmer colors represent areas of greater uncertainty in the simulation. These areas were those with no sampling and those with very different dental caries prevalence rates, demonstrating validity in the results indicated by the simulation.

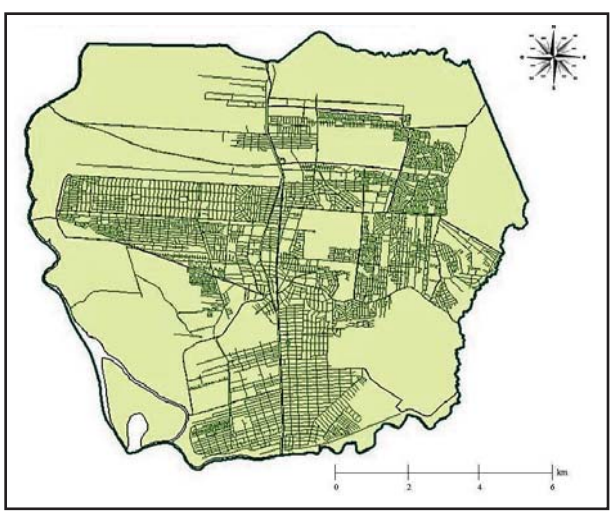

Fig. 1: Cartographic representation of the city of Canoas, southern Brazil.

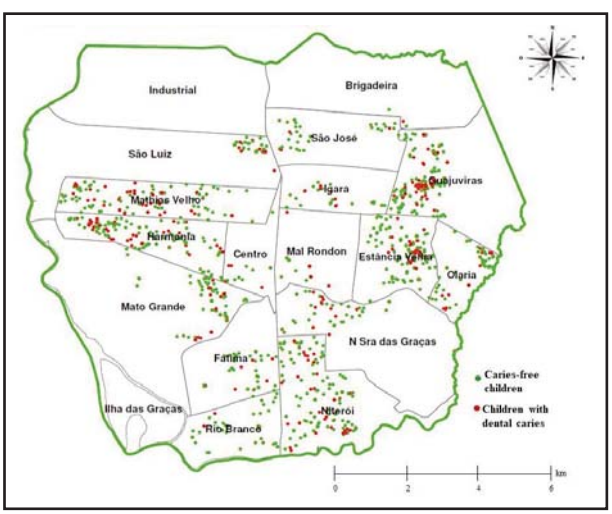

Fig. 2: Georeferencing of residences of children analyzed.

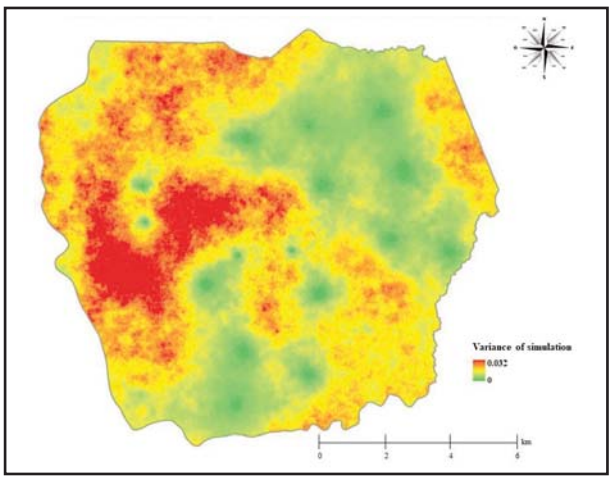

Fig. 3: Degree of uncertainty in the rates of dental caries experience simulated for each surface.

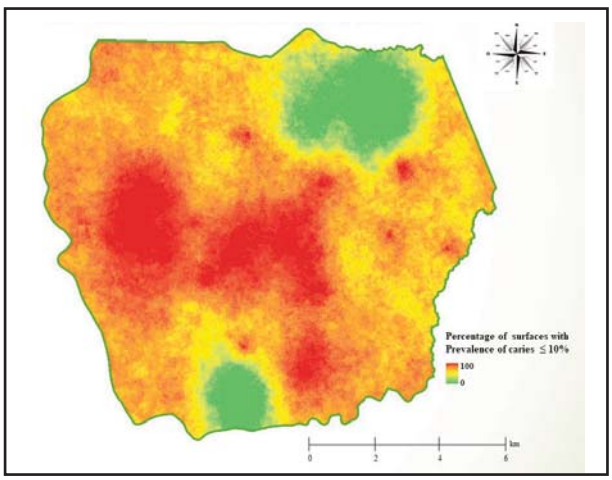

Fig. 4: Map of surfaces with lowest simulated rates of probability of dental caries.

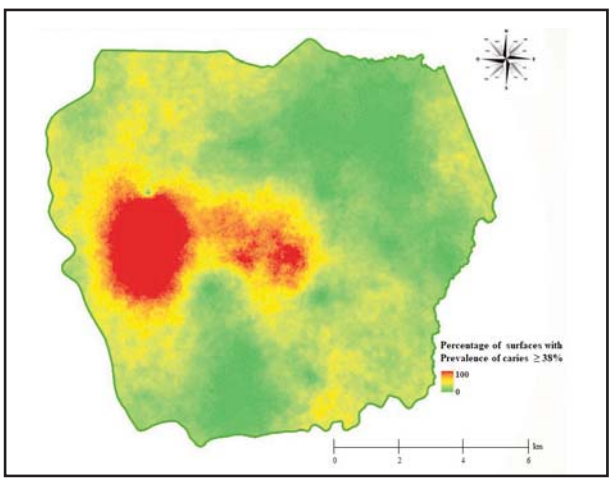

Fig. 5: Map of surfaces with highest simulated rates of probability of dental caries.

From the set of simulated maps, the distribution of probability values of dental caries prevalence was determined for each point on the map. These local distributions were divided into quintiles. The map of the 1st quintile (Fig. 4) shows that the northern and southern portions of the city had the lowest probability of dental caries occurrence, as represented by the coldest color (green). In the map of the last distribution quintile, the portions with the warmest colors are located in areas of the western periphery of the city and partially in the central region of the city, representing 1/5 of the sample and corresponding to a higher prevalence rate (38%; Fig. 5).

DISCUSSION

The spatial analysis of the distribution of dental caries among preschool children in the city of Canoas indicated the occurrence of intra-urban differentials, with the highest concentration of cases in the western portion of the city. This is the first study to investigate the spatial distribution of dental caries among a large sample of preschool children in a developing country. The findings demonstrate the importance of spatial analysis in understanding the polarization process of adverse health conditions and identifying more vulnerable groups.

Identifying spatial inequalities and visualizing them on maps enables health services to be qualified and organized. In addition to optimizing the allocation of financial and human resources based on the characteristics of each geographic area, this process is essential for guiding interventions aimed at reducing inequalities[11,13,24,25].

Certain features of the present study should be highlighted. The use of geostatistical modeling in the interpolation of data on dental caries enabled the probabilistic modeling of the uncertainty of the rate smoothing. Other studies estimate health data using different methods that incorporate the geographic position of the outcome, but without presenting the degree of uncertainty in the estimate[8,11,14].

The spatial continuity pattern of dental caries was only detected with the aggregated data. This was expected due to the behavioral diversity that may result in significant differences in caries experience among children who reside near each other. It is also important to stress that the geographic coordinate for the aggregated data per neighborhood was

determined based on the spatial location of all children in a given neighborhood, like a gravity center, and not through the geographic centroid of the neighborhood. Thus, the geographic location closest to the location of the study population was used.

Ordinary Kriging was used as the interpolation method. It differs from other interpolation methods in the manner in which the weights are attributed to different samples. In this case, the weights of an estimator are determined based on the spatial covariance obtained through the modeling of experimental variograms, thereby providing unbiased estimates with minimal variance[14]. Thus, this method enabled greater confidence in the estimation of the area with the greatest probability of the occurrence of dental caries: the westernmost periphery of the city. Other studies have used different estimation methods to detect geographic differences in oral health conditions in different populations[8,11].

Simulation models were also employed in the present study. Unlike estimation models, simulation models do not deliver the most probable image of the characteristics of an outcome, but rather, a setof equiprobable images with the same spatial variability depicted by the experimental data, thereby determining spatial uncertainty[14,16,18]. Methods that consider the degree of uncertainty in statistical models are important to the analysis of health data[16,18]. One map was thus created with the points of the lowest prevalence rates (up to 10%), representing the 1/5 of the distribution with the best oral health status, and another was created with points of the highest prevalence rates (> 38%), representing the 1/5 of the distribution with the worst oral health status. These maps reveal that the distribution of dental caries in the city of Canoas was unequal in the regions analyzed.

Although dental caries occurrence in Canoas appears to be related to socio-environmental features, with higher prevalence rates in areas with substandard living conditions, this study did not directly clarify mechanisms by which such disparities are generated. The socioeconomic gradient in children's oral health has been demonstrated in the individual and contextual level[26]. However, the mechanism underlying (socioeconomic) inequality in health is often complex[27,28]. There is further scope for evaluating the role of contextual and compositional factors that may explain inequalities in children's oral health[29].

As a reflection of socioeconomic conditions and subject to both social and political inequalities, the surrounding environment exerts an influence on the living conditions of its inhabitants. The evidence that inequalities in health have a spatial dimension is well established. There is growing understanding of the role that location plays in influencing individual and familial levels of exposure to health risks as well as opportunities for being healthy[30].

Geographic aspects may be related in different ways to aspects of oral health. Examples of these geographic aspects include location and forms of access to dental services, location of social equipment (schools, community centers and recreational areas), local infrastructure which can provide wellbeing (basic sanitation, electrical energy and public security) and the entire social support network.

The present study has some limitations that should be analyzed. The first is the lack of data on the location of all the preschool children, which is a common problem in developing countries, especially in communities with low socioeconomic levels. However, the likelihood of selection bias is low, since no significant difference was found in the socioeconomic level between the analyzed and non-analyzed children. Another limitation is the cross-sectional design, which does not allow conclusions to be drawn regarding the cause-and-effect relationship between exposure and outcome.

The present findings demonstrate the importance of identifying inequalities in the spatial distribution of diseases and visualizing this distribution on maps. Such visual resources enable a broader understanding of health data, which may help public administrators to plan health strategies in a given area. Further studies are needed to identify the contextual factors involved in the distribution of diseases. Instrumentalization through spatial epidemiology improves the ability of healthcare professionals and public health authorities to design, conduct and evaluate public health interventions.

The implementation of health promotion strategies based on changes in behavior has proven to be limited[29]. The present findings demonstrate 'upstream' structural and environmental risks, which may simultaneously serve as common causes for a variety of adverse health outcomes[5,30-32]. There is

growing consensus that oral health measures should be included in general health programs[33]. From the standpoint of public health, the common risk approach appears to be the most effective strategy.
In conclusion, the use of geostatistical methods to create equiprobable scenarios enabled the detection of areas with greater probability of dental caries in the study population. Such methods may help establish appropriate interventions and resource allocation, as well as improve our understanding of the influence of the surrounding environment on health status.

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Histological evaluation of subepithelial connective tissue grafts harvested by two different techniques. Preliminary study in humans

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ABSTRACT

Subepithelial connective tissue graft (SCTG) is an essential therapeutic tool in periodontal plastic surgery and implantology. The aim of this preliminary study was to observe and make a histological and histomorphometric comparison of the composition of subepithelial connective tissue grafts (SCTGs) harvested from the palatal mucosa by two different harvesting techniques: mucoperiosteal (lamina propria and complete submucosa including periosteum) and mucosal (lamina propria and a portion of the submucosa). The main hypothesis proposes that SCTG harvested with the mucosal technique contains a greater proportion of connective tissue proper (CTP) and a lower proportion of adipose tissue (AT) than the mucoperiosteal technique.

Twenty healthy patients who required SCTG for different purposes were selected and assigned to one of the two following groups: group A (n=10; mucoperiosteal harvesting technique) and group B (n=10, mucosal harvesting technique). The histological sample was obtained by removing a 2 mm thick slice from the most distal portion of the graft. The proportions of adipose tissue (AT), connective tissue proper (CTP) and vascular tissue (VT) were evaluated.

In group A, histomorphometric analysis showed that CTP accounted for 58.2% of the graft while AT accounted for 32.64%. In group B, the proportions of CTP and AT were 79.86% and 11.93%, respectively. The differences between groups were statistically significant for both tissues (p< .05). In contrast, no statistically significant difference was observed in the proportion of VT.

Within the limitations of this study, the results show that the SCTGs harvested by the mucosal technique contain a greater proportion of CTP and a lower proportion of AT than those obtained by the mucoperiosteal technique, whereas the proportion of VT does not differ.

Further long-term clinical and histological studies with more samples are needed to evaluate the clinical implications of SCTG composition.

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Keywords: *Periodontics; Grafts; Histology.*

Evaluación histológica de los injertos de tejido conectivo subepitelial del paladar mediante dos técnicas diferentes de obtención. Estudio preliminar en humanos

RESUMEN

El injerto de tejido conectivo subepitelial (ITCSE) es una herramienta indispensable en la cirugía plástica periodontal y la implantología.

El objetivo del presente estudio preliminar fue observar y comparar histológica e histomorfometricamente la composición de los injertos de tejido conectivo subepitelial (ITCSE) obtenidos de la mucosa palatina mediante dos técnicas diferentes: mucoperióstica (lamina propia y submucosa incluyendo el periostio) y mucosa (lámina propia y parte de la submucosa). La principal hipótesis postula que el ITCSE obtenido mediante la técnica mucosa contiene mayor proporción de tejido conectivo propiamente dicho (TCP) y menor proporción de tejido adiposo (TA) que el obtenido mediante la técnica mucoperióstica.

El presente estudio incluyó veinte pacientes sanos que requerían ITCSE por diferentes motivos, los cuales fueron distribuidos de forma equitativa en dos grupos: grupo A (n=10; técnica de obtención mucoperióstica) y grupo B (n=10; técnica de obtención mucosa). La muestra histológica se obtuvo removiendo una porción de 2 mm de ancho de la parte más distal del injerto. Se evaluó la proporción (%) de tejido adiposo (TA), tejido conectivo propiamente dicho (TCP) y tejido vascular (TV).

En el grupo A, el análisis histomorfométrico mostró que el TCP constituía el 58.2% del tejido mientras que el tejido adiposo constituía el 32.64%. En el grupo B, la proporción de TCP y AT fue 79.86% y 11.93%, respectivamente. Las diferencias observadas entre los grupos fueron estadísticamente significativas para ambos tejidos (p< .05). En cambio, no se observaron diferencias estadísticamente significativas en la proporción de TV.

Dentro de las limitaciones del presente estudio, los resultados mostraron que los ITCSE obtenidos mediante la técnica mucosa contienen mayor proporción de TCP y menor proporción de TA que los obtenidos con la técnica mucoperióstica, mientras que el TV permanece estable.

Se requieren estudios longitudinales clínicos e histológicos a largo plazo con mayor cantidad de muestras para evaluar las implicancias clínicas de la composición del ITCSE.

Palabras clave: *Periodoncia; Injertos; Histología.*

INTRODUCTION

Subepithelial connective tissue graft (SCTG) is currently considered an essential therapeutic tool in periodontal plastic surgery and implantology.[1-3]

It was initially used to increase the volume of the edentulous ridge and the width of keratinized gingiva.[4,5] Subsequently, it was used for numerous procedures such as root coverage,[6-13] soft tissue augmentation around dental implants and partially edentulous areas,[2,3,14-16] papilla reconstruction, and scar correction.[17-19]

SCTG has shown better aesthetic and biological behavior outcomes in different procedures than have other treatments such as free gingival grafts, allografts, and guided tissue regeneration.[1-3,20]

In order to minimize surgical trauma and reduce post-surgical discomfort, many authors have proposed different harvesting techniques. [6,7,9-12,21-24] They can be classified into two groups: mucoperiosteal techniques (lamina propria and complete submucosa including periosteum),[6,9,21,22] and mucosal techniques (lamina propria and a portion of the submucosa),[10,23,24]. Mucosal techniques have better post-surgical evolution at the palatal donor site, since the periosteal portion remains mostly attached to the bone plate, acting as a protective barrier and a source of vessels (supra-periosteal vessels), reducing wound healing time and patient morbidity.[10,23]

In the mucoperiosteal techniques, the deep portion of the submucosa, which is mainly composed of adipose and/or glandular tissue, is always included in the graft. Some authors[25] suggest that this tissue should be removed because it can interfere with the revascularization of the graft and it may work "as a barrier both to diffusion and vascularization".

Ouhayoun et al.[26] performed a histological and biochemical analysis of SCTG human samples and suggested that the deep portion of the connective tissue from the palate could not induce keratinization.

In the mucosal techniques, the graft can be obtained with a double-bladed scalpel which has parallel blades set 1.5 mm apart.[10,23,24] This technique enables a graft of homogeneous thickness to be obtained and the deep portion of the submucosa, which remains attached to the osseous plate, to be excluded.

Differences in the composition of the graft obtained by the mucosal technique (with a double-blade scalpel) were found in a human histological study.[27] While some samples consisted almost exclusively of lamina propria, others contained higher proportion of submucosa, which is mainly composed of adipose tissue. The authors observed that in all cases, the submucosa lies deeper to the lamina propria. This implies that if a thicker graft is taken by extending the dissection deeper, there is an increase only in the amount of submucosa, while the amount of lamina propria remains constant. It was also observed that the resulting portion of lamina propria could be greatly variable, ranging from 21.1% to 100% of the total composition of the graft. The results of this study suggest that grafts harvested from more superficial areas (closer to the epithelium) would increase the proportion of lamina propria within the graft. This approach, however, will increase the risk of including epithelium in the graft.

To date, no human study has been carried out comparing mucoperiosteal versus mucosal harvesting techniques. Therefore, the aim of the present preliminary study was to describe and compare – through histological and histomorphometric analysis – the composition of SCTG harvested from the palatal mucosa by a modification of the single incision technique (mucoperiosteal)[21] and a modification of the double-bladed scalpel technique (mucosal).[23,24]

The main hypothesis of this study proposes that SCTG harvested with the mucosal technique, excluding the deep portion of submucosa, contains a greater proportion of connective tissue proper and a lower proportion of adipose tissue than the SCTG harvested by the mucoperiosteal technique.

MATERIALS AND METHODS

Patient selection

Twenty patients who were referred to the Department of Periodontology, School of Dentistry, University of Buenos Aires (FOUBA), with procedures requiring SCTG for different purposes, were assigned to two groups according to their need for treatment: Group A: SCTG harvested through mucoperiosteal procedure,[21] in patients who mainly required ridge augmentations (n = 10), and Group B: SCTG harvested by mucosal procedure, [23,24] in patients who mainly needed root coverage procedures (n=10).

Average patient age was 41.5 years (18-65). All patients had good general health. Smokers, patients

with uncontrolled systemic diseases and anticoagulated patients were excluded from the study.
All patients in the study accepted the clinical procedures and signed informed consent approved by the FOUBA Ethics Committee (No. 029/14).

Surgical procedure: sample collection
All grafts were harvested by one experienced periodontist under magnified vision (10x) using an operative microscope (Zeiss ST, Carl Zeiss, Feldbach, Switzerland), from the palatal area comprised from canine to the first molar.
In both groups, the original technique[21,23] was modified: after the initial incision and before taking the graft, a full thickness detachment was performed up to 3 mm from the incision. This limited flap elevation was performed in both groups to allow better access and positioning of the blade, enabling the superficial incision to be placed parallel to the surface of the mucosa, avoiding the concave surface of the palate.[24]
The superficial incision was placed approximately 1 mm away from the epithelial surface, in order to avoid the inclusion of epithelium in the graft. The graft was harvested with a conventional scalpel in the mucoperiosteal (total thickness) technique and with a double-bladed scalpel in the mucosal (partial thickness) technique. Immediately after obtaining the SCTG, it was placed on a wooden tongue depressor without losing the reference of its original location in the palatal mucosa. The histological sample was obtained by removing a 2 mm thick slice from the most distal portion of the graft. A suture (Prolene 6-0 p1 Ethicon, Johnson & Johnson, Somerville, NJ, USA) was placed in the most coronal and superior portion of the graft (where the initial incision was made), which identifies the side and orientation of the sample (Fig. 1). All samples were immediately fixed in 10% formalin for histological analysis.

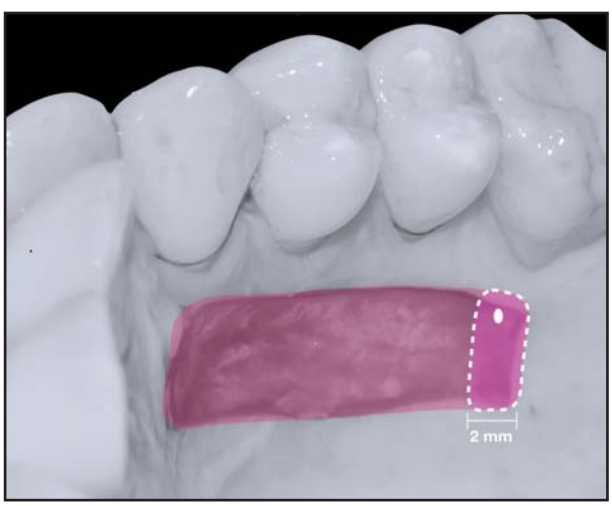

Fig. 1: Area of the subepithelial connective tissue graft is marked on a clinical image. A 2mm distal portion was used for histological evaluation. The white point represents the suture performed to indicate sample orientation (dotted line).

Histological processing
The length, width and thickness of the macroscopic sample was measured with a Vernier caliper. The samples were histologically processed to obtain 7 mm thick longitudinal sections, which were stained with hematoxylin-eosin and Masson´s trichrome. The sections were observed under light microscope.

Histological and histomorphometric analysis
All the sections were first scanned with a microscope (AXIO lab a1 Carl zZeiss) at 5x magnification at a resolution of 0.321 μm/pixel, by a digital virtual microscopy system (Carl Zeiss Zen Blue edition 2011). The most representative section was selected and digital JPEG images were obtained. Finally, tissue composition was analyzed with image analysis software (Image Pro Plus).
Histomorphometric analysis was performed by one of the authors, who was blinded regarding which harvesting technique had been used to obtain the graft. The following parameters were delimited on the microphotographs:

- CTP/TA (%): Connective tissue proper area = fraction of total area corresponding to connective tissue proper area
- AT/TA (%): Adipose tissue area = fraction of total area corresponding to adipose tissue area
- VT/TA (%): Vascular tissue area = fraction of total area corresponding to vascular tissue area

Total area (TA) value was the measurement of the area of the whole histological section. (TA= CTP + AT + VT).

Statistical analysis
Quantitative variables were described by Mean (M), standard deviation (SD) minimum (MIN) and maximum (MAX).
A grouped t-test was used to compare the percent of adipose tissue (AT), vascular tissue (VT) and connective tissue proper (CTP) between the two groups.

The data met the conditions of normality and homoscedasticity required for performing grouped Student's t-test. The assumption of normality was analyzed by the Shapiro-Wilk test with modifications. Homoscedasticity was analyzed by F test for equality of variances. The statistical value (t), the degrees of freedom (df) and the p-value were reported. A statistically significant result was considered when the *p*- value was less than .05. The 2016 version Infostat program was used.[28]

RESULTS

The results of the histomorphometric analysis, regarding the relative proportion of CTP, AT and VT in CTG harvested with mucoperiosteal and mucosal techniques are summarized in Table 1.

In group A (mucoperiosteal technique) the mean value for AT was 32.64%, VT was 8.05% and CTP accounted for 58.52% of the graft. In most samples, the grafts consisted of two different parts: the most superficial portion with dense connective tissue and the deep portion with adipose tissue (Figs. 2, 3). In others samples, the SCTG was almost entirely composed of submucosal tissue. Glandular tissue (GT, minor salivary mucosal glands) was present in one sample in Group A (7.64%; Fig. 4).

In group B (mucosal technique) the mean value for AT was 11.93%, VT was 8.03%, and CTP accounted for 79.86% of the graft. In some cases, the graft was mainly composed of connective tissue proper with zones of extremely dense collagen fibers (Fig.5). In other cases, the density of collagen fibers was moderate, with areas of loose connective tissue (Fig. 6). Epithelium (E) was present in two samples in Group B (1.18%, 0.57%; Fig. 6).

Statistically significant differences were found in the composition of the grafts according to harvesting technique. A higher proportion of CTP

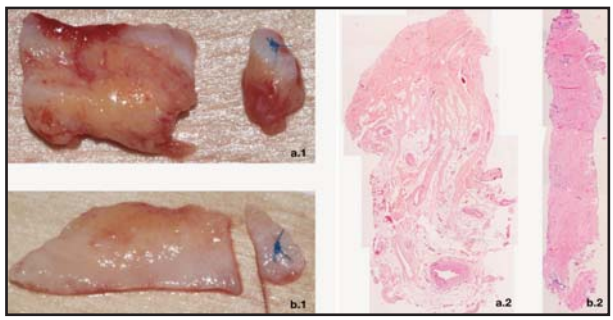

Fig. 2: Longitudinal sections of the grafts were obtained and stained with hematoxylin-eosin (H-E): Group A (mucoperiosteal technique). a.1: Sample collection. a.2: Longitudinal section (H-E stain, original magnification X5). Group B (mucosal technique). b.1: Sample collection. b.2: Longitudinal section (H-E stain, original magnification X5).

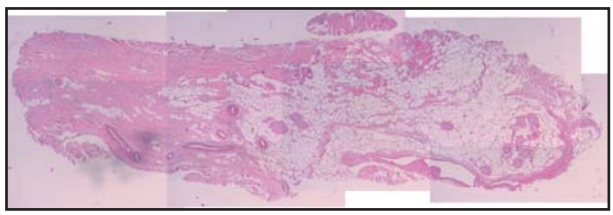

Fig. 3: Histological appearance of mucoperiosteal graft: (H-E stain, original magnification X5). A great proportion of adipose tissue and an increased diameter of vascular vessels are observed in the deeper area.

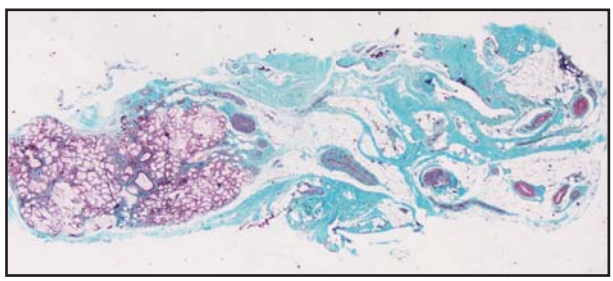

Fig. 4: Histological appearance of mucoperiosteal graft in which a minor salivary gland is observed (GT) (Masson´s stain, original magnification X5).

Table 1: Composition of the grafts: Mucoperiosteal versus mucosal technique.

	Group A: mucoperiostal technique (n=10)				Group B: mucosal technique (n=10)				Grouped t-test		
Component	Mean	SD	MIN	MAX	Mean	SD	MIN	MAX	T	df	p
CTP (% of TA)	58.52	9.78	45.71	78.5	79.86	10.11	63.34	92.47	3.6	9	0.003*
AT (% of TA)	32.64	10.2	12.36	47.38	11.93	8.43	0.97	31.12	-3.74	9	0.002*
VT (% of TA)	8.05	1.54	4.98	9.97	8.03	5.25	3.28	21.45	-.02	9	0.494

*Statistically significant difference, p < .05
AT= adipose tissue; CTP= connective tissue proper; df= degrees of freedom; MAX= maximum MIN= minimum; SD= standard deviation; T= statistical value; TA= total area; VT= vascular tissue.

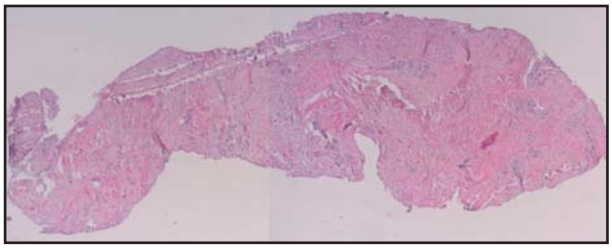

Fig. 5: Histological appearance of mucosal graft: graft composed only of lamina propria. (H-E stain, original magnification X5).

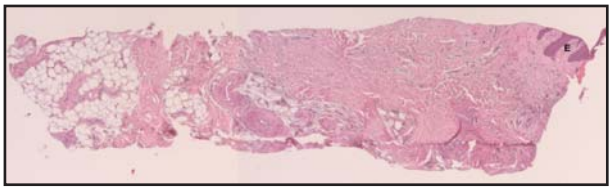

Fig. 6: Histological appearance of mucosal graft: Minimal epithelial tissue (E) and dense connective tissue can be observed in the most superficial portion. (H-E stain, original magnification X5).

and a lower proportion of AT were found in the mucosal technique than in the mucoperiosteal technique (p =.003 and .002, respectively).
No statistically significant difference was found between groups for the proportion of VT (p> .05).

DISCUSSION

Subepithelial connective tissue grafts are widely used in periodontal and peri-implant plastic surgery.[1-3] Although many harvesting techniques and modifications have been proposed since Edel in 1975,[4,6,7,9-12,21-24] there are few studies that describe the histological composition of the tissue harvested from humans.[26,27]
One recent histological study in fresh human cadavers[29] showed that the harvesting technique is important in the composition of SCTG. The authors concluded that SCTG harvested with de-epithelialized technique contains higher proportions of dense connective tissue and lower proportions of adipose tissue than SCTG harvested with split-flap technique (deeper area).
To date, there is no study in humans comparing the composition of the grafts harvested with two different "subepithelial connective tissue graft" techniques; i.e., without removing epithelium with the graft (such as free gingival graft or de-epithelialized free gingival graft). The aim of the present preliminary human study was to describe and compare the histological and histomorphometric characteristics of SCTG harvested from the palatal mucosa by two modified techniques: the single incision technique (mucoperiosteal – total thickness– technique)[21] and the double-bladed scalpel technique (mucosal –partial thickness– technique),[23,24] to evaluate whether the harvesting technique is an important factor in the relative composition of adipose tissue/connective tissue proper.

The palatal mucosa is composed of an epithelial layer, a lamina propria, a submucosa and the periosteum. The thickness of these tissues is relatively uniform for the epithelium and the lamina propria and highly variable for the submucosa.[11] The epithelial layer in the human palatal mucosa is about 0.5 mm thick and the lamina propria is about 1 mm.[30] The limited amount of lamina propria poses a surgical challenge when obtaining a subepithelial graft composed of collagen-rich connective tissue with the standard techniques. The main limitation lies in the curved shape of the palatal mucosa, which makes difficult to maintain the superficial incision close to the epithelial surface, especially in the most apical areas. For this reason, a modification of the standard technique was performed in this study, by elevating 3 mm full thickness mucosa in order to facilitate access with the blade.[24]
In the current study, the presence of lamina propria (connective tissue proper) was observed in both modified techniques. The main histological difference between the techniques was the amount of adipose tissue content, with 20.14 % for the mucosal vs. 41.48% for the mucoperiosteal technique, and the amount of connective tissue proper with 79.86% and 58.52%, for the mucosal and mucoperiosteal technique, respectively (p< .05). Although previous studies[25,26] have suggested that this tissue may not induce keratinization and may interfere with the revascularization of the graft, its clinical relevance has not yet been confirmed.
On the other hand, the amount of vascular tissue was similar in both groups (p< .05), although wider vessels were observed only in the deep submucosa of the mucoperiosteal group, which may partly explain the increase in bleeding observed while this technique is being performed. Glandular tissue was

found only in 1 case, representing 7.64% of the graft, while epithelium was present in two samples in the mucoperiosteal group in minimal proportions (1.18%, 0.57%) but was not found in the mucosal technique group. This could be important considering that, although some authors have suggested that the inclusion of epithelium in the graft does not affect clinical results; [6,9,27] others have reported complications as a result of epithelial cysts and edema.[31]

Within the limitations of the present study, we can conclude that the SCTGs harvested by the mucosal –partial thickness– technique contain a greater proportion of connective tissue proper and a lower proportion of adipose tissue than the mucoperiosteal –total thickness– technique, whereas the only tissue that remains stable is the vascular tissue.

Further long-term clinical and histological studies with a greater number of samples are needed to evaluate the clinical implications of SCTG composition.

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None

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Prevalence of periodontitis in a population of patients on dialysis in Colombia

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ABSTRACT

The aim of this study is to establish the prevalence of Chronic Periodontitis (CP) in patients with Chronic Kidney Disease (CKD) and to ascertain its relationship with several factors or indicators of micro inflammation. One hundred and thirty-five CKD patients on dialysis treatment were included. Biochemical parameters, clinical attachment level and pocket depth were recorded according of the American Academy of Periodontology and the CDC (CDC-AAP). Gingivitis and CP were recorded based on the biofilm-gingival interface (BGI) periodontal diseases classification. The rate of non-response to the survey was 10 percent. A total 2,636 teeth in 135 patients were examined, of whom 52.5% were males. Average age was 55.7 years (SD ± 1.32); 41.4% had a smoking history; 78/135 patients were on hemodialysis and 57/135 on peritoneal dialysis; 55.5% had been on dialysis for more than three years. Prevalence of gingivitis and periodontitis was 14.8%, 95% CI (9.7-21.9) and 82.2%, 95% CI (74.7 – 87.8), respectively; according to the BGI Index. Severity of CP was: No periodontitis, 14.0% 95% CI (9.1 - 21.1); mild, 11.1% 95% CI (6.7 - 17.7); moderate, 28.8% 95% CI (21.7 - 37.1); and severe, 45.9% 95% CI (31.6-54.47). Peritoneal dialysis and time on dialysis > 3 years increase the chance of having periodontitis, OR 11.0 95% CI (2.2-53.8) and OR 7.6 95% CI (1.1-50.2), respectively. In view of the high prevalence of CP in this population, programs designed to ensure better periodontal and gingival care in the population on dialysis need to be established.

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Keywords: *Chronic periodontitis; prevalence; peritoneal dialysis; renal insufficiency chronic.*

Prevalencia de enfermedad periodontal en una población de pacientes en diálisis en Colombia

RESUMEN

El objetivo de este estudio fue establecer la prevalencia de Periodontitis Crónica (PC) en pacientes con enfermedad renal crónica (ERC) en diálisis y determinar la relación de su presencia con algunos indicadores de micro inflamación. Un total de 135 pacientes con ERC en terapia dialítica fueron incluidos en este estudio. Se evaluaron parámetros bioquímicos, nivel de inserción clínica (NIC) y profundidad de sondaje (PS), de acuerdo con la Asociación Americana de Periodoncia y el CDC de Atlanta (CDC-AAP). También fue evaluada, la gingivitis y la PC de acuerdo con la clasificación interface biopelicula-encia (BGI). La tasa de no respuesta a la encuesta fue del 10%. Un total de 2636 dientes en 135 pacientes fueron evaluados, (52.5% hombres, edad promedio 55.7 ± 1.32), 56% con antecedente de tabaquismo. 78/135 en hemodiálisis y 57/135 en diálisis peritoneal, el 55.5 % con un tiempo en diálisis mayor a tres años. La prevalencia de gingivitis por la clasificación BGI fue del 14.8% IC 95% (9.7 - 21.9) y de periodontitis 82.2% IC 95% (74.7 – 87.8). La severidad de la PC fue: sin periodontitis 14.0% 95% IC (9.1 - 21.1); leve 11.1% 95% IC (6.7 - 17.7); moderada 28.8% 95% IC (21.7 - 37.1) y severa 45.9% 95% IC (31.6-54.47) La diálisis peritoneal y el tiempo en diálisis aumentaron la chance de tener PC: OR 11.0 95% IC (2.2-53.8) y OR 7.6 95% CI (1.1-50.2) respectivamente. Por la alta prevalencia de PC en esta población, es necesario establecer programas para asegurar el cuidado de la salud periodontal en esta población en diálisis.

Palabras clave: *Periodontitis crónica; prevalencia; diálisis peritoneal, insuficiencia renal crónica.*

INTRODUCTION

Chronic kidney disease (CKD) has become recognized as a key independent risk factor for several adverse health outcomes including cardiovascular disease (CVD)[1]. There is evidence that supports an inverse relationship between glomerular filtration rate (GFR) and degree of systemic inflammation in these patients[2]. Several epigenetic and genetic factors

influencing chronic inflammatory status have been identified, including reduced cytokine clearance, frequent infections, presence of oxidative stress, intestinal dysbiosis, periodontal disease, metabolic acidosis, deficit of vitamin D, and dialysis-related factors[2]. All these factors serve to perpetuate chronic inflammatory status, which leads to the occurrence of cardiovascular complications, protein-energy wasting, anemia and bone mineral disease; and to increased mortality rates in this population[2].

Acute phase markers are part of the innate immune response to Chronic Periodontitis and have been detected in systemic circulation. Platelet-lymphocyte ratio (PLR) and C-reactive protein are considered to be biomarkers of systemic inflammation[3,4].

The presence of urea in saliva and increased levels of blood urea nitrogen (BUN) have been associated with uremic stomatitis, which normally disappears after BUN values return to normal range[3, 5].

Patients with non-dialysis CKD, those receiving renal replacement therapy with dialysis and even patients with renal transplantation, experience changes in periodontal tissues. These patients usually have increased levels of plaque, calculus and gingival inflammation, as well as gingival hyperplasia and increased prevalence and severity of periodontal disease[5].

Chen et al. have shown a positive correlation between periodontal index, age and time on dialysis. Furthermore, they found an association between the presence of severe periodontal disease and low albumin levels in the population on hemodialysis, which might imply that systemic inflammatory status is a consideration in patients receiving renal replacement therapy[6].

According to a number of reports, 58.9% of prevalent hemodialysis patients have moderate to severe periodontitis[6]. In Thai population the prevalence of severe periodontal disease is 15.9% among the older population and greater prevalence of poor periodontal status in severe (23%) and moderate (25%) CKD (all groups had mean ages >50 years) is observed. The high prevalence of poor periodontal status in patients with severe and moderate CKD,was confirmed[7]. A study conducted in Colombia found that the prevalence of gingivitis among patients on dialysis ranged from 35% to 38%[8] .

The aims of this study are to estimate the prevalence of periodontal disease in the population on dialysis and to evaluate the severity and activity of periodontal compromise as a component of systemic inflammation, to establish which factors could be associated to this pathology.

MATERIALS AND METHODS

An analytical cross-sectional study was conducted, including patients aged 18 and above who had been on dialysis for more than 90 days, with at least six natural teeth, on chronic anticoagulation therapy within therapeutic range, who received care at seven renal clinics in Bogota. Patients who had taken antibiotics during the previous 3 months were excluded. A 2-stage sampling method was used. A probability proportional to size design was used in the first stage, and simple random sampling without replacement in the second stage.

Demographic and clinical variables such age, sex, cause of CKD, dialytic therapy, smoking history, time on dialysis, albumin and PRL were considered in this study. Data were obtained from the RTS Versia® electronic clinical history system and the periodontal assessments database. Periodontal measurements were performed by a single experienced periodontist using a Welch Allyn medical endoscope cold light source and a 15 mm long standard periodontal probe (UNC-15, Hu-Friedy. IL, USA). Measurements of plaque index (PI)[9], bleeding on probing (BOP), pocket depth (PD) and clinical attachment loss (CAL) were recorded at 6 sites per tooth. Root fragments and furcation measurements were excluded. The average value was calculated for each tooth using the measurements at six sites per tooth, and the average value was calculated per subject.

Silness and Loe plaque index was used to score the PI, i.e., 0: No plaque, 1: Presence of plaque only detectable by using the probe, 2: Moderate and visible plaque, 3: Abundance of plaque covering more than one third of the tooth surface.[9]

Bleeding sites were considered for the BOP, and an average value was determined for each subject. The pocket bleeding (PB) was calculated from the proportion of tooth surfaces with pockets per subject. An average value per subject was also calculated for CAL.

Periodontitis was diagnosed based on the Biofilm-Gingival Interface (BGI) index, the main independent variable[10].

Definition and severity of periodontitis were based on the CDC-AAP surveillance proposal, which was

also used to compare the findings of other studies conducted locally[11]. This database has quality controls in place to guarantee the soundness and confidentiality of the information. For the descriptive analysis, we used percentages for categorical variables and means or medians with their respective dispersion measures for continuous variables. Logistic regression was used to conduct multivariate analysis. The statistical analysis was performed using Stata 14® software. This study was conducted with the approval and oversight of an institutional research ethics committee. RTS Ruling number 003, May 17, 2016.

Table 1: Summary of descriptive data for the sample population

	n= 135	
Characteristics of the sample	**n**	**%**
Age [mean, SD] years	55.7 (1.32)	15.33
Male	71	52.59
Dialytic therapy		
Hemodialysis	*78*	*57.78*
Peritoneal dialysis	57	42.22
Smoking history	56	41.48
Time on dialysis		
Less than 1 year	*17*	*12.59*
1 to 3 years	*43*	*31.85*
More than 3 years	75	55.56
Cause of CKD		
High blood pressure	*51*	*37.78*
Diabetes Mellitus	*34*	*25.19*
Other	*21*	*15.56*
Obstructive	*17*	*12.59*
Glomerular	12	8.89
Albumin [mean; SD] gr/dl	4	0.44
PLR >= 140	77	72.64

CKD= Chronic Kidney Disease; SD= Standard Deviation; PLR: Platelet to Lymphocyte Ratio

RESULTS

A total 2,636 teeth from 135 patients were examined. Participants' mean age was 55.7 years (SD = 1.32); 52.5% were male; and time on dialysis treatment was longer than 3 years in 55.6% of patients. Details are provided in Table 1.

According to the BGI index, prevalence of gingivitis and periodontitis was 14.8%, 95% CI (9.70-21.95) and 82.2%, 95% CI (74.73-87.85), respectively. Prevalence of periodontitis according to the CDC-AAP severity classification was as follows: Healthy, 14.0%, 95% CI (9.1 - 21.1); mild, 11.1%, 95% CI (6.7 - 17.7); moderate, 28.8%, 95% CI (21.7 - 37.1); and severe, 45.9%, 95% CI (31.6-54.47). See Table 2.

We found that patients on peritoneal dialysis, OR 11.0 95% CI (2.2-53.8), who had been on dialysis for more than 3 years, OR 7.6 95% IC (1.1-50.2) had greater chances of developing periodontitis. Further details are provided in Table 3.

DISCUSSION

The prevalence of CP found in the study population is high, including the prevalence of severe periodontitis according to the CDC-AAP diagnostic criteria[11, 12]. Interestingly, nearly 86.0% of the study population had developed some degree of periodontitis. This result is higher than the 61.8% reported in the ENSAB IV (Spanish acronym for the fourth National Oral Health Study)[13]. In our study, the non-response rate to the assessment was

Table 2: Severity and activity of periodontal disease.

n= 135					
Severity of Periodontitis			**Activity of Periodontitis**		
Periodontal disease (CDC-AAP)	*n*	*%*	*BGI Index*	*n*	*%*
0. No periodontitis	19	14.07	1. Healthy	4	2.96
1. Mild	15	11.11	2. Gingivitis	20	14.81
2. Moderate	39	28.89	3. Mild pocket bleeding	10	7.41
3. Severe	62	45.93	4. Moderate pocket bleeding	20	14.81
			5. Severe pocket bleeding	81	60.00

Table 3: Logistic regression multivariate analysis.

Periodontitis	Odds Ratio	P value	[95% Conf. Interval]	
Smoking history	0.87	0.84	0.22	3.39
Female	0.38	0.16	0.10	1.47
Age; years	1.04	0.07	1.00	1.08
Peritoneal Dialysis: Hemodialysis	11.06	0.00	2.27	53.87
Less than 1 year	Ref			
1 to 3 years	5.04	0.11	0.70	36.43
More than 3 years	7.63	0.04	1.16	50.23
PLR	0.40	0.17	0.11	1.47
Albumin; gr/dl	0.98	0.98	0.25	3.89

PLR: Platelet to Lymphocyte Ratio

10%, which is not a high figure for dental assessment in patients with chronic disease. These patients did not attend their medical follow-up appointments.

According to the BGI index, periodontitis prevalence is also high in the "Severe pocket bleeding" category (60.0%) and may represent a source of systemic micro-inflammation, since this index accounts for bleeding and periodontal pocket activity. No other study was found assessing the presence of gingivitis and periodontitis in these patients as described in the present study.

Of the 2,636 teeth evaluated, mean bleeding on probing per examined subject was 65.6% ± SD 31.4%; which demonstrates a high level of periodontal tissue inflammation in this study sample. Nevertheless, no association was found between inflammatory market platelet-lymphocyte ratio (PLR) and presence of periodontitis, possibly because of the sample size. This suggests the need for further research on the potential association between CP and inflammation biomarkers such as interleukin 6 and highly sensitive C-reactive protein.

The multivariate analysis showed an association between treatment with peritoneal dialysis and duration on dialysis longer than 3 years and presence of CP. However, these estimators do not ensure the best accuracy, which could also be explained by the sample size, which was originally calculated to estimate the prevalence of periodontal disease. Severity and activity of CP in these patients were higher than reported in published studies on the general population.

CONCLUSIONS

In view of the high prevalence of CP in this population, programs designed to ensure better periodontal and gingival care in the population on dialysis need to be established. As a conclusion, the presence of periodontitis in these patients was higher than in the general population.

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Evaluation of 3Mix-MP and pulpectomies in non-vital primary molars

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ABSTRACT

Pulpectomies in primary molars are often hindered by several factors, including anatomical and physiological characteristics of posterior primary teeth and young patients' lack of cooperation with laborious treatments. This study was undertaken in search of easier but equally effective therapies that could eliminate infection, preserve the teeth and avoid extractions. The aim of the study was to estimate and compare clinical and radiographic success between pulp treatment with 3Mix-MP and pulpectomy with Maisto-Capurro paste in primary necrotic molars. A longitudinal prospective study was conducted at the Department of Comprehensive Pediatric Dentistry of the Faculty of Dentistry of the University of Buenos Aires (2015-2017). The study included 46 primary molars with necrotic pulp of children without immune or metabolic compromise. Children and their legal guardians provided assent and informed consent. Selected molars were randomly divided into 2 groups: G1: Pulpectomy treatment with Maisto-Capurro paste; and G2: Treatment with 3Mix-MP paste. Treatments were evaluated at 1, 3, 6, 12 and 18 months (intra and inter-rater agreement 0.92 and 0.84). Clinical success was considered to be the absence of any of the following: pain, sensitivity to percussion or palpation, swelling, fistula and non-physiological mobility, while radiographic success was considered to be: absence of internal or external non-physiological resorption, no progression or reduction of radiolucent periapical/interradicular lesion and evidence of bone regeneration. Percentages, 95% C.I., and CHI² were calculated for the comparison between groups. Overall clinical success was 91.5% and 87.5% (p=0.48) and overall radiographic success was 88.3% and 82.3% (p=0.31) for G1 and G2 respectively. No significant clinical or radiographic difference was found between groups. Both treatments showed similar clinical and radiographic behavior during the study periods.

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***Keywords**: 3Mix ointment, pulpectomy, primary dentition, dental pulp, endodontics.*

Evaluación de 3Mix-MP y pulpectomías en molares primarios no vitales

RESUMEN

Las pulpectomías en molares primarios se ven dificultadas frecuentemente por las características anatómicas y fisiológicas de éstos y por la escasa colaboración que brindan los pacientes de corta edad ante tratamientos tan laboriosos. En búsqueda de terapéuticas más sencillas, pero igualmente eficaces, que consigan eliminar la infección para conservar las piezas y evitar las exodoncias, se ha planteado como objetivo de este estudio: estimar y comparar la proporción de éxito clínico y radiográfico entre el tratamiento pulpar con 3Mix-MP y la pulpectomía con pasta de Maisto-Capurro en molares primarios con necrosis. Se realizó un estudio longitudinal y prospectivo en la Cátedra de Odontología Integral Niños de la Facultad de Odontología de la Universidad de Buenos Aires (2015 - 2017). Formaron parte del estudio 46 molares primarios con diagnóstico de necrosis pulpar, de niños sin compromiso inmunológico ni metabólico y que junto con sus responsables legales brindaron el asentimiento y el consentimiento informado. Los molares seleccionados fueron divididos aleatoriamente en 2 grupos: G1: Tratamiento de pulpectomía con pasta de Maisto-Capurro y G2: Tratamiento con pasta 3Mix-MP. Los tratamientos fueron evaluados al mes, 3, 6, 12 y 18 meses (concordancia intra-examinador 0,92 e inter-examinador 0,84), considerando como éxito clínico la ausencia de dolor, sensibilidad a la percusión y palpación, edema, fístula y movilidad no fisiológica; y como éxito radiográfico, ausencia de reabsorción interna o externa no fisiológica, no progresión o reducción de la lesión radiolúcida interradicular/periapical y evidencia de regeneración ósea. Se calcularon porcentajes, I.C 95% y CHI² para la comparación. El éxito clínico global fue de 91,5% y 87,5% (p=0.48) y el éxito radiográfico global de 88,3% y 82,3% (p=0.31) para G1 y G2 respectivamente, sin diferencias significativas entre ambos grupos. En los periodos estudiados ambos tratamientos mostraron comportamientos clínico y radiográfico semejantes.

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***Palabras clave:** Pasta 3Mix, pulpectomías, dentición primaria, pulpa dental, endodoncia.*

INTRODUCTION

Premature loss of primary molars can cause a series of consequences such as reduction of the space required for eruption of replacement teeth, mesialization of permanent first molars, problems with mastication and lingual interposition, among

others. The use of space maintainers is not always effective, especially when primary second molars are lost prior to the eruption of permanent first molars.

In pediatric dentistry, non-vital primary teeth often receive endodontic treatment with the aim of preserving those teeth in adequate anatomical-functional condition until the time of their normal exfoliation.

Pulpectomy is the endodontic therapy of choice. During this treatment, necrotic pulp tissue is removed, root canals are shaped and disinfected and an intracanal resorbable medication is placed[1]. In Argentina, Maisto-Capurro paste is the most frequently used endodontic material for filling root canals in primary teeth. It is an alkaline paste composed of calcium hydroxide and iodoform and is characterized by having the following properties: antimicrobial potential, biocompatibility, radiopacity and rapid resorption[2]. Several factors make pulpectomy treatment difficult to perform: the complex internal anatomy of root canals in primary molars,[3] which prevents their adequate mechanical/chemical preparation, physiological root resorption processes that modify the shape, position and size of the endodontic apex and factors related to cooperation and behavior in young children.

Less complex techniques are therefore needed, which would simplify operative steps, require less cooperation from the patient, and at the same time ensure proper disinfection of the root canal system. LSTR (Lesion Sterilization and Tissue Repair) therapy, developed at the University of Niigata, Japan, is proposed as a treatment option for primary molars with pulp necrosis. LSTR is based on the concept that repair is possible if lesions are adequately disinfected[4]. It uses the NIET (Non-Instrumental Endodontic Treatment) technique and a paste with proven antimicrobial efficacy: 3Mix-MP, which is a triple antibiotic paste (3Mix) containing metronidazole, ciprofloxacin and minocycline powder in macrogol and propylene glycol (MP) antibiotic carriers[5].

The ability of 3Mix-MP to disinfect carious lesions and infected root dentin lesions has been demonstrated in vitro, [5-7] including its efficacy against *E. Coli* [6] and *Enterococcus faecalis* and *faecium*[8]. Studies have also been performed on animals[9] and it has been proven that propylene glycol as a triple antibiotic paste carrier enables adequate penetration through dentin[10] and that its biocompatibility is similar to that of calcium hydroxide[11].

The aim of the current study was to estimate and compare clinical and radiographic success between pulp treatment with 3Mix-MP and pulpectomy with Maisto-Capurro paste in non-vital primary molars.

MATERIALS AND METHODS

This experimental, prospective, longitudinal study was conducted at the Department of Comprehensive Pediatric Dentistry at the Buenos Aires University School of Dentistry during 2015 – 2017, with approval from its Ethics in Research Committee (N° 0018/2015).

Sample selection and group formation

Teeth were selected through anamnesis and clinical-radiographic examination according to the inclusion and exclusion criteria specified below.

Inclusion criteria: primary molars diagnosed with pulp necrosis in patients of both sexes who visited for comprehensive dental care, and who signed assent and informed consent together with their legal guardians.

Exclusion criteria:

- Molars with more than 1/3 root resorption.
- Presence of periapical or interradicular radiolucent areas which could compromise the permanent tooth bud.
- Internal resorption.
- Perforation of the pulp chamber floor.
- Teeth with extensive crown destruction which would not allow subsequent restoration.
- Immunologically and/or metabolically compromised patients.

Selected molars were randomly assigned to 2 groups: G1 (control), treated with pulpectomy and Maisto-Capurro paste; G2 (experimental) treated with LSTR 3Mix-MP.

Tooth of patients who attended on even days were assigned to G1, and those who attended on odd days to G2. If the same patient had more than one molar appropriate for the study, such molars were assigned to different groups.

Professional team and evaluation criteria

Four professionals were trained and calibrated in the examination methods, materials and sequence of technical procedures (intra-rater agreement 0.92

and inter-rater agreement 0.84). They selected the teeth for the study, performed the procedures according to the protocol described in Table 1 and evaluated evolution clinically and radiographically at 1, 3, 6, 12 and 18 months.

Clinical success was considered to be the absence of any of the following: pain or sensitivity to percussion and palpation, swelling, fistula and non-physiological mobility, while radiographic success was considered to be: absence of internal or external non-physiological resorption, no progression or reduction of radiolucent periapical/interradicular lesion and evidence of bone regeneration.

Endodontic pastes preparation

1. Maisto-Capurro paste: equal parts by volume of calcium hydroxide and iodoform, with propylene glycol as carrier.
2. 3Mix-MP paste: the three antibiotics in this paste were purchased in their pure powdered form. For the preparation of the mixture they were proportioned in equal parts by volume (metronidazole: ciprofloxacin: minocycline=1:1:1). Carriers (macrogol and propylene glycol) were also used in a ratio 1:1 by volume. The antibiotic powder mixture was added to the carriers until a consistent, non-friable paste was obtained[12].

Both pastes were prepared at the time of use.

Statistical analysis

The records for the different variables were entered into a Microsoft Excel database and analyzed statistically with SPSS software (Statistical Packages for the Social Sciences 20.0; IBM, Armonk, NY, USA). Percentages were calculated with their respective 95% confidence intervals and Chi-square test with a significance level of 0.05 was used to compare evolution between groups.

RESULTS

Each group consisted of 23 primary molars in children whose mean age was 6.15 ± 1.38 and 6.3 ± 1.49 for G1 and G2, respectively.

Comparison between groups of clinical and radiographic characteristics of molars in baseline conditions showed no significant difference (p= 0.66) (Fig.1).

Table 1: Procedural protocol.

G1 – Maisto-Capurro Pulpectomy	G2 – LSTR 3 Mix-MP
• Preoperative periapical radiograph	
• Local anesthesia	
• Complete rubber dam isolation of operative field	
• Removal of carious tissue with sterile excavators or round burs at low-speed	
• Access opening of pulp chamber with round burs at high-speed and adequate water spray cooling	
• Removal of necrotic coronal pulp tissue using excavators	
• Visualization of the orifices of the root canals	
• Irrigation of pulp chamber with 1% NaOCl	• Irrigation of chamber with 2.5% NaOCl
• Radiographic determination of working length at 1-2 mm short from apex or from root resorption	• Drying with sterile cotton rolls
• Manual instrumentation using K-files not larger than size 30, with circumferential movement alternating with 1% NaOCl irrigation.	• Placing 3Mix-MP paste on floor of pulp chamber
• Canals drying with sterile paper points.	
• Canals filling with Maisto-Capurro paste	
• Cavity sealing with reinforced eugenol zinc oxide (IRM® Dentsply)	
• Postoperative periapical radiograph	
• Clinical follow-up at one week and rehabilitation with preformed steel crown (3M® ESPE) cemented with glass ionomer (Meron® Voco)	
• Clinical-radiographic follow-up at 1, 3, 6, 12 and 18 months.	

Table 2 shows the clinical and radiographic outcomes for the times evaluated. The success rates of both G1 and G2 declined over time.

At 18 months (as the result of patients non-compliance with follow-up appointments) 18 molars (78.2%) were checked in G1 and 17 molars (73.9%) in G2. Clinical success at 18 months was 16/18 (88.8%) in G1 and 14/17 (82.3%) in G2, and radiographic success at 18 months was 15/18 (83.3%) and 13/17 (76.4%) in G1 and G2 respectively.

Overall clinical success was 91.5% and 87.5% (p= 0.48) and overall radiographic success was 88.3% and 82.3% (p= 0.31) for G1 and G2 respectively, with no significant difference between groups.

DISCUSSION

The management of primary molars diagnosed with necrosis is a controversial issue in pediatric dentistry. Endodontic therapy is a reasonable conservative option to ensure normal tooth exfoliation or long-term survival of the primary tooth in cases of agenesis. It also reduces the need for extraction, which, in addition to being a traumatic experience for a young child, requires the use of prosthetic resources to maintain spaces, involving higher costs and commitment to periodical follow-up.

The AAPD[1] (American Academy of Pediatric Dentistry) considers pulpectomy to be the endodontic treatment of choice for necrotic primary molars. The review of the literature on this treatment showed

Table 2: Clinical and radiographic results for the evaluated periods.

		1 month		3 months		6 months		12 months		18 months		Overall success	
		C. S.	Rx. S.	C. S.	Rx. S.	C. S.	Rx. S.	C. S.	Rx. S.	C. S.	Rx. S.	C. S.	Rx. S.
G1	n	22/23	22/23	20/21	20/21	17/19	16/19	16/18	15/18	16/18	15/18		
	%	95.6	95.6	95.2	95.2	89.4	84.2	88.8	83.3	88.8	83.3	91.5	88.3
	95% CI	(77.0-99.8)	(77.0-99.8)	(75.1-99.8)	(75.1-99.8)	(63.7-98.6)	(57.5-98.6)	(65.2-98.6)	(55.5-96.4)	(65.2-98.6)	(55.5-96.4)	(87.2-94.7)	(80.5-95.4)
G2	n	21/23	21/23	20/22	19/22	18/20	16/20	15/18	14/18	14/17	13/17		
	%	91.3	91.3	90.9	86.3	90.0	80.0	83.3	77.7	82.3	76.4	87.5	82.3
	95% CI	(69.1-98.9)	(69.1-98.9)	(67.9-98.8)	(62.8-97.0)	(65.2-98.7)	(54.8-94.2)	(55.5-96.4)	(49.9-93.6)	(53.3-96.2)	(47.3-93.2)	(82.0-91.9)	(74.5-89.4)

Clinical and radiographic success at the different evaluation periods and overall success, without significant differences between groups
(C.S.: clinical success; Rx.S.: radiographic success.)

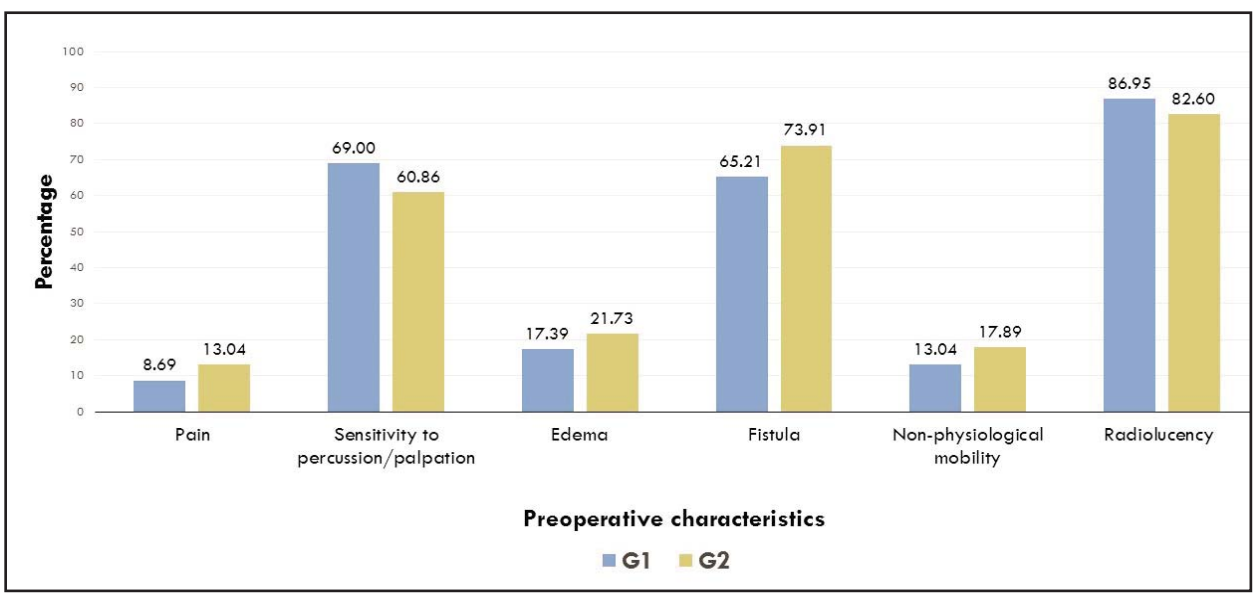

Fig. 1: Analysis of baseline clinical and radiographic characteristics for both groups. Preoperative characteristic percentages for each group (G1 and G2). No statistically significant difference was found between groups (p=0.66).

variable success rates, depending on the materials employed for filling the canals. The most frequently used are zinc oxide eugenol (ZOE), iodoform pastes (KRI®) and pastes with calcium hydroxide and iodoform (Vitapex®, Metapex®), among others.

Vitapex® has been widely studied and the literature reports clinical success rates of 100%[13-16] and radiographic success rates at 12 months of 90 to 100%[13,15-17]. We used Maisto-Capurro paste in this study because it is very similar to Vitapex® in composition and characteristics. However, our overall clinical and radiographic results (91.5% and 88.3%, respectively) were slightly lower than those mentioned above for treatments with Vitapex®. We assume that this difference may be owed to the fact that Vitapex® is an industrial paste, ready to be used and presented in a syringe application system which facilitates manipulation, whereas in the present study Maisto-Capurro paste was prepared at the time of use, the ingredients were dispensed by the operators, and it was applied within the canals using endodontic files (Fig. 2).

As mentioned, due to the complexity of the technique and the topographic characteristics of primary molars, pulpectomy is not always suitable to all children or all professionals. LSTR 3Mix-MP therapy is proposed as an alternative, especially for uncooperative children and low-resource areas. The procedure is simple, not time-consuming, and demands only one visit. It requires no mechanical instrumentation because the antimicrobial capacity of 3Mix-MP paste sterilizes the area, promoting lesion repair and preserving the primary tooth until normal exfoliation time[4] (Figs. 3 and 4).

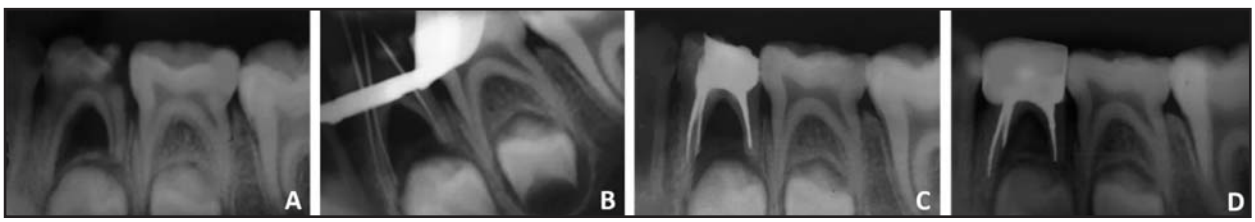

Fig. 2: Successful pulpectomy in a lower left first molar. A: Initial image: carious lesion involving pulp and interradicular radiolucency. B: Determination of working length. C: Immediately postoperative image. D: X-ray. 12 months postoperative.

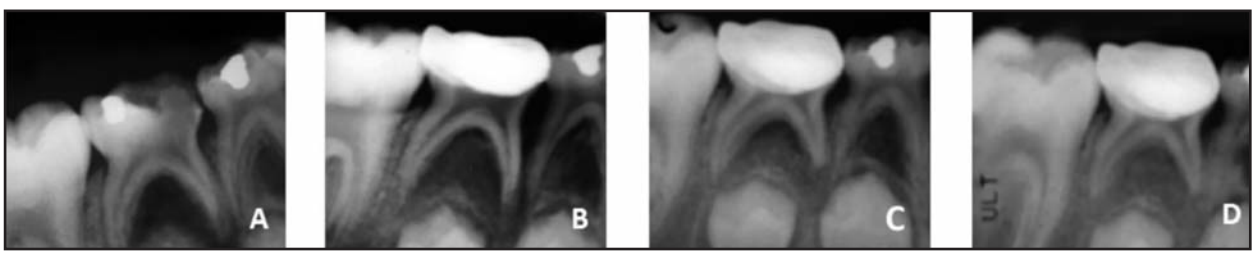

Fig. 3: Successful LSTR 3Mix-MP in a lower right second molar with carious lesion involving pulp and interradicular radiolucency. A: Initial image. B: X-ray. 6 months postoperative: shows non-progression of furcation lesion. C: X-ray. 12 months postoperative: reduction in interradicular radiolucency area and presence of new bone formation. D: X-ray. 18 months postoperative: complete resolution of bone lesion.

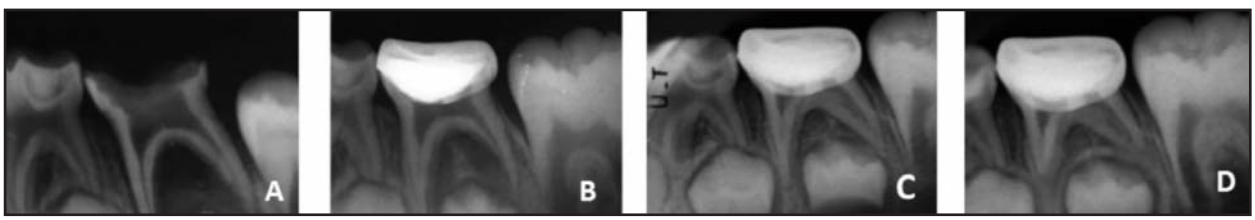

Fig. 4: Successful LSTR 3Mix-MP in a lower left second molar with carious lesion involving pulp and interradicular radiolucency. A: Initial image. B: X-ray. 6 months postoperative: reduction in interradicular radiolucency area and presence of new bone formation. C-D: X-ray. 12 - 18 months postoperative: complete resolution of bone lesion.

In 2016, we published a preliminary study on treatment with 3Mix-MP paste on 44 non-vital primary molars, using the same diagnostic and evaluation criteria as in the current study. Follow up at one month post-operatively showed only one clinically unsatisfactory treatment with persistence of fistula, and three radiographically unsatisfactory treatments with increase in the interradicular radiolucent area; with no further failure up to the final follow-up evaluation at 12 months[18].

Regarding to overall clinical success, in the current study we achieve a rate of 87.5% for LSTR 3Mix-MP, which is lower than the percentages reported by Prabhakar et al.[19], Nakornchai et al.[20] and Nanda et al.,[21] who obtained 93% to 100% for 12-month follow-up periods. This difference may be due to the fact that follow-up in our study was 18 months. Other authors such as Trairatvorakul & Detsomboonrat[22] and Duanduan et al.[23] achieved lower clinical success rates (75% and 84.62%, respectively) in longer follow-up periods. This suggests that the clinical success rate for LSTR 3Mix-MP therapy decreases as post-treatment time increases.

Radiographic success results are more heterogenous; Nanda et al.[21], Nakornchai et al.[20] and our study had success rates higher than 75%, while Trairatvorakul et al.[22] and Prabhakar et al.[19] had success rates lower than 40%. The disparity in success rates may be due to the different selection and evaluation criteria employed. Trairatvorakul classified teeth with no evidence of bone regeneration at 6 months as failures, in contrast to the rest of the clinical studies in which non-progression of the radiographic lesion was considered as success for all evaluation times.

In a prospective study on 50 teeth, Nakornchai et al.[20] compared 3Mix-MP and Vitapex® with 12-month follow-up, finding a clinical success rate similar to ours and a slightly lower radiographic success rate for Vitapex®. This may be because the study by Nakornchai et al.[20] included molars with unfavorable baseline conditions for pulpectomy treatments.

In a retrospective study, Duanduan et al.[23] compared pulpectomy treatments with Vitapex® and LSTR 3Mix-MP in 73 non-vital teeth for 6 to 72 months, finding no significant difference, either clinical or radiographic, between treatments (Vitapex 89% and 64.6%; 3Mix-MP 84.6% and 65.2%.). They also report that type of restoration and time between temporary and permanent restorations are significant factors in prognosis, concluding that LSTR therapy is simple, time-saving, places less psychological load on patients and should be especially considered for primary molars with poor prognosis for pulpectomy treatments.

Other researchers report slightly higher success rates with modifications to the antibiotic paste. Pinky et al. [24], Nanda et al.[21] and Doneira et al.[25] replaced metronidazole with ornidazole, which has longer-lasting action, better efficacy and lower metabolism than metronidazole.

According to Kayalvizhi et al.,[26] another factor impacting disparity in results may be the lack of standardization of the technique, the proportion of drugs and paste preparation.

Our study used equal parts of antibiotics, following Hoshino et al.[5], and 2.5% NaOCl as irrigant, following Nakornchai et al.,[20] due to its powerful antimicrobial activity and ability to dissolve organic tissue. Other authors employed saline solution or pulp chamber cleaners such as phosphoric acid or EDTA[4,19,21,22,24].

We consider it unnecessary to make small receptacles for the medication by enlarging the entrance to the canals (2 mm deep and 1 mm diameter) to contain more paste; given the porosity and permeability of the pulp chamber floor [27] which would facilitate rapid diffusion of 3Mix, in addition to the fact that it has been proven that propylene glycol, used as carrier in the paste, penetrates through dentinal tubules [10].

For the definitive restoration of treated molars, we used steel crowns, as did most authors. Steel crowns ensure a proper seal, preventing microfiltration, which is essential to repair[28].

CONCLUSION

For the times studied, both treatments had similar clinical and radiographic behavior.

Pulpectomy with 3Mix-MP could be a valid option for treatment of non-vital molars in young or uncooperative patients.

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Deproteinization of primary enamel with sodium hypochlorite before phosphoric acid etching

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ABSTRACT

The aim of this study was to evaluate the deproteinization of primary enamel by analyzing etching pattern types, with and without the application of 5% NaOCl before acid etching with 37% H_3PO_4. Fifteen extracted human primary molars were randomly selected for the present in vitro study; 1mm x 1mm blocks were prepared and divided into two groups (n = 21). These groups were treated as follows: Group A- Acid Etching with 37% H_3PO_4 gel for 15 s; Group B- 5% NaOCl for 60 s + Acid Etching with 37% $H_3PO_4$for 15 s. The specimens were prepared for scanning electron microscopy analysis. The images were evaluated for quality types I and II etching of the enamel surface using ImageJ software. Datasets were checked for normality by Kolgomorv-Smirnov test and the non-parametric unpaired Mann-Whitney test was applied. The mean surface area of type I and II etching pattern values was 1922.314 $\mu m^2$for Group A and 3840.473 μm^2Group B. We conclude that deproteinization with 5% NaOCl prior to acid etching can be used to increase the area of adhesion and the quality of the etching pattern.

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Keywords: *Dental enamel, tooth, deciduous, dental etching, sodium hypochlorite.*

Desproteinización del esmalte primario con hipoclorito de sodio antes del grabado con ácido fosfórico

RESUMEN

El objetivo del estudio fue evaluar la desproteinización del esmalte primario a través de los tipos de patrones de grabado, con y sin NaOCl 5% utilizado antes del grabado ácido con H3PO4 37%. Quince dientes primarios humanos extraídos se seleccionaron al azar para el presente estudio in vitro, se prepararon bloques de 1mm x 1 mm y se dividieron en dos grupos (n = 21). Estos grupos se trataron de la siguiente manera: Grupo A: Grabado ácido con H3PO4 37% en gel durante 15 segundos; Grupo B: NaOCl 5% durante 60 segundos + Grabado ácido con H3PO4 37% durante 15 segundos. Las muestras se prepararon para el análisis de microscopía electrónica de barrido. Las imágenes obtenidas se evaluaron principalmente por la calidad de los grabados tipo I y II de la superficie del esmalte primario, utilizando el software Image J. Los datos se analizaron en cuanto a su normalidad mediante la prueba de Kolgomorv-Smirnov, se utilizó pruebas no paramétricas: Prueba de Mann-Whitney no pareada. Como resultado, se encontró que el área de superficie media de los valores de patrón de grabado de tipo I y II para el Grupo A era 1922,314 μm^2 y el Grupo B era 3840,473 μm^2. Finalmente, llegamos a la conclusión de que se puede usar la desproteinización con NaOCl 5% antes del grabado ácido para aumentar el área de adhesión y la calidad del patrón de grabado.

Palabras clave: *Esmalte dental, diente deciduo, grabado ácido, hipoclorito de sodio.*

INTRODUCTION

For decades, the dental community has endeavored to obtain and use materials and techniques that increase the bond strength of restorative materials and orthodontic devices to the tooth enamel surface. A good bonding technique requires appropriate preparation of the enamel surface, which includes removing the pellicle and roughening the surface,

in a process called conditioning[1]. Enamel is conditioned using one of two techniques: (a) acid etching, which uses an acid gel to produce micro-etching and (b) the abrasive technique, using abrasive air and resulting in macro-etching[2].

The introduction of the acid etching technique in 1955 by Buonocore marked a milestone in dentistry[2]. This concept is based on the acid dissolution of the dental enamel surface to create micro-porosities on the surface, thereby improving micro-mechanical adhesion. Subsequent modifications and improvements have been made, including the dilution of phosphoric acid concentration from 80% to 37% and a timereduction from 60 s to 15 streatment[3]. Phosphoric acid etching removes a 10 μm to 50 μm layer from the enamel surface, while the rough surface increases from 10 μm to 200 μm[4, 5]. However, phosphoric acid alone does not produce a total adhesion surface. Espinosa et al.[6] found that phosphoric acid etched less than 50% of the enamel area, while Hobson et al.[7] found up to 69% of the surface intact, with only 2% of the ideal surface etched.

The adhesion of restorative materials and orthodontic devices to the enamel surface of the tooth depends on the type of agent used, the duration of the use of acid and the enamel surface[8]. Comparing the enamel of primary and permanent teeth, it is logical to expect differences in the quality of the etching process and in bond strength. Several studies have shown that this process is negatively affected by a high amount of organic structures and by the presence of a prism less layer on the enamel surface of deciduous teeth[9-11].There is therefore a need to modify the organic content of the enamel of primary teeth. This can be done by using a recently developed method: deproteinization.

Enamel deproteinization involves the removal of the organic content (proteins) from the enamel surface. NaOCl has been shown to degrade protein efficiently and to be capable of eliminating excess protein[12]. Proteins interfere in the establishment of a clinically successful acid etching pattern and their elimination improves the bond[13]. LA A study with electron microscopy by Espinosa et al.[12] showed that the use of 5% NaOCl improves both the quantity and the quality of the etching surface, suggesting that this method has the potential to optimize adhesion and improve bond strengths. This process duplicates the retentive surface of the enamel up to 94.47% and produces an increase in patterns I and II, which have greater retentive capabilities than the type III etching pattern, thus improving retention[12].

Several deproteinizing agents are currently used, such as papain gel from the papaya plant, bromelain enzyme from the leaves of the pineapple plant and calcium hypochlorite. Pithon et al.[14] used 10% papain gel as an enamel-protecting agent before the cementing process and achieved good results with respect to the bond strength of the cemented brackets with modified glass ionomer. Dayem and Tameesh[15] evaluated the deproteinizing effects of the enzyme bromelain, which decreased the filtration score of the adhesive system.

Deproteinization has the potential to become a convenient, non-invasive, low-cost method to increase the binding forces of restorative materials and orthodontic devices, which can be quickly and easily used in daily clinical practice[16].

However, few studies evaluating deproteinization of the enamel surface have found significant improvements in adhesion properties[17,18], so further studies are needed to elucidate this issue. The aim of this study was therefore to evaluate the deproteinization of primary enamel by analyzing etching pattern types, with and without the application of 5% NaOCl before etching with 37%H_3PO_4.

MATERIALS AND METHODS

Study Design

This study reports the findings of an experimental *in vitro* study. The samples consisted of fifteen asymptomatic primary molars without caries and with different degrees of root resorption, collected from children who visisted the Postgraduate Unit of the Faculty of Dentistry of the *Universidad Nacional Mayor de San Marcos*, Lima-Peru. Prior to tooth donation, signed parental consent and child assent agreement was requested. Teeth were extracted under clinical indication and immediately stored in saline solution. This study was approved by the Ethics Committee of the Faculty of Dentistry of the *Universidad Nacional Mayor de San Marcos*, Lima -Peru.

The fifteen teeth were washed with distilled water for 10 s, and then cut with a Giflex-TR diamond disc and a low-speed handpiece(NSK Ex 203C), discarding the roots. The buccal surface of the crown was divided into four 1 mm x 1 mm thickness

fragments[12]. All samples were stored in saline solution at 37 °C and then randomly assigned to two groups:

Group A: The enamel surface was etched with 37% H_3PO_4 (Scotchbond™ universal etchant gel; 3M ESPE, St Paul, MN, USA), applied for 15 s with a microbrush, washed with sterile water for 20 s, and then dried with compressed air[12].

Group B: The enamel surface was treated with5% NaOCl (Clorox®) applied with a sterile cotton swab for 60 s, washed with sterile water, then dried for 10 s, and etched with 37% H_3PO_4 (Scotchbond™ universal etchant gel; 3M ESPE, St Paul, MN, USA), applied for 15 swith a microbrush, washed with sterile water for 20 s then dried with compressed air[12]. This procedure was performed in the Postgraduate Unit of the Faculty of Dentistry of the *Universidad Nacional Mayor de San Marcos*, Lima-Peru.

Samples were analyzed with a scanning electron microscope (SEM,Faculty of Biology of *Universidad Nacional Mayor de San Marcos*, Lima-Peru, FEI model Inspect S50). All samples were sputter-coated with gold and taken to the SEM.A total 180 micrographs were taken at different magnifications: 150x, 500x, 4000x.

Two researchers received theoretical, practical and clinical specialized training and were calibrated for the evaluation of the SEM images. Intra-and inter-examiner reliability was assessed by kappa statistics (Kappa>0.80).

Etched area measurement protocol

The etched area, as defined by Silverstone, was measured using ImageJ[19] software, according to the following protocol:

1) The type of etching observed in each image was determined and marked according to the following color code: type I (green), type II (yellow), type III (red), type IV (blue), type V (orange).
2) With the aid of ImageJ software, the area corresponding to each color was measured.

Statistical analysis

The results of area measurements and type of etching were recorded in an electronic database with controlled entry fields in Microsoft Office Excel 2016. A total 42 images were analyzed, 21 from Group A and 21 from Group B. The data were analyzed with statistics and graphical tests using the Stata Statistical Software Release 14. College Station, TX: StataCorp LP.

Before determining significance, the variables were subjected to normality tests, using Kolgomorv-Smirnov graphical methods. It was found that the variables did not comply with this assumption of normality because the analysis with Stata Statistical Software (Release 14, College Station, TX: Stata Corp LP)gave a p value <0.05. Therefore, the nonparametric Mann-Whitney U test was used to determine the significance.

RESULTS

To evaluate the deproteinization of primary enamel through etching pattern types, the etching pattern was analyzed on a total 42 photographs, of which 21 corresponded to Group A (H_3PO_4 37%) and 21 to group B (NaOCl 5% +H_3PO_4 37%), at magnification 4000x. All samples were analyzed to determine whether their pattern was type I, II, III, IV or V. Table 1 shows average etched area in groups A and B, according to etching types. Only one sample from Group A and one sample from Group B had type V etching pattern. Only one sample from Group B had type IV etching pattern. No sample from Group A had type I etching pattern. Group A (1922.314 μm^2) hada lower etched area consistentwithtypes I and II compared to Group B (3840.47μm^2).The etched area of Group A exceeded the area of Group B, when the areas were of types III, IV and V. The etching type with the largest area in Group A was type IV, followed by type III. The etching types with the largest area in Group B were types I and II, almost in the same proportion. The area of type III in group A was three times greater

Table 1: Average etched surface in Groups A and B according to etching type (μm^2).

Group	Type I	Type II	Type III	Type IV	Type V
Group A	-	1922.314	3411.056	1989.716	4797.826
Group B	3870.061	3831.226	1066.414	458.037	450.939
Total	3870.061	3310.614	2169.775	1893.986	2624.383

than in Group B. Table 2 shows the type I and II etching surfaces per group.

Only 6 out of the 21 samples from Group A (28.57%) presented a type II etching pattern, with an average of 1922.314 μm^2, while all samples from Group B presented a type I or II etching pattern, with an average of 3840.473 μm^2. Figures 1 and 2 show that Group B has a larger area of type I and II compared to the mean (Fig. 1) and compared to the median (Fig. 2).

The Mann-Whitney U test showed significant differences between groups ($p = 0, 0004$). Table 3 shows the etching pattern of the surface (types III, IV and V) per group.

All samples from Group A had type III, IV or V surface etching patterns, while in Group B, this percentage was 85.71%. However, the type III, IV or V etched area was four times smaller in group B than in Group A. Figs. 3 and 4 show that Group A has a larger area of type III, IV or V, compared to the mean (Fig. 3) and to the median (Fig. 4).

Mann-Whitney U test showed significant differences in the etched area between the two groups, ($p< 0.001$). Figs. 5 and 6 show some examples of the etching patterns found in this study.

DISCUSSION

Studies have shown that enamel etching patterns depend on the type of substance used and its concentration, as well as the time of etching and the composition and morphology of the surface of the enamel to be etched[6]. Good adhesion of composite materials requires a good amount of etching to ensure maximum retentive capacity, as well as a good etching pattern (types I and II). Phosphoric acid has long been used for this purpose. However, H_3PO_4alone does not produce a total adhesion surface[7]. Espinosa et al.[6] found that H_3PO_4etched

Table 2: Descriptive statistics of etched surface type I and II per group (μm^2).

Group	N	Mean	Standard Deviation	Minimum	Maximum	Median	Interquartile range
Group A	6	1922.31	574.490	1278.72	2599.196	1868.21	1149.605
Group B	21	3840.47	754.799	2502.12	4797.826	3939.90	1021.807

Table 3: Descriptive statistics of etched surface type III, IV and V per group (μm^2).

Group	N	Mean	Standard Deviation	Minimum	Maximum	Median	Interquartile range
Group A	21	4248.59	935.07	2198.63	4797.83	4797.83	1278.72
Group B	18	1116.91	693.95	107.88	2295.70	1069.35	1049.80

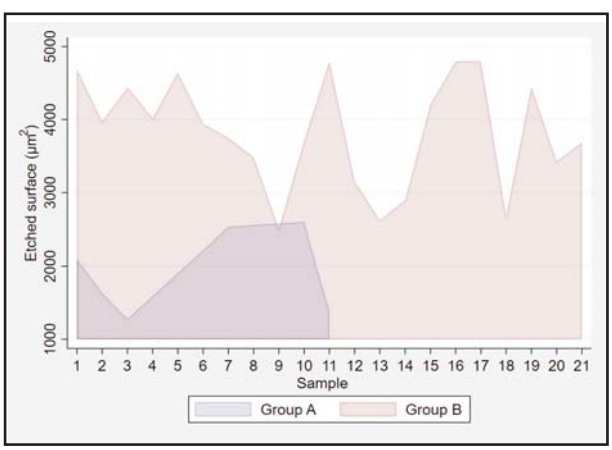

Fig. 1: Type I and II etched surfaces per group (μm^2).

Fig. 2: Box plots for type I and II etched areas per group.

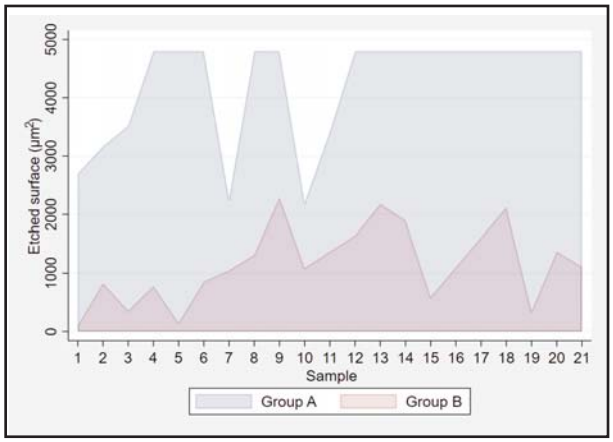

Fig. 3: Type III, IV and V etched areas per group (µm²).

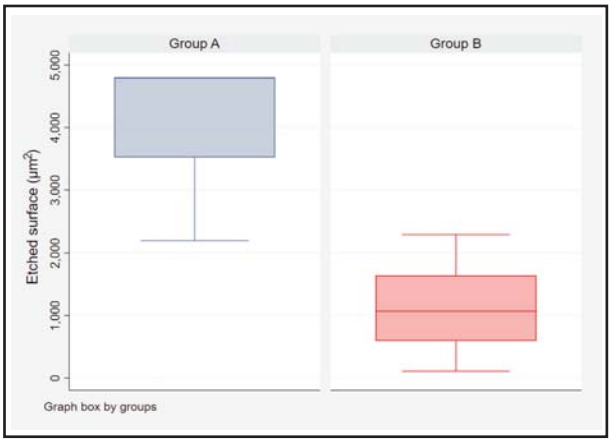

Fig. 4: Box plots for types III, IV and V etched areas per group.

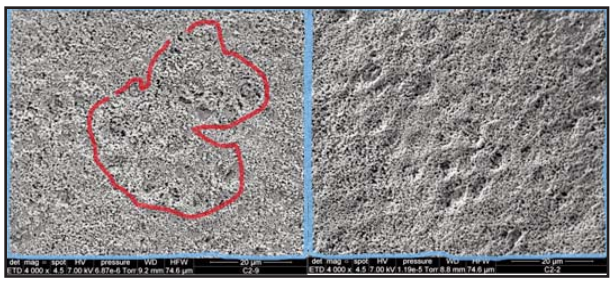

Fig. 5: Example of etching pattern types III (red) and IV (blue) found in Group A.

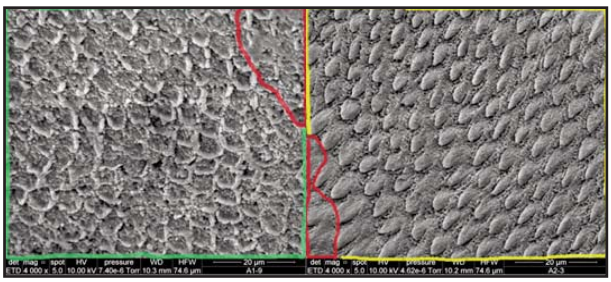

Fig. 6: Example of etching pattern types I (green) and II (yellow) found in Group B.

less than 50% of the enamel area, while Hobson et al.[7] found up to 69% of the surface intact, with only 2% of the surface ideally etched. In our study, phosphoric acid used alone produced 11.44% of etching surface type I and II patterns (considered the ideal etching types) with respect to the total area of all samples analyzed. This highlights the fact that the H_3PO_4alone did not produce a single sample with a type I etching pattern.

This poor etching action is a consequence of phosphoric acid acting only on the mineralized surface (inorganic material) and not on the organic material that usually coats the enamel. So, a previous process to remove this organic material is needed before starting the etching protocol. Etching is often hampered by the presence of proteins seated between the enamel crystals. In order to overcome this difficulty and obtain a greater retentive etching, Espinosa et al.[12] and Ananthi et al.[20] developed a pre-treatment for all the enamel to be etched based on enamel deproteinization with 5.25% NaOCl for one minute. This step has been successful in etching enamel surface, increasing the etched retentive surface by more than 50%[12,20].

We found that in group B (5% NaOCl +37% H_3PO_4), 80.04% of the total area was etched in all of the samples with type I and II patterns. This was higher than the etched area in group A (only 37% H_3PO_4). Moreover, only some of the Group A samples had type I or II etching patterns, with an average of 1922.314 µm², while all the Group B samples had etching types I or II, with an average of 3840.473 µm². In other words, the type I and II area etched by 37% H_3PO_4 combined with sodium hypochlorite was much larger than the type I and II area etched by H_3PO_4 alone. These differences were statistically significant.

The opposite occurred for etched surface types III, IV and V by group. Types III, IV and V are considered weaker etching patterns. Group A had a larger etched area for each of these types (III, IV and V). Etching patterns Type III, IV or V were four times higher in group A than Group B. In addition, in Group B, only one sample was found with

type IV etching and only one sample with type V etching, clearly showing that 5% sodium hypochlorite and 37% phosphoric acid almost exclusively generated the best etching patterns (I and II) in the samples.

These results agree with Ananthi et al.[20] who also found a larger type I and II etching surface when $H_3PO_4$was used in combination with NaOCl (45051.34 μm^2) compared to H_3PO_4alone (39608.18 μm^2), and with the study by Sharma et. al.[16] who found a greater amount of type I and II patterns in the group deproteinized with 5.25% NaOCl in a study to determine the effect of deproteinization using sodium hypochlorite on the bond strength of orthodontic brackets.

Ekambaram et al.[21] have even observed type I and II etching patterns in hypomineralized teeth after pre-treatment with 5% NaOCl. These data also agree with an earlier study conducted by Espinosa et al.[12],who found the largest etched area with type I and II patterns when the combination was used for 30 s(559.681 μm^2) and for 60 s (718.244 μm^2), compared to the area etched when phosphoric acid alone was used (368,689 μm^2). The difference in the numerical values can be explained by the differences in the magnification and the calibration used. For example, Espinosa et al.[12] used samples with 500x magnification, while we used 4000x magnification.

Our results do not agree with Ramakrishna et al.[17], who concluded that there was no significant improvement in the deproteinization of enamel after acid etching with respect to the appearance of Type I-II etching patterns. Neither do our results agree with Bhoomika et al.[18], who found no significance in etching pattern values in a group treated only with phosphoric acid, while another group treated with sodium hypochlorite and phosphoric acid had53.58%. Bhoomika et al.[18]even claim that the use of 37% H_3PO_4for15 s is still the best method for the pre-treatment of enamel.

The best retention area is obtained with Type I and I etching patterns, which have larger, deeper retentive areas compared to other types of patterns which do not haveneat, deep morphology, and lack the micro-mechanical retention that guarantees proper adhesion of restorative resins.

Another way to evaluate and compare the effect of deproteinization is through tensile tests performed on teeth that have been subjected to any of the etching procedures. The mechanical tests confirm that deproteinization before etching significantly improves the values of shear bond strength both in permanent teeth and in immature primary teeth, as it increases the surface area of the adhesion of the composite material tothe dental surface and presents better etching patterns, thereby increasing the adhesion strength of orthodontic appliances[5,8,16,21-24]. However, other researchers such as Poggioet al.[25] and Monjarás-Avila[26]did not find significant effects of the deproteinization of the enamel with NaOCl on shear bond strength.

Taken together, these data indicate that the deproteinization of the tooth structure before acid etching increases the etched area of type I and II patterns, which are considered the best etching patterns. This, in turn, enables better adaptation of the material to be bonded and longer survival of restorations. The latter was demonstrated in the study by Espinosa et al.[6], which found a lower loss in percentage of sealing material in molars with deproteinization compared to teeth that had not received this treatment, when the teeth were checked six months after treatment.

Shear tests have also found the same phenomenon, showing more cohesive failures when enamel is deproteinized before acid etching [11]. Additionally, Ekambaram et al.[22] have found that deproteinization of the enamel with NaOCl results in some cohesive failures within the enamel structure.

It should be noted that other studies have used software outside the field of medicine to measure etching, which implies certain limitations. For instance, AutoCAD is a software used to develop 3D structures, which is widely used in engineering and requires quite a long learning time. Photoshop software, even though it provides tools for manipulation, is also not designed for the field of medicine. In this study, we used the ImageJ program to determine the recorded area. ImageJ is a free software which is widely used in the field of biomedicine for densitometry morphometric analyses. It can be used to calculate area, perimeter or length of particles contained in a digital image[19].

Finally, the clinical implications of these findings are important. Based on the results (a greater amount of type I and II etching after the use of NaOCl and H_3PO_4),deproteinization of the surface of enamel with 5% NaOCl for 60 s before acid

etching can be recommended to improve the adhesion of restorative resins, as well as orthodontic appliances, providing dentists with a non-invasive, effective and affordable method.

CONCLUSIONS

The use of the conventional method with $H_3PO_4$37%on primary enamel has limitations, since it produces small areas of type I and II patterns with respect to the total area. A significant improvement both in the etched area and in the etching pattern can be achieved when 5% NaOCl is applied before 37%H_3PO_4etching.Deproteinization with 5% NaOCl before acid etching offers a non-invasive, efficient, affordable method to improve the adhesion of materials to primary tooth structure.

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Subgingival microbiological profile of periodontitis patients in Dominican Republic

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ABSTRACT

Several studies have tried to associate the presence of different pathogens with the onset and progression of periodontitis, reporting a wide variety of results from different populations and environments. The aim of this study was to determine the main periodontal pathogens present in the subgingival biofilm of Dominican patients with periodontitis, by using specific microbiological culturing techniques. Periodontitis patients were selected after a full-mouth periodontal evaluation, and assigned to different periodontitis groups based on percentage of affected locations. Subgingival samples were collected and analyzed by means of specific culture techniques. Anaerobic counts, frequency of detection and proportions of target pathogens were calculated. Variables were analyzed by means of Student's T-test or chi-square test. Twenty-nine subjects were recruited, of whom 17 were diagnosed with generalized periodontitis (GenP) and 12 with localized periodontitis (LocP). The most prevalent bacterial species in both groups was Prevotella intermedia (94.1% in GenP and 91.7% in LocP), followed by Porphyromonas gingivalis (88.2% in GenP and 83.3% in LocP). Total microbiota in subgingival samples was 1.3 x10^7 colony-forming units (CFU)/mL (standard deviation, SD= 1.5 x10^7) and 9.6x10^6 CFU/mL (SD= 1.1 x10^7) in GenP and LocP subjects, respectively, though differences were not statistically significant (p= 0.222). The highest counts were observed for P. gingivalis in both groups, with mean concentration 2.5x10^6 CFU/mL (6.1x10^6) in GenP and 2.9x10^6 CFU/mL (5x10^6) in LocP, with no statistically significant difference (p=0.879). These results suggest that relevant periodontal pathogens are found with diversity and abundance in the subgingival microbiota of adult Dominican patients with periodontitis.

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Key words: *microbiology, culture techniques, Dominican Republic, periodontitis.*

Perfil microbiológico subgingival de pacientes con periodontitis en República Dominicana

RESUMEN

Varios estudios han tratado de asociar la presencia de diferentes patógenos con el inicio y la progresión de la periodontitis, mostrando una gran variedad de resultados en diferentes poblaciones y entornos. El objetivo del presente estudio fue determinar los principales patógenos periodontales presentes en la biopelícula subgingival de pacientes dominicanos con periodontitis, utilizando técnicas específicas de cultivo microbiológico. Los pacientes con periodontitis se seleccionaron después de una evaluación periodontal de boca completa y se asignaron a diferentes grupos de periodontitis según el porcentaje de localizaciones afectadas. Las muestras subgingivales fueron recolectadas y analizadas mediante técnicas de cultivo específicas. Se calcularon los recuentos anaeróbicos, la frecuencia de detección y las proporciones de los patógenos seleccionados. Las variables se analizaron mediante la prueba T de Student o la prueba de chi-cuadrado. Se reclutaron veintinueve sujetos, 17 diagnosticados como periodontitis generalizada (GenP) 12 con periodontitis localizada (LocP). La especie bacteriana más prevalente en ambos grupos fue Prevotella intermedia (94.1% y 91.7%, respectivamente) y seguida de Porphyromonas gingivalis (88.2% y 83.3%, respectivamente). La microbiota total en muestras subgingivales fue 1.3 x10^7 unidades formadoras de colonias (CFU)/mL (desviación estándar, SD= 1.5 x10^7) y 9.6x10^6 CFU / mL (SD= 1.1 x10^7) en sujetos GenP y LocP, respectivamente, pero no hubo diferencias estadísticamente significativas (p= 0.222). Los recuentos más altos se observaron para P. gingivalis en ambos grupos, con una concentración media de 2.5x10^6 CFU/mL (6.1x10^6) en GenP y 2.9x10^6 CFU/mL (5x10^6) en LocP, sin diferencias estadísticamente significativas (p= 0.879). Estos resultados sugieren que se encuentran patógenos periodontales relevantes con diversidad y abundancia en la microbiota subgingival de pacientes adultos dominicanos con periodontitis.

Palabras clave: *microbiología, técnicas de cultivo, República Dominicana, periodontitis.*

INTRODUCTION

Periodontal diseases are a group of diseases of infectious origin that occur with the inflammation of the tissues supporting the teeth. They are currently highly prevalent worldwide[1,2], and represent one of the main factors leading to tooth loss[3-6].

The primary etiological factor of periodontitis is the presence of bacteria organized in biofilms, which develop as interactive communities of microorganisms. The relationships between the bacteria embedded in a biofilm can be symbiotic, when there is a beneficial relationship among the bacteria that make up the biofilm and between them and the host, or dysbiotic, when there is a change in the community of microorganisms that leads to the development of pathology[7].

Several studies, mainly based on microbiological culturing for analysis of the samples, have tried to associate the presence of different pathogens with the diagnosis of periodontitis, reporting a wide variety of results from different populations and environments[8-11]. One of these studies by Sanz *et al.* (2000)[11] compared patients with periodontitis in Spain and the Netherlands, finding significant differences between the microbiological profiles. *Aggregatibacter actinomycetemcomitans* was found to be more prevalent in Dutch than in Spanish patients (23% versus 3%), whereas *Porphyromonas gingivalis* was found to be more prevalent in Spanish than in Dutch patients (65% versus 37%). These differences may be explained by patients' genetic profiles or as a result of the difference in the use of antibiotics between these two countries[12].

In Latin America, the "red complex" of bacteria (*P. gingivalis, Tannerella forsythia* and *Treponema denticola*) is found in high levels in patients with periodontitis [13, 14, 9, 10.] In Brazil, black-pigmented bacteria of the species *Porphyromonas* was detected in patients with periodontitis (89.4%), gingivitis (30%) and healthy patients (8%)[13]. In another study of the Brazilian population, *Porphyromonas* has prevalence of 74% in patients with periodontitis[14].

In the Dominican Republic to date, only two studies have been conducted analyzing subgingival microflora of Dominican patients with periodontitis, and they report different results. Slots et al.[15] performed the first study in which direct microscopic examination revealed that nonmotile organisms and cocci made up 85% of total microorganisms, while spirochetes only accounted for 3%. Non-selective culturing showed 53% Gram-negative organisms, 15% *Fusobacterium nucleatum*, 7% black-pigmented anaerobes and 10% *Parvimonas micra*. In contrast, a recent study using polymerase chain reaction (PCR) found prevalence of "red complex" bacteria, with approximately 90%, in patients with periodontitis, especially *T. forsythia*, differing from reports published for other Latin American countries[16].

The primary aim of the current study was to determine the main periodontal pathogens present in the subgingival biofilm of patients with periodontitis in the Dominican Republic, by using specific microbiological culturing techniques. The secondary aim was to compare these pathogens between patients with generalized and localized periodontitis.

MATERIALS AND METHODS

Study design

This cross-sectional study was approved by the institutional ethical committee of Pontificia Universidad Católica Madre y Maestra (PUCMM). All participants signed written informed consent. Study procedures were conducted according to the Declaration of Helsinki, the UNESCO Universal Declaration and the requirements of the Dominican Republic legislation.

Participants

Patients at the PUCMM dental clinic (Santo Domingo, Dominican Republic) who met the inclusion criteria were invited to participate in the study. All subjects provided informed consent. The screening period lasted from January 2014 to August 2015.

Inclusion criteria were: (1) age 18 years or older; (2) non-smokers; (3) at least 15 teeth present; (4) had not received periodontal treatment in the 12 months prior to the study; (5) were free of systemic diseases that could affect the tissue response (such as diabetes or immune diseases); (6) presented periodontitis, defined as the presence of at least 3 interproximal non-adjacent sites with probing pocket depth (PPD) of 4 mm or greater and (7) radiographic evidence of alveolar bone loss. Exclusion criteria were: (1) pregnant women and (2) having taken antibiotics and/or anti-inflammatory drugs in the previous month.

Clinical outcomes

A full-mouth clinical examination was performed on each patient and the following parameters were recorded at six sites per tooth using a North

Carolina periodontal probe (Hu-Friedy, Chicago, IL, USA): (1) Plaque index (PlI) in percentage of sites, following O'Leary[17]; PPD; recession (REC) and clinical attachment loss (CAL) in millimeters; and bleeding on probing (BOP) as present/absent 30 seconds after probing, following Ainamo & Bay[18]. Based on their periodontal information (proportions of affected sites), patients were assigned to the generalized or localized periodontitis groups.

Microbiological sampling

In each patient, four sampling sites were selected, one in each quadrant, choosing those most accessible and with deepest probing depth and bleeding on probing[19]. At the selected sites, supragingival plaque was removed and the sites were dried with sterile cotton roles and air. Two consecutive sterile paper points (#30, Maillefer, Ballaigues, Switzerland) were inserted as deep as possible into the pocket, and left in place for 10 seconds. The paper points were transferred to a vial containing 2 ml of reduced transport fluid[20], and pooled with all the other paper points. The vial was kept at 4 ºC and sent to the laboratory at the Complutense University of Madrid (Spain) and processed within 24-36 hours.

Culture analyses

Vials were vortexed (30 seconds), serially diluted in phosphate-buffered saline, and plated on two different media: (1) blood agar medium (Blood Agar Base II®, Oxoid, Basingstoke, United Kingdom) supplemented with 5% horse blood, haemin (5 mg/l) and menadione (1 mg/l), and (2) Dentaid-1 medium[21]. After 4-14 days of anaerobic incubation (80% N_2, 10% CO_2 and 10% H_2), the plates were examined for the identification of *A. actinomycetemcomitans, P. gingivalis, P. intermedia, T. forsythia, P. micra, Campylobacter rectus, F. nucleatum, Capnocytophaga* spp. and *Eikenella corrodens*, based on different microbiological procedures: colony morphology, Gram-staining, catalase test, N-benzoyl-dL-arginine-2-napthylamide, indole and alpha -glucosidase activity; and standard biochemical test (RapIDTM ANA II System; Remel, Lenexa, KS, USA). Bacterial counts were expressed in colony-forming units (CFU) per mL of the original sample and total anaerobic counts were calculated, as well as counts of the detected periodontal pathogens. In addition to the quantitative microbiological data, the frequency of detection and proportions for each bacterial species were calculated.

Statistical analysis

Sample size calculation could not be performed. A convenience sample of 29 subjects was therefore selected based on previous microbiological studies[8-11]. The primary outcome variable was total anaerobic count (CFU/mL). Secondary outcome variables included all other microbiological variables, including frequency of detection of target pathogens, counts of each study pathogen, proportions of flora of each pathogen, and all clinical variables (PPD, BOP, REC, PlI, CAL). A subject-level analysis was performed for each study parameter. Data were expressed as means and standard deviation (SD), prevalence and proportions (%) for all variables. Total anaerobic counts and counts of each study pathogen were log transformed to fit a normal distribution. After evaluating the normality of the distribution (assessed by the Shapiro Wilk test), differences between different periodontal diagnosis groups were compared by Student´s T or Mann-Whitney U-test for quantitative variables, and chi-square or Fisher tests for categorical variables. The level of statistical significance was set at $p<0.05$. A statistical software package IBM® SPSS Statistics 25.0 (IBM Corporation, Armonk, NY, USA) was used for data analysis.

Table 1: Demographic data for GenP and LocP periodontitis groups.

	GenP (n= 17)	LocP (n=12)	*P value*
Age (years) *[mean (SD)]*	42.3(9.9)	46.2 (10.7)	*0.31*
Sex *[n (%)]*			*0.14*
Male	5 (29.4)	7 (58.3)	
Female	12 (70.6)	5 (41.7)	

SD: standard deviation; GenP: generalized periodontitis; LocP: localized periodontitis

RESULTS

Patient sample description

Twenty-nine patients were recruited, of whom 17 were diagnosed with generalized periodontitis (GenP) and 12 with localized periodontitis (LocP). Table 1 shows patient demographics. No statistically significant difference was found between groups. Mean age was 42.3 and 46.2 years, and percentage of males was 29.4% and 58.4% in the GenP and LocP groups, respectively.

Periodontal outcome variables

Table 2 shows clinical outcome variables. PPD and BOP values were significantly higher in GenP patients than in LocP patients ($p<0.05$). Mean PPD was 2.9 mm (SD=0.6) in the GenP versus 2.1 mm (SD=0.2) in LocP subjects. Similarly, BOP percentages were significantly higher in the GenP group (60.7% [SD=26.4%] versus 33.0% [SD=17.1%]). Table 3 shows the frequency distribution of PPD.

Table 4 shows data from the sampling locations. Plaque was 100%, BOP 85.7% and 62.5%, and mean PPD 5.8 and 5.0 mm in GenP and LocP, respectively. Statistically significant differences were found for PD values between groups ($p=0.037$).

Table 2: Mean values and standard deviation (SD) for clinical variables.

	GenP (n= 17)	LocP (n=12)	*P value*
	Mean (SD)	Mean (SD)	
Number of teeth	21.6 (4.1)	21.7 (3.7)	*0.969*
Number of sites	129.6 (24.7)	130.0 (22.05)	*0.968*
PII (%)	59.9 (32.1)	58.9 (32.5)	*0.720*
PPD (mm)			
All	2.9 (0.6)	2.1 (0.2)	*<0.001**
Upper	3.3 (0.7)	2.4 (0.3)	*<0.001**
Lower	2.5 (0.6)	1.9 (0.3)	*0.006**
Interproximal	3.2 (0.6)	2.4 (0.2)	*<0.001**
Vestibular/lingual	2.2 (0.5)	1.7 (0.3)	*0.019**
REC (mm)			
All	0.3 (0.7)	0.3 (0.9)	*0.793*
Upper	0.1 (0.7)	0.4 (0.9)	*0.372*
Lower	0.4 (0.7)	0.3 (0.9)	*0.806*
Interproximal	0.1 (0.7)	0.2 (0.9)	*0.929*
Vestibular/lingual	0.5 (0.7)	0.7 (0.9)	*0.554*
CAL (mm)			
All	3.2 (1.2)	2.5 (0.9)	*0.150*
Upper	3.4 (1.3)	2.8 (1.1)	*0.206*
Lower	3.0 (1.2)	2.3 (1.0)	*0.145*
Interproximal	2.5 (0.7)	1.9 (0.6)	*0.035**
Vestibular/lingual	2.1 (0.5)	1.8 (0.6)	*0.417*
BOP (%)			
All	60.7 (26.4)	33.0 (17.1)	*0.004**
Upper	68.3 (21.4)	38.1 (19.4)	*0.001**
Lower	54.0 (34.6)	27.9 (15.9)	*0.022**
Interproximal sites	60.8 (28.7)	32.4 (20.0)	*0.007**
Vestibular/lingual	60.6 (27.2)	34.1 (16.5)	*0.006**

BOP: bleeding on probing; CAL: clinical attachment loss; PPD: probing pocket depth; PII: plaque index; REC: recession; SD: standard deviation; GenP: generalized periodontitis; LocP: localized periodontitis; *: Statistically significant differences ($p<0.05$).

Subgingival samples

Table 5 shows data on detection of pathogens from subgingival samples, including their frequency of detection, mean concentrations and proportions.

The most prevalent bacterial species in GenP subjects were *P. intermedia* (94.1%) and *F. nucleatum* (94.1%) followed by *P. gingivalis* (88.2%). In LocP patients *P. intermedia* (91.7%) and *P. gingivalis* (83.3%) were the most prevalent bacteria, followed by *F. nucleatum* (58.3%). Statistically significant differences were only found for *F. nucleatum* ($p=0.019$).

Total microbiota counts in subgingival samples were 1.3×10^7 CFU/mL (SD=1.5×10^7) and 9.6×10^6 CFU/mL (SD=1.1×10^7) in GenP and LocP subjects, respectively, but differences were not statistically significant ($p=0.222$). The highest counts were observed for *P. gingivalis* in both groups, with a mean concentration of 2.5×10^6 CFU/mL (6.1×10^6) in GenP groups and 2.9×10^6 CFU/mL (5×10^6) in LocP, followed by *P. intermedia* [with mean concentration 7.2×10^5 CFU/mL (1.4×10^6) in GenP

Table 3: Frequency distribution of probing depth in both groups.

	GenP (n= 17)	LocP (n=12)	*P value*
PPD (% of sites)			
< 4 mm	71.4 (17.5)	90.6 (4.9)	<0.001*
4-6 mm	25.9 (15.9)	8.6 (4.7)	<0.001*
> 6 mm	2.7 (2.5)	0.8 (1.9)	0.040*

PPD: probing depth; GenP: generalized periodontitis; LocP: localized periodontitis; *: Statistically significant differences ($p<0.05$).

Table 4: Mean values and standard deviation (SD) of clinical variables at sites selected for microbial sampling.

	GenP (n= 17)	LocP (n=12)	*P value*
	Mean (SD)	Mean (SD)	
PII (%)	100 (0)	100 (0)	
PPD (mm)	5.8 (1.1)	5.0 (0.8)	*0.037**
BOP (%)	85.7 (23.8)	62.5 (38.3)	*0.081*
REC (mm)	0.2 (1.0)	0.2 (0.9)	*0.963*

BOP: bleeding on probing; PII: plaque index; PD: probing depth; REC: recession; SD: standard deviation; GenP: generalized periodontitis; LocP: localized periodontitis; *: Statistically significant differences ($p<0.05$).

Table 5: Frequency of detection (%), mean counts (in colony forming units, CFU/mL) and mean proportions of microbiota (%) of periodontal pathogens in subgingival samples.

	- Aa	- Pg	- Pi	- Fn	- Cr	- Ec	- Tf	- Capno	- Pm
Prevalence n (%)									
- GenP	- 2 (11.8)	- 15 (88.2)	- 16 (94.1)	- 16 (94.1)	- 2 (11.8)	- 6 (35.3)	- 10 (58.8)	- 2 (11.8)	- 3 (17.6)
- LocP	- 1 (8.3)	- 10 (83.3)	- 11 (91.7)	- 7 (58.3)	- 1 (8.3)	- 2 (16.7)	- 5 (41.7%)	- 1 (8.3)	- 4 (33.3)
p value	*0.765*	*0.708*	*1.000*	*0.019**	*0.763*	*0.408*	*0.362*	*1.000*	*0.403*
Concentration CFU/ml [Mean (SD)]									
GenP	$2.1x10^4$	$2.5x10^6$	$7.2x10^5$	$1.5x10^5$	$2.3x10^4$	$3.0x10^5$	$4.5x10^5$	$1.2x10^4$	$9.4x10^3$
	($8.7x10^4$)	($6.1x10^6$)	($1.4x10^6$)	($2.2x10^5$)	($6.6x10^4$)	($5.8x10^4$)	($8.4x10^5$)	($4.8x10^4$)	($2.6x10^4$)
LocP	$5 x10^2$	$2.7x10^6$	$2.7x10^5$	$2.1x10^5$	$3.3x10^4$	$1.6x10^4$	- $3.1x10^5$	$4.5x10^4$	$3.9x10^4$
	($1.8x10^3$)	($5x10^6$)	($4.1x10^5$)	($3.8x10^5$)	($1.2x10^5$)	($3.6x10^4$)	($5.9x10^5$)	($1.5x10^5$)	($7.7x10^4$)
P value	*0.222*	*0.403*	*0.403*	*0.238*	*0.397*	*0.599*	*0.478*	*0.523*	*0.551*
Mean proportions of microflora % [Mean (SD)]									
- GenP	- 0.6 (2.7)	- 6.3 (11.2)	- 2.2 (6.8)	- 0.4 (0.7)	- 0.01 (0.03)	- 0.5 (1.7)	- 1.2 (3.7)	- 0.01 (0.03)	- 0.1 (0.7)
- LocP	- 0.1	- 3.2 (5.7)	- 0.7 (1.2)	- 2.0 (4.4)	- 0.02 (0.08)	- 0.03 (0.09)	- 0.02 (0.03)	- 0.5 (1.8)	- 0.3 (0.6)
- p value	*0.84*	*- 0.74*	*0.49*	*0.44*	*0.91*	*0.39*	*0.30*	*0.91*	*0.44*

-Aa: Aggregatibacter actinomycetemcomitans; Pg: Porphyromonas gingivalis; Pi: Prevotella intermedia; Fn: Fusobacterium nucleatum; Cr: Campylobacter rectus; Ec: Eikenella corrodens; Tf: Tannerella forsythia; Capno: Capnocytophaga spp.; Pm: Parvimonas micra.
-GenP: generalized periodontitis; LocP: localized periodontitis; SD: Standard deviation; *: Statistically significant differences ($p<0.05$).

groups and $2.7x10^5$ CFU/mL ($4.1x10^5$) in LocP] and *T. forsythia* [with mean concentration $4.5x10^5$ CFU/mL ($8.4x10^5$) in GenP groups and $3.1x10^5$ CFU/mL ($5.9x10^5$) in LocP]. Statistically significant differences were not found between groups for any individual bacteria counts. In terms of proportions, similar mean values were found between groups without statistically significant differences between them.

DISCUSSION

In the present study, subgingival samples from 29 Dominican patients with periodontitis were analyzed using microbiological culturing in specific non-selective and selective media. The bacterial species with highest frequency of detection were *P. intermedia, P. gingivalis* and *F. nucleatum* (93.1%, 86.2% and 79.3%, respectively). No difference was detected when localized and generalized periodontitis patients were compared. There are few previous studies on the Dominican population using cultures. The results reported by Slots et al. [15] are consistent with ours (*P. gingivalis, F. nucleatum* and *P. intermedia* as the most prevalent species), but the frequencies of detection are different, almost certainly attributable to the use of different transport media [reduced transport fluid (RTF) or Viability Medium, Göteborg, Anaerobically prepared (VMGA) III]. Collins *et al.*[16] studied gingivitis, periodontitis and healthy participants from the Dominican Republic using specific PCR, finding frequencies of 93.3% for *P. gingivalis,* 53.3% for *P. intermedia* and proportions greater than 80% for other species such as *F. nucleatum, P. micra and E. corrodens.* They detected these three species in a greater number of patients compared to the current study, possibly due to the lower detection limits of molecular techniques compared to culture.

In the general population, the reported prevalence (86.2%) of *P. gingivalis* agrees with the current evidence, with this species being one of the most frequently detected species in subgingival samples in patients with periodontitis. In Spain, this bacterial species was found in 64.5%[11] and 77.8%[10] of the population. In Chile and Colombia, its prevalence has been reported as 83.8% and 65.9%, respectively. All these studies used similar methodologies and the populations analyzed had similar clinical features. In all cases, the samples were processed between 24 and 48 hours after being taken.

In our study, *P. intermedia*, a Gram-negative bacillus, was also found in a high percentage of cases, having been recovered in the cultures from 93.1% of the

subjects. These findings are similar to those from Spain and Chile, where the occurrence of *P. intermedia* was 97.2% and 72.5%, respectively. However, in a Colombian population the frequency was lower, at 19.4%[10]. These differences could be attributed to the different lifestyles of people living in each geographical location or to the clinical presentations of periodontitis[22,23]. On the other hand, differences could also be explained, not only by genetic differences[24] , but also by epigenetic differences, which could be related to variations in the abundance and distribution of microbial species in populations[25].

It should be highlighted that *T. forsythia* was detected in more than 50% of the patients in the present study, but in 90% of the subjects in the PCR-based study[16]. The greater frequency of detection using molecular methodologies evidences the greater sensitivity of the PCR technique[26-28], though it should also be considered that detection and abundance of this anaerobic species, which is difficult to culture, could have been underestimated due to the time elapsed between sampling and processing. However, although *T. forsythia* may be partly underestimated, its detection by culture highlights its high frequency in Dominican patients compared to other populations, with reported frequencies of 16.2%, 39.0% and 36.1%, in Chile, Colombia and Spain, respectively.

A. actinomycetemcomitans has been associated by various authors, such as Jardim *et al*.[29] or Sulugodu *et al*.[30], with a higher rate of progression in periodontitis. In the present study, its occurrence was 11.8% and 8.3% for GenP and LocP, respectively, which was low compared to other studies, such as Mullally *et al*.[31], which reported a detection frequency of 19%, and higher in patients with LocP.

It should be considered that results for bacterial abundance may be a direct consequence of the sampling and processing protocol. The volume of transport medium in which the sample was placed, and from which dilutions are subsequently prepared for inoculation into the culture media, as well as the number of sites from which the sample was collected, will reflect the total counts of viable cells present in these samples. Both these factors may make comparisons between studies difficult. However, in the present study, the most abundant species in relative terms were *P. gingivalis* and *P. intermedia,* followed by *F. nucleatum* and *T. forsythia*, similarly to Slots *et al*. [15], with the exception of *T. forsythia,* which was not evaluated.

The advantages of detecting bacteria by means of culture techniques are that it enables determination of number of viable cells in a sample, detection of species that could be present unusually, analysis of susceptibility to antimicrobials and characterization of the microbiota associated with oral diseases[32]. Microbiological culturing techniques are fundamental and basic diagnostic methods widely used as research tools in molecular biology. In addition, these methods are still considered the "gold standard" methods of reference in periodontal microbiology, to which other microbiological identification procedures are compared[33]. However, culturing can be affected by the handling and processing of the sample, especially in the current study, in which sampling and analysis were performed in different countries. These aspects, along with the relatively small sample size, should be considered as potential limitations of the study.

In the present study, periodontal diagnosis was initially based on the 1999 classification of periodontal diseases[34]. In the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions, the terms "aggressive" and "chronic" have disappeared, and the condition "periodontitis" should be now categorized with a multidimensional system of stages (I, II, III, IV) and grades (A, B, C). The stages will capture the severity of the disease and the anticipated complexity of the therapy, while the grades consider the identification of risk factors that may impact general health and the progression of the disease[35-37]. This new approach may allow the development of meticulous treatment strategies according to the specific needs of each patient (precision medicine). With regard to the extension and distribution of periodontitis, minor differences are expected with the new classification. To determine a case of LocP or GenP, the presence of <30% or >30%, respectively, of affected sites was used in the 1999 classification[34], while with the 2018 approach, LocP is described as <30% of the teeth involved and >30% for GenP.

The strength of this study is that samples were analyzed in an experienced laboratory, which has already processed samples from distinct geographical populations (Spain, The Netherlands, Chile, Colombia) in different international research projects. This enables comparison of the results of

the present study (and population) to previous studies which followed identical methodology and were analyzed in the same laboratory[10,11]. It was possible to detect and identify nine bacterial species that are part of the subgingival microbiota and that are closely associated with the dysbiotic phenomena that trigger periodontal diseases. Therefore, this study constitutes an approximation of the bacterial composition in both diversity and abundance of the subgingival microbiota of patients with periodontitis in the Dominican Republic.

CONCLUSION

Within the limitations of the present study, the subgingival microbial composition in patients with periodontitis from Dominican Republic showed a high frequency of detection of *P. intermedia, P. gingivalis* and *F. nucleatum.* Our data suggest that periodontal pathogens in the subgingival microbiota of adult Dominican patients with periodontitis have overall similar diversity and abundance to those in other geographical populations, but with higher frequency of detection of *T. forsythia*.

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CORRESPONDENCE

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Molar incisor hypomineralization: Analysis of asymmetry of lesions

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ABSTRACT

Clinically, Molar-Incisor Hypomineralization (MIH) lesions are not distributed symmetrically, and their severity varies even within the same arcade. Aim: To estimate the frequency of asymmetries in hypomineralized lesions on permanent molars and incisors of children with MIH. Methods: Three pediatric dentists, calibrated following the diagnostic criteria of Mathu-Muju and Wright (2006) (Kappa 0.87) identified presence and severity of opacities on molars and incisors of patients with MIH. Six pairs of teeth (permanent maxillary and mandibular first molars, central and lateral incisors) were evaluated in each patient. Degree of lesion severity (0–none, 1–mild, 2–moderate, 3–severe) was recorded for each tooth. For each pair containing any affected teeth, asymmetry of presence (one tooth in the pair with lesion and the other intact), asymmetry of severity (both teeth with lesions but with different degrees of severity) or symmetry of severity (both affected teeth with the same degree of severity) were evaluated. The recorded values were entered into a database to calculate percentages, 95% confidence intervals and Chi-Square test for comparisons. Results: The sample consisted of 475 of the 1032 pairs of teeth evaluated in the 172 patients included in the study, mean age 11± 2.2 years, and 50% female. Asymmetry was found for 67.5% (63.1 - 71.7) of the pairs of the studied teeth. There was a significant relationship between asymmetries and symmetries (p= 0.038). A total 50.1% of the pairs were asymmetrical for presence of opacities. Of these, 62.2% scored severity 1 (mild). Symmetry of severity was found for 32.5% of the lesions. Among the pairs of affected teeth, the most frequently observed degrees of lesion severity were mild and moderate, with the exception of lower molars, in which 49% had severe lesions. Conclusions: In this study, MIH lesions were asymmetrical both in presence and severity for all tooth types.

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Keywords: *Dental enamel; molar incisor hypomineralization; tooth abnormalities.*

Hipomineralización Molar Incisiva: Análisis de la asimetría de las lesiones

RESUMEN

Clínicamente las lesiones de Hipomineralización Molar Incisiva (HMI) no tienen una distribución simétrica variando su severidad inclusive en la misma arcada. Objetivo: Estimar la frecuencia de asimetrías en lesiones de hipomineralización en molares e incisivos permanentes de niños afectados con HMI. Métodos: Tres odontopediatras calibrados (Kappa 0,87) según los criterios de diagnóstico de Mathu-Muju y Wright (2006), registraron presencia y severidad de opacidades en molares e incisivos de pacientes con HMI. En cada paciente se evaluaron 6 pares de piezas dentarias permanentes: primeros molares, incisivos centrales y laterales de ambos maxilares. Para cada una de las piezas se registró el grado de severidad de la lesión (0–intacto, 1–leve, 2–moderado, 3–severo). Para cada par con alguna de sus piezas afectadas, se analizó si presentaba asimetría de presencia (una pieza del par con lesión y la otra intacta), asimetría de severidad (ambas piezas con lesión pero con distinto grado de severidad) o simetría (ambas piezas afectadas con el mismo grado de severidad en la lesión). Se calcularon porcentajes, I.C. del 95% y CHI² para las comparaciones. Resultados: La muestra quedó conformada por los 475 pares de piezas dentarias que presentaron lesión entre un total de 1032 pares de piezas analizadas en los 172 pacientes incluidos en el estudio (edad media 11 ± 2,2 años, 50% género femenino). El 67,5% (63,1- 71,7) de los pares de piezas dentarias estudiados presentaron relación de asimetría. La relación entre frecuencia de asimetrías y simetrías fue significativa (p= 0,038). Del 50,1% de los pares que presentó asimetrías en presencia, un 62,2% mostró grado de severidad 1(leve) en una de sus piezas. Entre los pares afectados, las lesiones leves y moderadas fueron las más observadas en todos los grupos dentarios, a excepción de los molares inferiores que revelaron un 49% de lesiones severas. Conclusiones: En este estudio las lesiones de HMI presentaron, en su mayoría, algún tipo de asimetría (de presencia o de severidad) en todos los grupos dentarios.

Palabras clave: *Esmalte dental; hipomineralización molar incisiva; anomalías dentarias.*

INTRODUCTION

Hereditary, environmental and local factors can cause structural defects in the dental enamel of primary and/or permanent teeth. Depending on the amelogenesis period affected, these defects will be quantitative if such factors act during the protein

matrix secretion phase, or qualitative if they act during maturation or mineralization processes.

Until a decade ago, the three developmental defects most frequently cited in the literature were amelogenesis imperfecta, endemic fluorosis and hypoplasia.

Amelogenesis imperfecta includes a series of clinically and genetically heterogenous hereditary disorders with low prevalence[1]. According to the classification by Witkop, C.J. Jr.[2], there are 4 types: hypoplastic, hypomaturation, hypocalcified and hypoplasia-hypomaturation associated to taurodontism. In addition to phenotypic criteria, more modern classifications include genetic criteria, molecular defects and biochemical results, when known[3]. Although Witkop's classification is still the most often used, it is based on phenotype as primary discriminating factor, and inheritance mode as secondary discriminating factor. There is no classification correlating phenotype/genotype, so it has recently been proposed that inheritance should be the primary classification factor[4].

Dental fluorosis is considered to be a geochemical disease[5] resulting from excess fluoride intake during the odontogenesis period, which translates clinically, depending on its severity, into white, usually symmetrical opacities with diffuse borders (mild fluorosis), ranging to dark brown stains with enamel erosion (moderate to severe).

Finally, hypoplasias are quantitative enamel defects caused by factors acting on the initial phase of matrix secretion, causing a deficit in the quantity of adamantine structure. They may present as shallow or deep fossae with vertical or horizontal grooves, with partial or total absence of enamel[6].

Research in recent years, particularly in pediatric dentistry, has focused on Molar-Incisor Hypomineralization (MIH), considered to be an "emerging disease" because it has recently acquired epidemic character[7]. It presents as an anomaly in tissue translucency, with demarcated white/yellow/brown colored areas, without alteration of enamel thickness, which may sometimes disintegrate, giving rapid rise to caries. In contrast to amelogenesis imperfecta and endemic fluorosis, which are considered hypomaturation defects due to high level of residual amelogenins, MIH is typified as hypocalcification with a normal level of residual amelogenins[8].

Worldwide MIH prevalence calculated from 79 studies in 36 countries is currently 15%[9]. Previous papers published by our team reported the prevalence of MIH in Buenos Aires City[10] and evaluated preventive strategies with different alternatives[11].

The high frequency of MIH and its impact on needs for treatment have made it a silent public health problem, and it is the enamel anomaly that involves highest social cost[12].

Although publications on MIH have increased dramatically, neither its etiology nor the best preventive and restorative strategies are yet clear. Another as yet unresolved issue is that although it is a chronological defect, clinically, MIH lesions are not distributed symmetrically. Within the same tooth type in a single patient, lesions either may not appear or may present different degrees of severity, ranging from mild opacities to post-eruptive breakdown.

The aim of this study was to estimate the frequency of asymmetries in hypomineralization lesions in permanent molars and incisors of children affected with MIH.

MATERIALS AND METHODS

An observational, prospective, cross-sectional study was designed, which included all children with MIH seeking dental care the Department of Comprehensive Pediatric Dentistry at the School of Dentistry of Buenos Aires University and at three private practices in the same area, from March to September 2017, who provided agreement and whose legal guardians provided consent. The study was approved by the Institutional Ethics Committee (FOUBA 26092012-28).

Three pediatric dentists calibrated following the diagnostic criteria of Mathu-Muju and Wright[13] (2006) (Kappa 0.87) identified presence and severity of opacities on permanent molars and incisors. Six pairs of teeth were evaluated in each patient: first maxillary molars (1.6 and 2.6), first mandibular molars (3.6 and 4.6), central maxillary incisors (1.1 and 2.1), lateral maxillary incisors (1.2 and 2.2), central mandibular incisors (3.1 and 4.1) and lateral mandibular incisors (3.2 and 4.2). For each of the 12 teeth, degree of lesion severity was recorded (0–none, 1–mild, 2–moderate, 3–severe). For each pair, it was determined whether there was symmetry (both teeth affected by the same degree of severity in the lesion) (Fig. 1), asymmetry of presence (one tooth in the pair with lesion and the other without) (Fig. 2) or asymmetry of severity (both teeth with lesions, but with different degrees

of severity) (Fig. 3). Pairs of teeth without clinical lesions were not included. The data were entered into an Excel spreadsheet and analyzed statistically with software R. Percentages, 95% confidence intervals and Chi-Square were calculated.

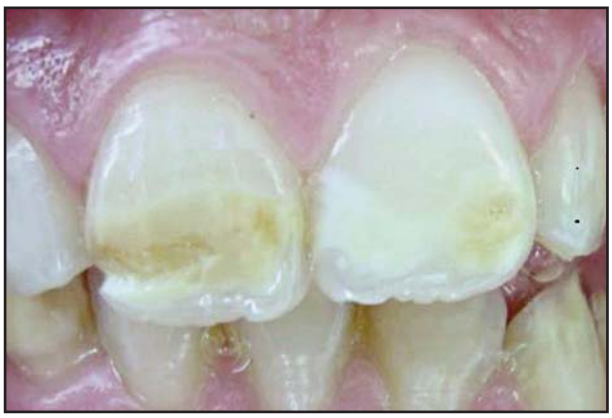

Fig. 1: Symmetry of presence.

RESULTS

The sample consisted of 475 pairs of teeth with lesions out of a total 1032 pairs evaluated in the 172 patients included in the study (mean age 11±2.2 years, and 50% female).

Of the pairs of teeth studied, 67.5% (63.1 - 71.7) showed asymmetry. Analysis of asymmetrical lesions showed that most asymmetries were due to the presence of unilateral defects, with a total 50.1% of the pairs revealing asymmetry of presence. There was a significant relationship between asymmetries and symmetries (p=0.038) (Table 1).

No significant difference was found at 0.05 confidence level between percentage of teeth affected on right side (34.98%) and left side (34.01%) (p= 0.6433). There was, however a small but significant difference (p=0.002) between percentage of affected maxillary (37.69%) and mandibular (31.30%) teeth. Total sample (2064 teeth) was used to calculate and compare these percentages.

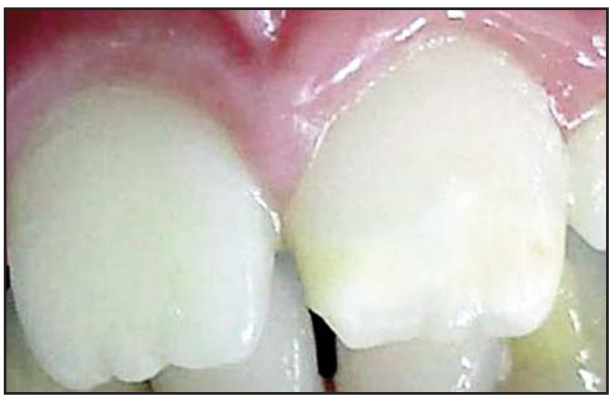

Fig. 2: Asymmetry of presence.

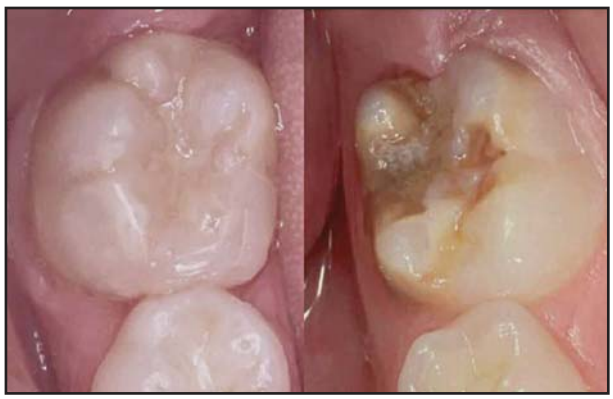

Fig. 3: Asymmetry of severity.

Table 1: Number and percentage of symmetries and asymmetries per tooth.

	Cases		Asymmetry Total		Asymmetry Presence		Asymmetry Severity		Symmetry	
	No.	%	No.	%	No.	%	No.	%	No.	%
UM	139	29.3	91	65.5	50	36.0	41	29.5	48	34.5
LM	149	31.4	98	65.8	66	44.3	32	21.5	51	34.2
UCI	81	17.0	51	63.0	46	56.8	5	6.2	30	37.0
ULI	35	7.3	27	77.1	25	71.4	2	5.7	8	22.9
LCI	45	9.5	36	80.0	34	75.6	2	4.4	9	20.0
LLI	26	5.5	18	69.2	17	65.4	1	3.8	8	30.8
Total	475	100	321	67.5	238	50.1	83	17.4	154	32.5
CI 95%				(63.1-71.7)		(45.5-54.7)		(14.1-21.2)		(28.2-36.8)

UM: upper molars; LM: lower molars; UCI: upper central incisors; ULI: upper lateral incisors; LCI: lower central incisors; LLI: lower lateral incisors.

Lower central incisors had the highest percentage of asymmetrically distributed lesions (80%). No significant difference was found in the symmetry/asymmetry ratio between different tooth types (p=0.95).

The combinations that included a tooth with mild severity were the most frequent. Mild severity was observed in 62.2% of the pairs with asymmetry of presence and in 55.8% of pairs with symmetrical lesions. Of the pairs with asymmetry of severity, the most frequent combination was mild-moderate (1-2), with 43.4%. Lower molars had 49% severity in pairs with symmetry, being the group of teeth with the highest degree of severity for both symmetrical and asymmetrical lesions (Table 2).

DISCUSSION

Enamel is formed during a defined period of odontogenesis, known as amelogenesis. It is an extremely complex, genetically controlled process, and ameloblasts are particularly sensitive to environmental changes during this phase[14]. Dental anomalies are caused by complex interactions among genetic, epigenetic and environmental factors during the period of dental development, which is multifactorial, multidimensional, multilevel and progressive over time, but involves critical periods[15]. Even when a specific mutation of a single gene or one important environmental factor has been identified in a patient with a dental anomaly, detailed exploration of phenotype may reveal variations among affected individuals in the same family, between dentitions in the same individual, and even among different teeth in the same dentition[15]. The stage of amelogenesis at which a given tooth germ is at a particular moment in time when the insult occurs is critical for the type and location of the defect. The teeth mainly affected by MIH, though not the only ones, are permanent incisors and first molars. Permanent incisor enamel forms between approximately 3 months and 5 years of age, while first molar enamel forms at about the 8th month of intrauterine life and continues to the age of 4 years. It is therefore believed that the factors causing the disease would act during those periods, with the first 10 months of life being critical [16].

In contrast to hypoplasias which present symmetrically, except when they respond to an identifiable environmental factor (e.g., and infection or trauma to a primary tooth which could cause an alteration in the replacement tooth), in MIH, as shown by the results obtained in the current study, lesions are asymmetrical for both presence and severity. The reason for this is not clear, and the literature contains no paper explaining this situation. With regard to asymmetry of frequency, Padavala and Sukumaran[17] (2018), in a study on only 22 children with MIH, report that teeth on the right side are more affected. This is not consistent with the current study, in which no significant difference was found between sides upon considering a much larger sample. With relation to the presence of disease in upper and lower jaws, our results also contradict the same study, which reports that lower teeth are more affected.

MIH etiology seems to be multifactorial with associated genetic predisposition associated to one or

Table 2: Number and percentage of each possible combination of severity in teeth for each type of asymmetry or symmetry.

	Asymmetry of presence							Asymmetry of severity							Symmetry						
	0-1		0-2		0-3		*Total*	1-2		2-3		1-3		*Total*	1-1		2-2		3-3		*Total*
	N°	%	N°	%	N°	%	*N*	N°	%	N°	%	N°	%	*N*	N°	%	N°	%	N°	%	*N*
UM	22	44.0	18	36.0	10	20.0	*50*	16	39.0	15	36.6	10	24.4	*41*	18	37.5	16	33.3	14	29.2	*48*
LM	24	36.4	21	31.8	21	31.8	*66*	11	34.4	12	37.5	9	28.1	*32*	19	37.3	7	13.7	25	49.0	*51*
UCI	38	82.6	8	17.4	0	0.0	*46*	4	80.0	0	0.0	1	20.0	*5*	30	100.0	0	0.0	0	0.0	*30*
ULI	22	88.0	3	12.0	0	0.0	*25*	2	100.0	0	0.0	0	0.0	*2*	7	87.5	0	0.0	1	12.5	*8*
LCI	28	82.4	6	17.6	0	0.0	*34*	2	100.0	0	0.0	0	0.0	*2*	7	77.8	2	22.2	0	0.0	*9*
LLI	14	82.4	3	17.6	0	0.0	*17*	1	100.0	0	0.0	0	0.0	*1*	5	62.5	3	37.5	0	0.0	*8*
Total	*148*	*62.2*	*59*	*24.8*	*31*	*13.0*	*238*	*36*	*43.4*	*27*	*32.5*	*20*	*24.1*	*83*	*86*	*55.8*	*28*	*18.2*	*40*	*26.0*	*154*

UM: upper molars; LM: lower molars; UCI: upper central incisors; ULI: upper lateral incisors; LCI: lower central incisors; LLI: lower lateral incisors.

more environmental factors acting during a specific period of amelogenesis of a specific tooth. Brook[15] suggests that this may be explained by the multidimensionality of the process of molecular and cellular interactions and their outcomes which occur during the etiology of dental anomalies. The different tooth germs are at different stages of development at a particular time, so an insult during a specific time period could cause different defects in different teeth, depending on the specific formation of that tooth germ.

Symons and Gage[18] claim that in genetic anomalies, all quadrants should be affected in the same way, although severity could increase due to a variation in gene penetration.

The current study, as a first approach to the subject, and in order to facilitate interpretation of results, used the Chi-square test for comparisons. A further study could be analyzed using mixed models, to consider possible correlation among teeth in one patient.

MIH is a disease that still gives rise to controversies in the literature, especially because its exact etiology is unknown to date. When causal factors are identified, the reasons for asymmetries in lesions will likely be explained.

CONCLUSIONS

In this study, most MIH lesions presented some type of asymmetry (asymmetry of presence or of severity) for all tooth types.

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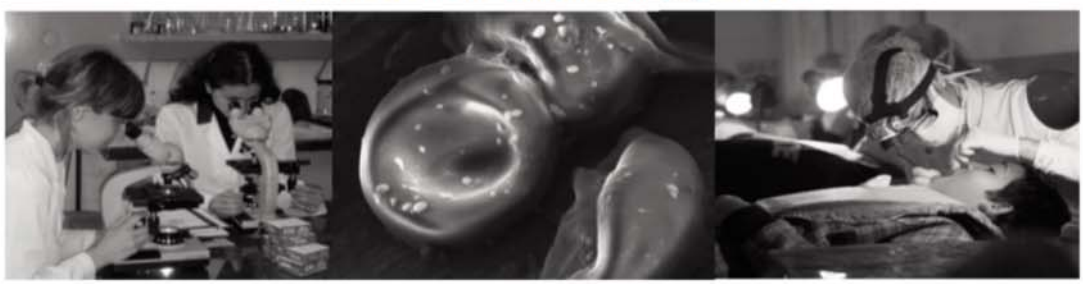

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