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# *ACTA ODONTOLOGICA LATINOAMERICANA*

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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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## IN MEMORIAM



**Prof. Dr. María Beatriz Guglielmotti**  
(1950 -2016)

*We deeply regret to announce the passing of Dr. Guglielmotti last April 26. She was one of the most enthusiastic and helpful members of our journal's Editorial Committee. A brain tumor quickly ended a life dedicated to dental science and university management.*

*Dr. Guglielmotti was a member of the Researcher's Career at the National Council for Scientific and Technical Research (CONICET, Argentina), being an outstanding researcher in the field of biocompatibility of dental implants/ bone substitutes and bone biology.*

*Since 2002 she was Full Professor of the Department of Oral Pathology, School of Dentistry, Buenos Aires University.*

*Throughout her fruitful life's work as teacher and researcher, she published numerous papers in international journals, made many presentations at congresses, taught graduate and postgraduate courses, directed PhD and Master's theses and directed researchers and fellows.*

*From a very young age, she divided her time among scientific activities and university management tasks, having held several positions, including Dean of the School of Dentistry at Buenos Aires University from 2006 to 2014 and Vice-Rector of Buenos Aires University from 2008 to 2010.*

*Her teachers, colleagues, disciples, students and especially her friends will always remember her great capacity for work and her boundless, selfless generosity in sharing and teaching her knowledge.*

### **Prof. Dra. María Beatriz Guglielmotti (1950 -2016)**

*Con profundo pesar, despedimos el pasado 26 de Abril a la Dra. Guglielmotti, uno de los miembros más entusiastas y colaboradores del Comité Editorial de nuestra revista. Un tumor cerebral rápidamente puso fin a una vida dedicada a las ciencias odontológicas y a la gestión universitaria.*

*La Dra. Guglielmotti fue miembro de la Carrera del Investigador del Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET, Argentina) destacándose como investigadora en temas de biocompatibilidad de implantes dentales- sustitutos óseos y biología ósea.*

*Desde el año 2002 ocupaba el cargo de Profesora Titular de la Cátedra de Anatomía Patológica de la Facultad de Odontología, Universidad de Buenos Aires.*

*Su fecunda labor como docente e investigadora ha dado lugar a numerosas publicaciones en revistas internacionales y presentaciones a Congresos, dictado de cursos de grado y posgrado, dirección de tesis de doctorado y maestría y dirección de investigadores y becarios.*

*Desde muy joven, compartió sus actividades científicas con tareas de gestión universitaria, ocupando diversas posiciones hasta el cargo de Decana de la Facultad de Odontología, UBA, en los años 2006 a 2014 y Vicerrectora de la Universidad de Buenos Aires entre 2008 y 2010.*

*Sus maestros, colaboradores, discípulos, alumnos y especialmente sus amigos, recordarán siempre su gran capacidad de trabajo y su inmensa y desinteresada generosidad en compartir e impartir sus conocimientos.*

## REPAIRABILITY OF AGED RESIN COMPOSITES MEDIATED BY DIFFERENT RESTORATIVE SYSTEMS

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### ABSTRACT

The aim of this study was to evaluate the shear bond strength of resin composite repairs with and without aging of the surface to be repaired, using different adhesive systems and resin composites. Ninety specimens were prepared: 10 for the Control Group (GC - without repair); 40 for Group I (GI - repairs after 7 days) and 40 for Group II (GII - repairs after 180 days). Groups I and II were divided into 4 subgroups of 10 specimens each, according to the adhesive system and composite resin used: A) Adper Scotch Bond Multipurpose + Filtek Z350 XT; B) Adper Single Bond Plus + Filtek Z350 XT; C) Adper Scotch Bond Multipurpose + Esthet-X; D) Adper Single Bond Plus + Esthet-X. The specimens were tested for

shear strength in a universal testing machine. The results were analyzed by two-factor one-way ANOVA and Fisher's post hoc tests ( $\alpha=0.05$ ). The control group had better performance than the other groups. There was no significant difference when comparing different adhesive systems and composite resins. Repairs performed at 7 days were better than those performed at 180 days. The composite repairs decreased the mechanical strength of the restoration. Aging of the resin substrate may decrease repair bond strength over time, regardless of the type of adhesive systems and resin composites used.

**Key words:** Composite resins, adhesives, aging.

## REPARO DE RESINAS COMPOSTAS ENVELHECIDAS E MEDIADAS POR DIFERENTES MATERIAIS RESTAURADORES

### RESUMO

Avaliar a resistência de união ao cisalhamento de reparos de resina composta com e sem envelhecimento da superfície a ser reparada, utilizando diferentes sistemas adesivos. Noventa corpos de prova foram confeccionados sendo: 10 para o Grupo Controle (GC - sem reparo); 40 para o Grupo I (GI - reparos após 7 dias) e 40 para o Grupo II (GII - reparos após 180 dias). Para os reparos, os grupos GI e GII foram subdivididos em 4 subgrupos com 10 corpos de prova, variando o sistema adesivo e a resina composta: A) Adper Scotch Bond Multipurpose+ Filtek Z350XT; B) Adper Single Bond Plus+ Filtek Z350XT; C) Adper Scotch Bond Multipurpose+ Esthet-X; D) Adper Single Bond Plus+ Esthet-X. Os corpos de prova foram submetidos a uma força de cisalhamento em uma máquina de

ensaio universal (EMIC). Os resultados foram analisados pelo teste estatístico Anova dois fatores, seguido pelo teste de Fisher's. Observou-se melhor comportamento do grupo controle sobre os demais grupos, além disso, os reparos realizados aos 7 dias foram superiores aos dos realizados em 180 dias. Não houve diferença significativa quando se comparou diferentes sistemas adesivos e resinas compostas. Os reparos de resina composta diminuem a resistência mecânica da restauração. O envelhecimento do substrato de resina pode diminuir a resistência ao reparo ao longo do tempo, independentemente do tipo de sistemas adesivos e resinas compostas utilizados.

**Palavras-chave:** Resinas compostas, adesivos, envelhecimento.

### INTRODUCTION

Despite significant developments in composites and restorative techniques, restorations can still sometimes fail. Repairing restorations is a minimally invasive approach, which preserves part of the material, thus preventing a repetitive restoration cycle<sup>1</sup>.

Although it is possible and recommendable to repair composite restorations, there are still some problems that need to be resolved. The literature contains studies on different repair techniques for composite resin restorations<sup>2-6</sup>. The repair is achieved by chemical bonding between the filler

particles and the organic matrix through the use of adhesive systems, and the surface to be restored may require roughening<sup>7</sup>.

There is no clear consensus regarding whether or not the waiting time until repair interferes with the bond strength of the material, although the aging of the composite is considered detrimental to the process of chemical bonding<sup>8</sup>. There is a wide range of available composites and adhesive systems to choose from, and when a dentist repairs a restoration done by someone else, it is not always possible to obtain all the information about the restorative materials used<sup>4,9</sup>.

The aims of this study were to (a) analyze whether there is any difference between repaired and non-repaired resin composite; (b) compare repairs using composites which are the same as or different from

the substrate in early and aged repairs using different types of adhesive systems; and (c) measure whether aging decreases the repair bond strength.

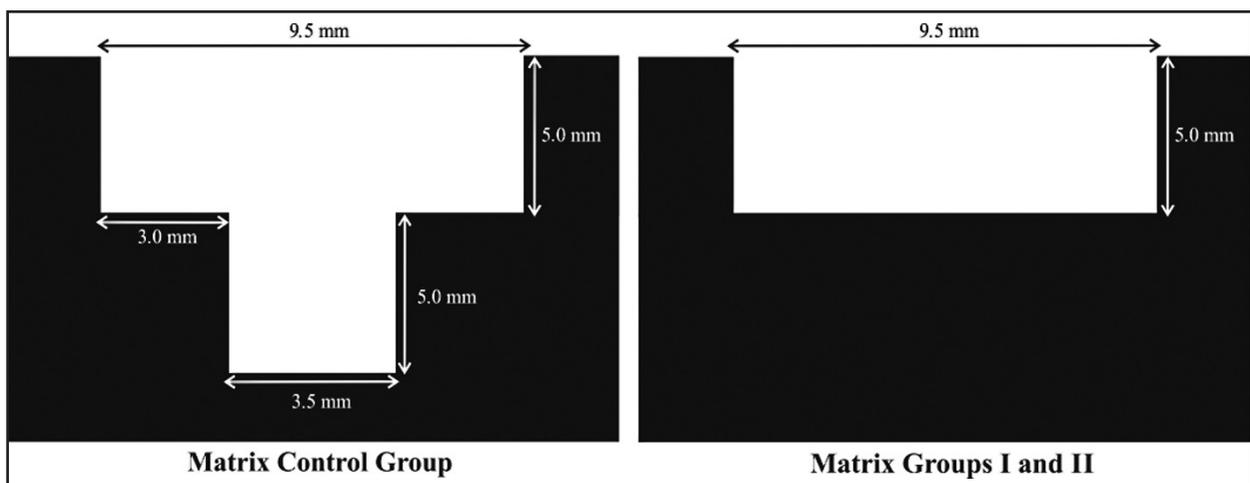
## MATERIALS AND METHODS

The study factors were the materials used for repairs on eight levels (different combinations of adhesive systems and composite resins) and the time factor on two levels, with repairs performed after 7 and after 180 days. The response variable was the shear bond strength of the resin composite repairs.

Table 1 shows the materials used in this study. The specimens were made in a Teflon mold 9.5 mm in diameter and 5 mm deep. For the control group, a cylindrical protuberance, 3.5 cm diameter and 5 cm tall was added to the center of the mold (Fig. 1). A total 90 specimens were prepared (10 specimens per group).

**Table 1: Brand name, composition, lot number and manufacturer of the materials used in this study.**

Materials	Composition	Batch#	Manufacturer
Filtek Z350 XT	BisGMA, UDMA, BisEMA, TEGDMA, nanosilica filler, zirconia/silica particle agglomerates	7GM 7CA 6GR	3M/ESPE, St. Paul, MN, USA
Esthet-X	BisGMA, modified urethane, BisEMA, TEGDMA, aluminum borosilicate fluoride glass, silanized Barium	0510232 0510281 0801282	Dentsply Caulk, Milford, DE, USA
Adper Single Bond Plus	Priming resin Bis-GMA, HEMA, polyalkenoic acid, water, ethanol, dimethacrylates pH – 4.5	7KR	3M/ESPE, St. Paul, MN, USA
Adper Scotch Bond Multipurpose	Etchant: 35% phosphoric acid, silica thickener Adhesive: Bis-GMA, HEMA, tertiary amines, and photo-initiator	6PL	3M/ESPE, St. Paul, MN, USA



*Fig. 1: Matrix for preparation of specimens.*

The control group and the substrates to be repaired were made using Filtek Z350 XT resin (3M ESPE, St. Paul, MN, USA). The cohesive strength of the nanofilled resin composite was used as control. The resin composite was applied in increments of 2 mm, which were polymerized for 40 seconds at 500mW/cm<sup>2</sup> (Ultraled – Dabi Atlante SA, Ribeirão Preto, SP, Brazil).

The 80 test specimens were divided into two groups of 40 and stored in distilled water at 37°C for 7 days (Group I) or 180 days (Group II) before being repaired. Groups I and II were divided further into four subgroups, for which different adhesive systems and resin composites were used in the repair (Table 2).

After the storage periods, the specimens were embedded in acrylic resin and the external surfaces of the composite resins were roughened using #320 grit sandpaper (3M Brazil, Sumaré, SP, Brazil) in a polishing machine (Arotec Ind. e Com, Cotia, SP, Brazil). The roughened surface was washed in an ultrasonic tank for 10 minutes (Cristófoli, Campo Mourão, PR, Brazil) and air-dried before phosphoric acid etching at 37% (Condac 37-FGM Joinville, SC, Brazil) for 20 seconds. The specimens were washed again and dried with air jets. Adhesive tape (3M Brazil, Sumaré, SP, Brazil) was placed on the surface of the specimens, leaving a central perforation 3.5mm in diameter, and with the aid of micro-brush, the adhesive system was applied and light-cured for 20 seconds.

To insert the new portion of composite resin, the specimens were fixed to a device and positioned against a Teflon mold (3.5 mm wide by 5 mm high) with a central perforation matching the delimitation

of the tape. Resin composite increments approximately 2 mm thick were inserted and cured for 40 seconds, after which the assemblies were removed from the device. The specimens thus obtained were used as simulations of repairs (Fig. 2).

For mechanical testing, the specimens were subjected to a shear bond test using a universal testing machine EMIC (EMIC DL-1000, EMIC Equipamentos e Sistemas de Ensaio Ltda, São José dos Pinhais, PR, Brazil) at a crosshead speed of 0.5 mm/min (Fig. 2). The fractured surfaces were examined using a binocular microscope to assess failure modes (Stemi SU 11, Zeiss, Oberkochen, Germany) at 40× magnification. Failures were classified as adhesive (fracture on the adhesive interface of the resin portions), cohesive (fracture within one of the two resin portions), or mixed (simultaneous occurrence of adhesive and cohesive fractures).

The samples were gold sputtered (Balzers SCD-050 sputter coater, OC Oerlikon Corporation AG, Pfäffikon, Switzerland) and analyzed under scanning electron microscope (Evo LS15, Carl Zeiss, Oberkochen, Germany). All samples were scanned at 40 to 45× magnification, and then the most

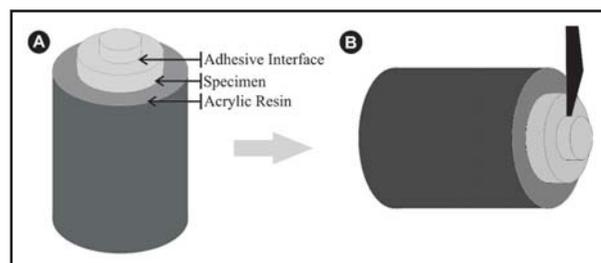


Fig. 2: Repair accomplished with resin composite (A). Measuring shear strength (B).

**Table 2: Distribution of the groups according to the combination of materials tested.**

Groups		Adhesive System	Composite Resin	Storage period before repair
GI	GC	----	Filtek Z350 XT	----
	A	Adper Scotchbond Multiuso	Filtek Z350 XT	7 days
	B	Adper Single Bond		
	C	Adper Scotchbond Multiuso	Esthet X	
	D	Adper Single Bond		
GII	A	Adper Scotchbond Multiuso	Filtek Z350 XT	
	B	Adper Single Bond		
	C	Adper Scotchbond Multiuso	Esthet X	
	D	Adper Single Bond		

representative area of each specimen was selected and magnified at 1000 $\times$ .

The results of the mechanical tests were analyzed and submitted to one-way ANOVA and Fisher's test for multiple comparisons, with a significance level of 5%.

## RESULTS

In the control group, there was prevalence of cohesive-type fractures, and significantly higher shear strength than in the other groups.

No statistically significant difference was found among the different adhesive/resin composite systems used for repair when they were evaluated in each storage period.

**Table 3: Average values and standard deviation of the shear bond strength of resin composite repairs.**

Groups	Shear bond strength (Mean/Standard deviation)
GC	18.71 ( $\pm$ 3.10) <sup>A</sup>
GI-A	14.35 ( $\pm$ 6.06) <sup>B</sup>
GI-B	12.43 ( $\pm$ 2.08) <sup>BC</sup>
GI-C	13.47 ( $\pm$ 5.75) <sup>B</sup>
GI-D	12.43 ( $\pm$ 3.98) <sup>BC</sup>
GII-A	9.26 ( $\pm$ 4.34) <sup>CD</sup>
GII-B	7.86 ( $\pm$ 3.04) <sup>D</sup>
GII-C	6.27 ( $\pm$ 1.08) <sup>D</sup>
GII-D	7.07 ( $\pm$ 2.61) <sup>D</sup>

Groups with the same letter do not show statistically significant differences ( $p \geq 0.05$ )

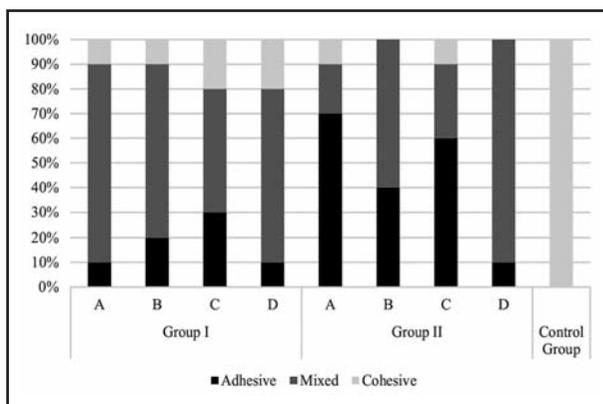


Fig. 3: Distribution of the failure modes according to the variables, after mechanical testing.

The groups repaired after 7 days had statistically higher bond strength than the groups repaired after 180 days, except for GII-A, for which the results were similar to GI-B ( $p=0.0736$ ) and GI-D ( $p=0.0729$ ) (Table 3).

All specimens in the control group had cohesive failures. There were more adhesive fractures after 180 days' storage, except in GII-D, which had the same number of adhesive failures but no exclusively cohesive failure (Fig. 3). Figure 4 shows representative SEM images of each type of failure.

## DISCUSSION

There is concern that high-quality evidence does not yet exist to support restoration repair<sup>10</sup>. However, some clinical studies demonstrate the success of restoration repair when performed appropriately<sup>11</sup>. The view must be taken that the replacement of a restoration is contraindicated when most of the restoration concerned is intact. Repairing restorations enables the adoption of minimal intervention approaches to dental restorations<sup>1</sup>.

Shear strength has been widely used in mechanical tests to verify adhesion to tooth structure or to

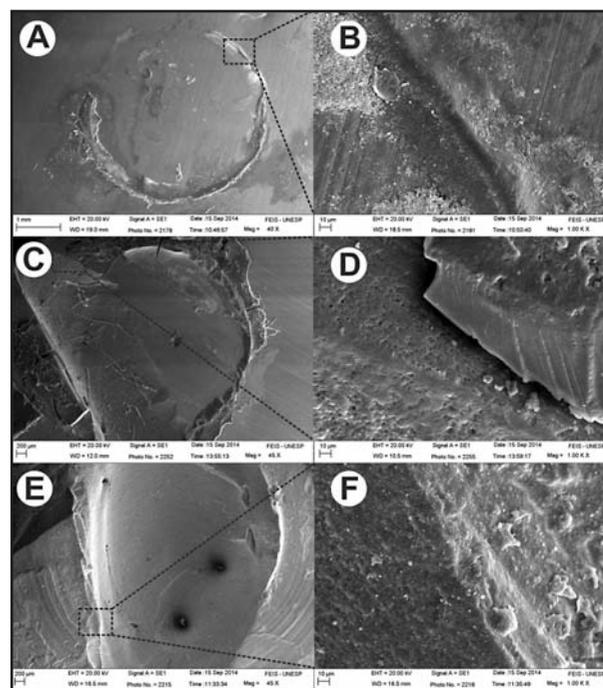


Fig. 4: Scanning electron microscopy of resin surfaces with different failure modes (A,B) Adhesive from GI-C group; (B,C) Mixed from GI-B group; (E,F) Cohesive from control group.

restorative materials, because it is similar to the forces clinically obtained in restorations<sup>12,13</sup>. Microtensile bond strength has also been used because it provides more uniform stress distribution on the relatively small adhesive interface<sup>14</sup>.

The cohesive strength of non-repaired resin composite is expected to be higher than that of a repaired specimen<sup>2,15</sup>. Ilie et al.<sup>15</sup> reported repair strength equivalent to 35.4% to 90.9% of the cohesive strength of the original composites, in agreement with the results of our study, which found a similar interval, ranging from 35.5% to 76.8% of the cohesive strength in the control group. Our results showed that using a resin composite different from the original one made no significant difference in the bond strength of the repairs performed after 7 or 180 days' storage. Other studies have also reported that different repair resins did not significantly affect the results under either aged<sup>13,15</sup> or non-aged conditions<sup>13,16</sup>.

Baur and Ilie<sup>4</sup>, however, report that it was not the same to repair resin composites with the same material or in combination with other materials. They advise clinicians to keep careful records on the material they have used. However, when a repair is not performed by the same professional, it is difficult to identify the resin used in the previous filling technique. Adhesion between materials probably depends much more on the basic chemical interaction of materials and micromechanical retention than on the specific constituents incorporated by each manufacturer<sup>13</sup>.

Our results demonstrated that using hydrophobic adhesive (Adper Scotch Bond Multipurpose) after 180 days' storage provided similar results to using a hydrophilic system (Adper SingleBond Plus) after 7 days' storage, since GII-A showed similar results to GI-B and GI-D. Another study using same adhesive systems also demonstrated that the hydrophilicity of the intermediate agent did not affect the initial composite repair strength and silver nitrate deposition; however, spotted silver nitrate deposits were seen in specimens bonded with the hydrophilic system (Adper SingleBond Plus) after being stored six months in water<sup>5</sup>. Cavalcanti et al. report that the type of bonding system did not influence microleakage at the composite-repair interface<sup>17</sup>.

Various methods have been described for artificially aging a substrate material before repair<sup>18</sup>. It has

been shown that aging methods produce significant differences on the composite-composite repair strength<sup>18</sup>. A storage period of 180 days was used in order to simulate possible changes occurring in composites exposed to humid environments, such as water absorption and leaching of the resinous components<sup>9</sup>. The longer it is after the restorative procedure, the lower will be the values of bond strength of repair resin composite<sup>6,19</sup>. This consideration was confirmed in our study, with shear bond strength decreasing significantly in specimens aged for 180 days before completing the repairs.

The specimens in Group II, which were stored in distilled water for a longer period of time, probably lost some of the free carbon present in these materials<sup>8</sup>, favoring the breakdown and hydrolysis of polymers and silane bonds<sup>20</sup>. This process is also influenced by the reduction in the number of free methacrylates, which are essential to the bonding process to the composite<sup>8,9</sup>.

In our study, the composite surface was roughened based on previous results<sup>5</sup>. Clinically, the use of diamond tips favors the formation of a debris layer (smear layer) which can compromise the bonding, thus, the use of phosphoric acid favors bonding between the restorative materials<sup>21</sup>. Within this context, micromechanical interlocking produced by roughening is crucial to establishing a strong bond between the old with the new resin composite<sup>7</sup>; since chemical bonding may be hindered, possibly due to the small amount of available monomers, as mentioned above<sup>3</sup>.

Although the micro-retentive features establish a greater surface area, this does not allow close contact between old and new resin composite portions, and thus requires the application of an adhesive system to decrease the surface energy of the old resin and establish excellent surface wetting<sup>5</sup>. It can also promote a better chemical interaction between the composites<sup>22</sup>.

However, there is no clear consensus in the literature regarding the indication of the type of treatment to be performed on the surface of the old resin for subsequent repair<sup>10</sup>. Kimyai et al.<sup>23</sup> reported that surface treatment with air abrasion and laser Er, Cr: YSGG provided higher bond strength than treatment with diamond tips; however, the bond strength obtained by using diamond tips was higher when no treatment was performed. Bonstein

et al.<sup>7</sup> found that the surface treatment of the old resin with diamond drills resulted in higher bond strength than treatment with air abrasion. However, other studies found no difference between the different types of surface treatments<sup>2</sup>.

Regarding the failure mode, there was predominance of mixed-type failures after 7 days' storage. Adhesive-type failures tended to increase after aging, possibly due to the decrease in the adhesive strength of the repair. Other studies evaluating the bond strength of composite resins also report predominantly mixed failure<sup>2</sup>.

High bond strengths have been correlated with cohesive fracture patterns, whereas at low bond strengths, an increased incidence of adhesive fracture modes has been observed<sup>4</sup>. Ozcan and Pekan<sup>24</sup> report that the incidence of cohesive failures was more common when the substrate and the adherent were of the same composite type, whereas when they differed, adhesive failures were more frequent. This trend was not observed in our study. In general, there is no consensus on type of failure mode. Some studies report no cohesive failure for repaired groups<sup>2</sup>, in contradiction to others that report cohesive<sup>6</sup> or adhesive failures modes<sup>15</sup>. Such differences may arise from the different methodologies employed.

The subject of the difficulty in interpreting the bonding performance of adhesion has been discussed. Scherreret al.<sup>14</sup> reported that all broken specimens showing cohesive failure should be discarded because they are not representative of interface bond strength, but rather, reflect a mixture of mechanical properties of the different materials involved (*i.e.* dentin, restorative resin). However,

adhesion of repaired resin composites involves substrates with similar mechanical properties, since Filtek Z350 XT and Esthet-X showed similar flexural strength<sup>25</sup>. The few cohesive failures observed in our study suggest that the adhesive strength at the interface exceeded the cohesive strength of the underlying composite resin, and thus, the repair as such cannot be considered the weakest link.

Within the limitations of this study, it can be seen that the adhesive systems and composite resins used for carrying out the repairs did not affect the values of shear bond strength, although prolonged storage significantly reduced the bond strength of the repaired specimens.

The clinical relevance of this study is that it shows that in cases where resin composite restorations are very old, the effectiveness of bond repair resin is not enough to maintain the expected longevity in the restorative procedure. In cases of recent need for repairs, the repetitive cycle of restorations could be avoided, regardless of the materials used in the repair procedures.

It is impossible to replicate in the laboratory the different conditions that a restorative material undergoes in the oral cavity, being one of the limitations of *in vitro* studies. Further randomized controlled trials are needed to investigate the repair of resin composite and explore qualitatively the views of patients on repairing versus replacement, and investigate themes around pain, anxiety, time and costs.

Within limitations of this study, it can be concluded that aging of the resin substrate may decrease the repair bond strength over time, regardless of the type of adhesive systems and resin composites used.

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## STUDY AND ANALYSIS OF INFORMATION TECHNOLOGY IN DENTISTRY IN LATIN AMERICAN COUNTRIES

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### ABSTRACT

Dentistry increasingly uses Information and Communication Technology (ICT), which has impact on teaching, research, the profession and dental care in general. However, there is a lack of valid information on ICT resources and use in Latin America. This was a descriptive, cross-sectional, multi-center, interdisciplinary study, the aim of which was to conduct a survey on how extensively ICT is used in Dentistry in Latin American countries by enquiring into two primary components: 1) use of ICT in student training and 2) use of ICT by professionals in consulting rooms and services. Two questionnaires on ICT were prepared: one for teachers/researchers and another for students/professionals. We received 94 answers from teachers/researchers at universities in the region providing information on ICT resources for teaching (type and implementation) and 221 answers from professionals (personal

use and use in healthcare). Data are presented as absolute relative frequencies and analyzed quantitatively as percentages. Results: 1) Teachers highlight ICT as an instrument for development, democratization and fairness in access to knowledge for higher education. 2) ICT supports collaborative learning and generates other innovative resources (e.g. simulators). 3) Innovations in telemedicine and experiences with electronic clinical history were identified in Brazil, Uruguay and Colombia. These results are a basis upon which to reach a consensus regarding a set of ICT indicators which are comparable at regional level and serve as input to unify the design and implementation of ICTs experiences in both teaching and dental care in Latin America.

**Key words:** Dental informatics, communication media, oral health, dental research.

## ESTUDIO Y ANÁLISIS DE LA INFORMÁTICA ODONTOLÓGICA EN PAÍSES DE LATINOAMÉRICA

### RESUMEN

La Odontología utiliza en forma creciente las Tecnologías de la Información y la Comunicación (TIC) impactando en la enseñanza, la investigación, la profesión y la atención odontológica en general. Sin embargo, no se cuenta con información válida sobre los recursos e utilización de las TIC en Latinoamérica. La investigación representó un estudio descriptivo, de corte transversal, multicéntrico e interdisciplinario, cuyo objetivo fue realizar un relevamiento del grado de informatización en Odontología en países latinoamericanos indagando dos componentes principales en el empleo de las TIC: 1) en la formación de estudiantes y 2) por parte de los profesionales en consultorios y servicios. Se realizaron cuestionarios a referentes TIC: uno para docentes/investigadores y otro para estudiantes/profesionales. Se recibieron 94 respuestas de docentes/investigadores de universidades de la región dando cuenta de los recursos TIC para la enseñanza (tipo e implementación) y 221 de profesionales

(personal y en la asistencia clínica). Los datos recogidos fueron analizados cuantitativamente con tabulación en porcentaje y se presentan en frecuencia relativa absoluta. Resultados: 1) Los docentes enfatizan a las TIC como instrumento de desarrollo, democratización y equidad en el acceso al conocimiento en educación superior. 2) Las TIC apoyan el aprendizaje colaborativo y generan otros recursos innovadores (ej: simuladores). 3) Se identificaron innovaciones en telemedicina y experiencias con historia clínica electrónica en Brasil, Uruguay y Colombia. Los resultados constituyen una base para consensuar un conjunto de indicadores TIC comparables a nivel regional y sirven como insumo para unificar el diseño e implementación de las experiencias TIC tanto en enseñanza como en la asistencia odontológica en Latinoamérica.

**Palabras clave:** Informática odontológica, Medios de comunicación, Salud oral, Investigación odontológica.

### INTRODUCTION

The rapid development of Information and Communication Technology (ICT) poses major challenges to the health sector in Latin America.

There is a need to evaluate how adopting ICT and using it efficiently can contribute to responding to those challenges. Within the field of health, dentistry has been using ICT increasingly in

teaching, research, extension, the profession and dental care in general. However, there is a lack of valid information on ICT resources and use in Latin America enabling the establishment of groups and networks that could contribute to responding to the challenges of teaching, research and dental care in the region. Guillén and Monteagudo<sup>1</sup> claim that health researchers need to have at least a minimum level of knowledge of new technologies in order to carry out their activity. Peña<sup>2</sup> believes that research in biomedicine and health ultimately depends on researchers' ability to include and use technological developments in their daily work. Including ITC in Dentistry calls for changes in management, teaching and professional work, or, in more general terms, in lifestyle. Computers, networks and in particular the Internet, have had great influence on the ways in which information and knowledge are generated, managed and made known. Worldwide, ICT has changed training processes, whether in distance learning or as support for face-to-face learning. As centers of knowledge production and transmission, Institutions of Higher Education cannot ignore the enormous potential of ICT as a resource for graduate and post-graduate training, leading to substantial renovation or transformation of the ends, means and organization of the educational system<sup>3</sup>.

In 1999, Ehrmann S.C.<sup>4</sup> foresaw that there would be major transformations in upcoming years regarding the conception and practice of university teaching, suggesting that they would lead to a veritable pedagogical revolution. Axt M.<sup>5</sup> suggests that this passage to an information and communication society is similar what happened during the transition to literate societies with printing, recognizing four comparable features: (a) although technological progress is quantitative, it is especially qualitative (in terms of new cognitive demands); (b) once the process of technological diffusion begins, it appears to be irreversible; (c) a given development appears as a process of continuous extension and sophistication and (d) advantages and disadvantages go together, creating situations that require control by societies. It should be noted that there are certain features in the conceptions held by knowledge societies that go beyond those held by information societies. Although both types of society consider that ICT provides a qualitative leap, knowledge societies have an egalitarian background which respects

cultural diversity, and aim to reduce the digital divide between different countries. Both conceptions are based on the assumption that knowledge is key to the development of societies; thus, today, more than ever before, educational systems face previously unimaginable transformations. While information societies are based on technological progress, knowledge societies hold notions that include social, ethical and political dimensions.

Higher Education in Latin America is undergoing changes caused the widespread growth of teaching and the range of dynamics imposed by globalization. In this context, new communication and information technologies and increasing demand for access to education have major roles. Quality assurance, new post-graduate programs and virtual education are some of the tools created by a university system seeking to respond to new demands imposed by the international context<sup>6</sup>. The challenge is for universities to innovate, not only in technology, but also in their pedagogical conceptions and practices, which would involve changing the global university teaching model: changes in organization, ways of working, human interaction, the role of the teacher, learning activities and processes, forms of classroom organization and modes of access to knowledge. This requires universities to establish new organizational models and resources (combination of virtual and face-to-face learning). M. Silva<sup>7</sup> says, "... it should be noted that the distinction between "face-to-face" and "distance" will become less and less relevant as digital technologies become more widespread. Both modalities will coexist: use of the internet, multimedia supports and the traditional classroom with teacher and students face-to-face. The student will have a classroom at school or university, and also a website for the subject with exercises and new proposals, which will be the virtual classroom". Maraschin C. and Axt M.<sup>8</sup> analyze how teacher-student practices and their relationship to knowledge are changed by new devices. They enquire into how relationships, learning and knowledge can be affected by coexisting with technology, and emphasize an intricate, dynamic relationship between knowledge and technology. Information technology is not perceived simply as a means for learning or knowing something, but as an intrinsic, constitutive part of the way of knowing itself.

In recent years, the dental profession has included ICT in healthcare activities. ICT is increasingly

used for administrative activities at offices, clinics and healthcare services, as well as for activities for promoting health and health education, for marketing and for continuing education in dentistry. The aim of this study was to survey the degrees of computerization in the field of dentistry in Latin American countries by enquiring into two primary components (domains) in the use of ICT: (1) use for training students (graduate and postgraduate) and (2) use by professionals at dentists' offices and healthcare services. As a hypothesis, it was considered that input from local reference persons for countries in the Latin American Region (LAR) of the International Association for Dental Research (IADR) would provide valid information regarding ICT use in Latin America from a previously unexplored perspective, which would enable a consensus to be reached on a set of comparable ICT indicators in the region. Two questionnaires were prepared on ICT: one for teachers/researchers and another for students/professionals.

## METHOD

A descriptive, cross-sectional, multi-center, interdisciplinary study was conducted, based on questionnaires on two domains with relation to ICT (approved by the Ethics Committee, UFRGS, Brazil-CAAE: 12381613.7.0000.5347). The study began in February 2012 according to defined guidelines and preliminary agreements on different components of the research process, professional activities and dental care in particular. Permanent online exchanges were maintained among members of the research team, and four face-to-face meetings were held in Montevideo (Uruguay) and Porto Alegre (Brazil) (April and September 2012; August 2013 and April 2014). Relevant university actors and professionals from Latin American countries were defined as "key informers". They were asked about demographic data (population, number of dentists, number of dental schools and reference persons on the subject in their countries). Inclusion criterion was to be a country in the Latin American Region (LAR) of the International Association for Dental Research (IADR): Argentina, Brazil, Chile, Colombia, Costa Rica, Ecuador, Paraguay, Peru, Uruguay and Venezuela, which answered the questionnaires (Paraguay was excluded because it did not meet this criterion). Two questionnaires were prepared in Spanish and Portuguese containing

multiple choice and open-ended questions about what, how and why ICT was used. Questionnaires were e-mailed to the reference persons for ICT identified by the key informers for each country: one questionnaire for teachers and researchers (A) and another for students and healthcare professionals (B):

A. Questionnaire in Spanish and Portuguese for teachers, researchers and undergraduate and graduate students

(<https://docs.google.com/forms/d/12MFIFHXSy5tO9PGR-ErGs3PgwnHlzBqCFErwlcDXLY/viewform>)

and

[https://docs.google.com/forms/d/1o5g0WFBRAp237SkH0RUnl\\_cPbLYRNKIw6jRku-QwCfz/viewform](https://docs.google.com/forms/d/1o5g0WFBRAp237SkH0RUnl_cPbLYRNKIw6jRku-QwCfz/viewform))

B. Questionnaire in Spanish and Portuguese for ICT users at dental offices, clinics and/or services

(<https://docs.google.com/forms/d/1Ndn4CF3ZxRCuekABQfpiLaHPDIOrcLHKEFeTUswZhYg/viewform>)

and

<https://docs.google.com/forms/d/1FvDAPNzpdI4vLAzqLxB6bXk3sEdUZ3W-RbvLU9fzrGE/viewform>)

Data collected were analyzed quantitatively by percentage. They are presented in relative and/or absolute frequencies.

## RESULTS

Table 1 shows the number of key reporters, ICT reference persons and total number of answers to questionnaires, distributed by country.

Total number was considered as having answered the questionnaire; thus, as some reference persons did not answer all the questions, there are a few situations in which the sum of answers to a specific question does not match 100% of questionnaires answered.

### A) TEACHERS/RESEARCHERS

We received 94 answers from teachers and researchers at universities in the region: Argentina 18, Brazil 32, Chile 6, Colombia 3, Costa Rica 8, Ecuador 2, Paraguay 0, Peru 10, Uruguay 9, Venezuela 6. Teacher and researcher profile was: (a) age: 36 were younger than 40 years, 30 were 40 to 50 years old and 28 were over 51 years old; (b)

**Table 1: Frequency distribution in absolute values for number of answers from key informers and ICT reference persons to questionnaires per country.**

COUNTRY	KEY REPORTERS	ICT REFERENCE PERSONS	TOTAL ANSWERS
ARGENTINA	20	26	27
BRAZIL	25	63	221
CHILE	10	7	6
COLOMBIA	6	6	3
COSTA RICA	3	7	7
ECUADOR	3	7	3
PARAGUAY	6	1	-
PERU	3	11	14
URUGUAY	9	20	15
VENEZUELA	2	19	9

graduation year: 36 graduated before 1990, 32 graduated between 1990 and 2000 and 25 graduated after 2001; postgraduate degree: 82 have a postgraduate degree and 12 do not. With regard to specific ICT knowledge: 43 consider it is sufficient and 51 consider it insufficient; 60 took training courses within the past 5 years and 34 did not. Some general results on university resources and activities are shown in Table 3, e.g. number of computers, virtual classroom, distance education courses (DE). For number of computers at the educational institution, the answers were: 27 had 1 to 10 computers, 34 had 11 to 50 computers, 31 had 51 to 100 computers and 11 had over 100 computers. With regard to the question of whether distance education (DE) was used as an educational methodology, 47 answered yes, accounting for 50%.

Tables 2, 3, 4 and 5 show the answers from reference university teachers about use of ICT resources for teaching: type, implementation and difficulties in use.

## **B) PROFESSIONALS AND/OR CLINICIANS**

We received 211 answers from professionals in the region. Their profile was: (a) age: 179 were younger than 40 years, 20 were 40 to 50 years old and 12 were over 51 years old; (b) graduation year: 11 graduated before 1990, 17 graduated from 1990 to 2000 and 182 graduated after 2001; postgraduate degree: 82 have a postgraduate degree and 129 do not; 79 work in public service and 132 work in private dental offices. With regard to frequency of ICT use: 32.22% answered always, 54.44% often, 6.77% occasionally and 6.67% never. With regard

to type of Internet used: 10% use 3G (cell phone), 78.89% use broadband, 0.37% use dial-up, 10% none and 0.74% does not know. With regard to connection between health centers/clinics: 15.93% respond they have, 79.63% do not and 4.44% do not know.

Tables 6 and 7 show the answers from university professional reference persons about use of ICT resources for personal and clinical use.

## **How does ICT help the teaching and learning process?**

Answers highlight that ICT is an instrument for development, democratization and fairness in access to knowledge in Higher Education, enabling knowledge to be conveyed in different ways which are more dynamic and encourage association and teamwork. ICT supports collaborative learning, i.e. participation of two or more persons in performing a task, with the aim of the participants constructing knowledge by exploring, discussing, negotiating and debating. Here, the teacher acts as guide and facilitator, introducing the subject, and students discuss, create and modify content by using resources available online. It has been found that virtual settings facilitate exploration and individual search for information and knowledge, and that collaborative learning increases student participation and motivates student learning; the student is no longer a passive receiver, but becomes the protagonist of his/her own learning. Moreover, research skills and construction of student's own learning are reinforced, competition is left aside,

**Table 2: Frequency distribution in absolute values for answers on use of ICT resources at Latin American Schools of Dentistry.**

Enrolment, courses, exams	Access to didactic material	Results of exams and evaluations	Group work	Training	Institutional communications
69	73	55	53	64	67

**Table 3: Frequency distribution in absolute values for answers on type of ICT resources for teaching Dentistry.**

PC	Projector	Interaction device	Video conference	Digital board	Moodle	CDs, Dropbox	Virtual classroom
84	88	9	7	20	2	4	80

**Table 4: Frequency distribution in absolute values for answers on communication media used by Latin American university teachers.**

Telephone	E-mail	Virtual platform	Paper	Chat	Other
23	77	14	11	4	5

**Table 5: Frequency distribution in absolute values for answers on difficulties in implementing ICT by university teachers.**

Lack of resources	Lack of training	Lack of student interest	Lack of teacher incentive	No access	Lack of time	Preconception with ICT	No difficulty
25	40	9	7	5	6	15	4

**Table 6: Percentage of personal ICT equipment.**

Smart phone	PC	Web cam	Scanner	Tablet	Printer	Screen in reception	Digital biometry	At least one resource
54.07	96.19	58.51	60	30.74	83.70	7.04	4.44	96.30

**Table 7: Percentage of clinical ICT equipment.**

Laser	Intraoral camera	Digital camera	Digital photopolymerizer	Tablet	Printer	Screen in reception	Ultra sound/prophylaxis	Dental equipment monitor	Implant digital motor
90.3	87.41	58.51	60	30.7	83.70	7.04	63.33	15.19	18.15

and students listen to each other and cooperate actively to complete the final project. ICT generates new learning contexts and in addition, can be used to represent phenomena by means of models and simulations that enable the abstract to be visualized<sup>9</sup>.

#### **Relevant experiences at the institution with ICT use (teaching and/or research)**

Answers refer to a series of resources and methods which can be grouped into: (a) Electronic clinical history (for healthcare, research, extension); (b)

Institutional repositories: teaching materials, news, documents (e.g. Universidad Mayor, Chile<sup>10</sup>); (c) Management support: enrolment, formation of student groups, evaluation results; (d) Teaching support: questionnaires, tests, videos, photographs, google doc, webquest, moodle platform, youtube, live clinical procedures transmitted in real time, virtual training classrooms with teacher forums; postgraduate distance courses; (e) Communication: chat, forums, e-mails, social networking, blogs, tutoring, presenting theses by videoconference; (f) Groups: telehealth, teleconsultation (second opinion), web conferences for discussing clinical cases (teachers and students), video conferences among several universities; projects with researchers from different cities; virtual professorship (international relations and regional integration).

## DISCUSSION

Considering the exploratory character of this study, the results can be used as a basis to reach a consensus on a set of ICT indicators that would be comparable on a regional level and could be used as input to unify the design and implementation of ICT experiences, both in teaching and in dental care. ICT is a tool for learning and teaching, representing an opportunity as well as a challenge. We propose that ICT could be instrumental in improving the learning and teaching process, without intending to assign any intrinsic transformational capacity to ICT alone. Latin America and the Caribbean have been at the cutting edge in recent years, with the fastest growth rates in the world for incorporating technology and connectivity (IDB, 2012)<sup>11</sup>, even though there is still a long way to go to ensure fair, universal access. To date, it has not been easy to connect this huge investment and progress to better and fairer development, or, for educational systems, to better student learning outcomes. The ECLAC report Social Political Series, N° 171<sup>12</sup> states that the design and implementation of ICT policies in the different countries in the region vary widely. In general, it can be said that countries are better at designing policies than at implementing them. Outstanding countries are Mexico, Panama, Uruguay and Cuba, which have relatively high rates of ICT policy definition and implementation. In contrast, Bolivia, Colombia, Paraguay and Ecuador tend to have low policy definition and implementation rates. Finally,

Argentina, Chile, Costa Rica, El Salvador, Nicaragua, Peru and Dominican Republic have higher rates of definition than implementation. This reflects a scenario in which many countries have the necessary definitions but implementation is pending. According to the abovementioned report, although the most likely reason for this situation is lack of resources to invest in implementing policies, it could be assumed that in some cases this lack is added to the inherent difficulty in implementing these initiatives, i.e., lack of institutional capacity to put designs into practice. Various authors and international agencies<sup>13-15</sup> recognize the importance of certain conditions of ICT context, access and use for producing concrete impact.

In agreement with the above, the results of this study show that most universities in Latin America have made progress in ICT infrastructure and in providing Internet resources and access. However, there is a need to improve teacher training in order to increase the use of ICT in teaching, especially in graduate courses, where ICT is mainly used to store subject materials, and in interactive and/or distance teaching, which is currently limited to optional or postgraduate courses. Analysis of university curriculum and ICT implementation in different areas and/or subjects shows that there is a variety of resources. The field of pathology is outstanding in several universities in the region, with innovations in teaching method (microscopy) and services (exam results). There is also relevant use of equipment in the field of imaging, with results transforming clinical and paraclinical dentistry practice. Many teachers and researchers relate the difficulties in using ICT to "lack of resources" and "lack of training" (Table 5). The 2012 SITEAL report<sup>16</sup> (System of Educational Trends in Latin America) The Digital Divide in Latin America, says that despite the relevance of Internet use for accessing knowledge in a globalized world, Internet access in Latin America is not yet democratic and access opportunities are very uneven: in Bolivia, El Salvador, Guatemala, Honduras and Paraguay, less than 5% of the total population has Internet access, while in Costa Rica, Chile, Brazil and Uruguay, 19 to 30% has Internet access. Latin American student home Internet access varies considerably by country. Students in Guatemala, Honduras, Bolivia and Paraguay have the least home Internet access (2 to 4%). Uruguay has the best relative situation,

with approximately 4 out of 10 students having home Internet access. In Brazil, Chile and Costa Rica 1 out of 4 students has home Internet access. Thus, according to the country where they live, some students have a likelihood up to 17 times higher of Internet access at home than their peers in other countries. University student Internet skills are variable and seem to depend on public policies at primary and secondary school. The abovementioned document from the Regional Bureau for Education in Latin America and the Caribbean (OREALC/ UNESCO Santiago 2013) says that new generations experience the omnipresence of digital technology intensely, to the point that it might even be modifying their cognitive skills. Indeed, these are young people who have not known the world without Internet, and much of whose experience is mediated by digital technology. Some people claim that these youths are developing distinctive skills such as acquiring much information outside teaching centers, taking decisions rapidly and being used to receiving almost instantaneous responses to their actions, having surprising multitasking ability, being highly skilled in multimedia and apparently learning in a different way. Teaching centers thus face the need to innovate in pedagogical methods if they wish to attract and inspire new generations. Are current pedagogical methods useful for motivating them? Activities and pace need to be adapted to the attitudes and features of new students who are used to accessing digitalized information, not only information on paper; enjoying moving images and music, in addition to text; are comfortable multitasking; and learn by processing discontinuous rather than linear information.

At dental offices and clinics, use of ICT resources has provided various benefits for dentists in areas such as management, diagnosis and professional marketing. This study found that high percentage of respondents (96.19%) has a PC at the dental office or service, especially for administration and patient databases. Current technology is powerful enough to provide real benefits to dental practice, with increasing importance of high-tech equipment; digital cameras and video cameras, intraoral cameras, digital imaging equipment, scanners, 3D technology, digital printing and laser application are all relevant resources for diagnosis, planning and treatment evaluation. However, these ICT resources are used little at dentists' offices except for those who have a

postgraduate degree (Tables 6 and 7). In endodontics, apex locators have increased accuracy in measuring root length and perfected canal filling. In orthodontics and surgery, ICT enables prior analysis of the result of a therapeutic plan and improvement of patient communication and understanding. In periodontology, tissue bioengineering has impact on regenerative therapies by enabling reconstruction of tissues damaged or destroyed as a result of disease, and aims to develop tissues similar to tooth, bone, mucosa and skin by means of regenerative cells.

### ICT innovations highlighted in this study:

**1. Cyclops Group (Brazil)**<sup>17</sup>. Development of telemedicine technology for Public Health, especially applied to remote contexts where there is broadband communication. Thus in Santa Catarina Province (Brazil), this group set up a platform to support diagnosis in cooperation with the provincial government.

**2.** The Ministry of Health in Brazil, in the context of the Single Health System (Sistema Único de Saúde, SUS), is developing two projects using ICT:

**2.1. The Telehealth Program**<sup>18</sup> for distance learning for professionals in family healthcare teams. Healthcare centers are connected via Internet to university academic teams, which support local decision making through formative second opinions.

**2.2. Universidade Aberta (UNASUS)**<sup>19</sup> provides ongoing education and training for workers in the Single Health System by connecting family healthcare teams to specialists at universities through videoconferencing.

**3. Electronic clinical history.** Although 81.43% of professionals in the region answered they do not use electronic clinical history, there is nevertheless a positive trend among researchers in the region towards creating and executing projects using it in order to improve information and knowledge exchange among clinics, universities, basic healthcare units, etc. Three of them have a high degree of implementation:

**3.1. Project REDIENTE**<sup>20</sup>, **Uruguay.** Clinical record in an online-accessible database, with national vocation and local management by dentists at their individual offices or institutions. Patients keep a card with a copy of their clinical history, which connects all events in a coherent documentary thread. REDIENTE enables epidemiological studies, evaluation of dental

care quality, and follow-up, and respects laws on patient data privacy protection.

**3.2. Project DENTSIO<sup>21</sup>, Colombia.** An application designed exclusively for iPad, to enter and manage patients' clinical histories quickly, easily, economically and intuitively. DENTSIO administers dental practice using dental record data, images, X-rays, etc.

**3.3. Project mobile phones<sup>22</sup>, Brazil.** A multi-center research project conducted experimentally by a group of professionals from Healthcare Centers with the aim of improving the quality and efficiency of services provided by the Basic Healthcare Units. Cell phones store information as mobile electronic records. They serve as a tool to support dental care by accessing patient background or other information, and are also able to cross-check data.

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This study provides perspectives for various lines of action with the aim of increasing exchange of experience, knowledge and tools among universities, services and other institutions in the different countries in the IADR Latin American Region, supporting research, establishing a permanent communications network among LAR/IADR divisions and sections, and promoting networking opportunities among researchers with innovative works on similar subjects. With regard to support for teaching dentistry in the region, it shows the need for universities to invest in ICT resources and to train teachers, students and officials in ICT resources and methodologies. It also shows the need to promote distance education by perfecting extension services and healthcare team training in distant areas in order to improve the quality of dental care and thereby community health.

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# CRANIOFACIAL PAIN CAN BE THE SOLE PRODROMAL SYMPTOM OF AN ACUTE MYOCARDIAL INFARCTION. AN INTERDISCIPLINARY STUDY

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## ABSTRACT

We recently found craniofacial pain to be the sole symptom of an acute myocardial infarction (AMI) in 4% of patients. We hypothesized that this scenario is also true for symptoms of prodromal (pre-infarction) angina. We studied 326 consecutive patients who experienced myocardial ischemia. Intra-individual variability analyses with respect to ECG findings and pain characteristics were performed for those 150 patients who experienced at least one recurrent ischemic episode. AMI patients ( $n=113$ ) were categorized into two subgroups: "abrupt onset" ( $n=81$ ) and "prodromal angina" ( $n=32$ ). Age, gender and risk factor comparisons were performed between groups. Craniofacial pain constituted the sole prodromal symptom of an AMI in 5% of patients. In those who experienced two ischemic episodes, women were more likely than men to experience craniofacial pain in both

episodes ( $p<0.01$ ). There was no statistically significant difference between episodes regarding either ECG findings or the use of the two typical pain quality descriptors "pressure" and "burning".

This study is to our knowledge the first to report that craniofacial pain can be the only symptom of a pre-infarction angina. Craniofacial pain constitutes the sole prodromal AMI symptom in one out of 20 AMI patients. Recognition of this atypical symptom presentation is low because research on prodromal AMI symptoms has to date studied only patients with chest pain. To avoid a potentially fatal misdiagnosis, awareness of this clinical presentation needs to be brought to the attention of clinicians, researchers and the general public.

**Key words:** Myocardial infarction, myocardial ischemia, facial pain.

## EL DOLOR CRÁNEO-FACIAL PUEDE SER EL ÚNICO SÍNTOMA PRODRÓMICO DE UN INFARTO AGUDO DE MIOCARDIO. ESTUDIO INTERDISCIPLINARIO

### RESUMEN

En un estudio previo encontramos que un dolor en la región cráneo-facial puede ser el único síntoma de un infarto agudo de miocardio (IAM) en el 4% de los casos. En el presente trabajo la hipótesis fue que este escenario es cierto también para la angina pre-infarto o angina prodrómica.

En el estudio se incluyeron 326 pacientes consecutivos con isquemia cardiaca sintomática. Se realizó un análisis intra-individual con respecto a características del dolor y hallazgos electrocardiográficos en los 150 pacientes que presentaron episodios recurrentes de isquemia cardiaca. Los pacientes con infarto agudo se categorizaron en dos grupos: "comienzo abrupto" ( $n=81$ ) y "angina prodrómica" ( $n=32$ ). Se realizaron comparaciones entre grupos con respecto a edad, género y factores de riesgo cardiovasculares. El dolor en la región cráneo-facial constituyó el único síntoma prodrómico (pre-infarto) de un IAM en el 5% de los casos. En aquellos pacientes que experimentaron dos episodios isquémicos, las mujeres tuvieron

una mayor prevalencia de dolor cráneo-facial en los dos episodios ( $p<0.01$ ). No se detectaron diferencias estadísticas entre episodios con respecto a hallazgos electrocardiográficos o al empleo de los descriptores verbales del dolor de origen cardiaco "opresivo" y "quemante".

Este es el primer estudio de investigación en documentar que el dolor en la región cráneo-facial puede ser el único síntoma de una angina pre-infarto. En efecto, esto ocurre en uno de cada 20 casos de IAM. El reconocimiento de esta presentación clínica es baja debido a que históricamente los criterios de inclusión de los estudios de angina pre-infarto incluyeron únicamente pacientes con dolor de pecho. Para evitar el error diagnóstico con consecuencias fatales para el paciente, es importante que esta información llegue tanto a los clínicos como al público en general.

**Palabras clave:** Infarto de miocardio, isquemia cardiaca, dolor facial.

## INTRODUCTION

Coronary disease is the leading cause of death in developed countries, but its clinical and epidemiological characteristics are not fully understood<sup>1</sup>. Craniofacial pain as the sole symptom of myocardial ischemia has been overlooked as a research topic until recently, when we revealed a prevalence of this potentially fatal condition of 6% among angina patients<sup>2</sup>. We also found craniofacial pain to be the sole symptom in 4% of acute myocardial infarction (AMI) patients. Application of this percentage to the number of coronary heart disease patients in the US<sup>3</sup> indicates that more than 28,000 patients per year might experience craniofacial pain as the sole symptom of an AMI. The corresponding number for the UK would be 6,800<sup>4</sup> and for Sweden 750<sup>5</sup>. The risk of missed diagnosis and death in AMI patients without chest pain is high<sup>6-9</sup>.

Overlooking variation in symptoms of a medical condition is likely to affect the results of decision-making models<sup>10</sup>. Most studies on variation in symptom presentation during myocardial ischemic episodes, including AMI, have been based on inter-individual comparisons. Only a few studies have focused on intra-individual variability and they have mainly been limited to ECG findings regarding the frequency and duration of transient ischemic episodes<sup>11</sup>, alterations of autonomic nervous activity during angina<sup>12</sup> and the intra-individual variability in plasma levels of markers of cell damage<sup>13</sup>. Intra-individual variation in the presentation of craniofacial symptoms during recurrent acute ischemic episodes has not been investigated. Prodromal (pre-infarction) angina constitutes one or more acute myocardial ischemic episodes prior to an AMI, which can occur several hours, weeks or months before an AMI<sup>14,15</sup>. Prodromal angina was shown to be a strong predictor of improved survival and limited infarct size<sup>16,17</sup>. The physiological mechanism underlying the protective role of prodromal ischemia is known as “ischemic preconditioning”<sup>18</sup>. It was previously assumed that the window of protection of the preconditioning mechanism lasts only for a few days<sup>19</sup> but a recent clinical study suggests that it can last for weeks<sup>20</sup>. The inclusion criteria for clinical research on prodromal angina have specified patients with chest pain/discomfort with or without other symptoms<sup>12,16,17,21-24</sup>.

The possibility of prodromal angina presenting as craniofacial pain alone has so far not been addressed. The aim of this study was to elucidate the prevalence of craniofacial pain as the sole symptom of prodromal angina and to analyze the intra-individual variations of pain characteristics and ECG findings in patients who experienced an AMI with prodromal symptoms.

## MATERIAL AND METHODS

### *Study populations*

Patients with a verified episode of myocardial ischemia were derived from 404 subjects who were consecutively admitted with signs and/or symptoms of myocardial ischemia to three cardiology units in Montevideo, Uruguay. Twenty patients could not be interviewed due to death or prompt dismissal for follow-up treatment elsewhere. The remaining 384 patients were examined and interviewed. Patients were excluded when myocardial ischemia was not verified (n= 25), craniofacial pain was due to a verified non-cardiac origin (n=18), ischemia was asymptomatic (n=9) or the patients had severe heart failure (n=3), psychiatric disorders or confusion (n=3). A total 326 patients met the criterion of having symptomatic myocardial ischemia, with or without AMI. Of these, 176 patients experienced only one symptomatic episode of myocardial ischemia, while 150 patients experienced at least two episodes within five months of each other. In the latter group of 150 patients, the last two episodes of symptomatic myocardial ischemia were included in the intra-individual variability analyses.

Two AMI subgroups were defined according to previous definitions<sup>16,17</sup>: “AMI with abrupt onset” and “AMI with prodromal angina”. Fig. 1 illustrates patient subgroups with information on gender and age. Sample size calculations were made using the OpenEpi program. A minimum sample size of 73 AMI patients was needed to estimate the prevalence of craniofacial pain as the sole symptom of prodromal angina.

### *Data collection and analysis*

Data were collected on demographic details, health history, risk factors, ECG findings and pain characteristics for each ischemic episode according to the methodology described in detail in our previous reports<sup>2,25</sup>. Myocardial ischemia and AMI were diagnosed by cardiologists according to

the American College of Cardiology definitions. Both ECG changes and biochemical marker evidence were analyzed. The locations of ECG findings (ST-segment elevation or depression) were categorized as anterior (leads V1 to V4), inferior (leads II, III, aVF) and lateral (leads I, aVL, V5 to V6) according to acknowledged criteria<sup>26</sup>. Ischemia related symptoms that were experienced within five months prior to the occurrence of an AMI were regarded as prodromal<sup>14,15</sup>.

Age, gender and risk factor comparisons were performed between AMI groups. Intra-individual comparison was carried out in the patients with prodromal angina with respect to ECG findings, pain characteristics and effect of physical activity on pain.

### Statistical analysis

The Wilcoxon test was used to compare mean ages between groups. Univariate chi-square analyses were used to assess gender differences between groups. A multivariate logistic regression model was used to assess possible associations between the presence of prodromes and craniofacial pain (dependent variables) and risk factors, age and gender.

McNemar's test was used to assess the significance of the differences between intra-individual episodes when the variable was dichotomous (e.g. AMI, pain relief at rest, etc). For intra-individual analysis of multinomial variables (e.g. quality of pain, site of ischemia) the Marginal Homogeneity test was used. The "coin" and "stats" packages of the R software were used to perform the statistical analysis<sup>27,28</sup>. The Wilcoxon signed-ranks test was used to analyze intra-individual variations in pain intensity.

### Ethics approval

The Ethics Committees at the Universidad de la República and the Hospital Central de las Fuerzas Armadas, Uruguay, approved the study protocol. Informed consent was obtained from each patient included in the study.

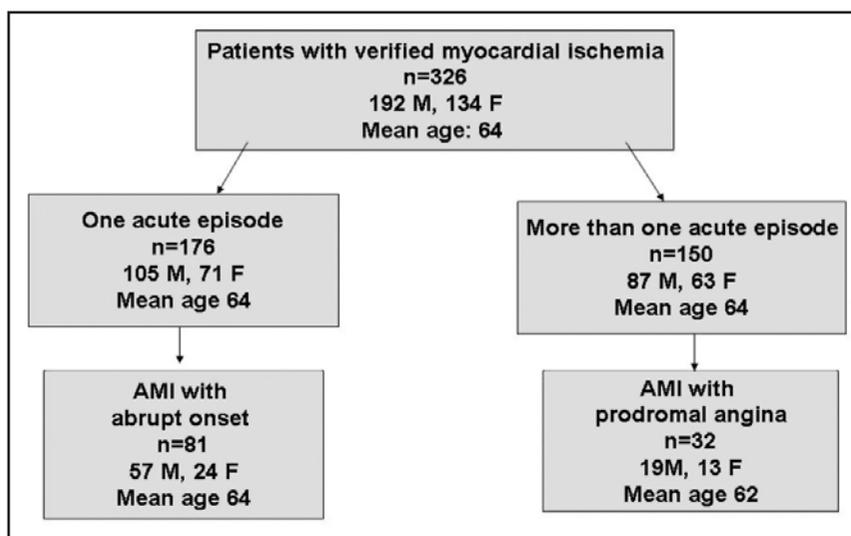


Fig. 1: Flowchart of patients and subgroups with information on age and gender. AMI: Acute myocardial infarction.

## RESULTS

### *Craniofacial pain as the sole prodromal symptom of an acute myocardial infarction (AMI).*

AMIs were experienced by 120 patients, 81 from the "AMI with abrupt onset group" and 32 from the "AMI with prodromal angina group", with seven patients being excluded as they suffered recurrent AMIs.

Pain in the craniofacial area constituted the sole prodromal symptom of an AMI in 5% of patients (three men, three women, mean age 68 years). The pain locations reported by these patients were throat, right jaw, left jaw, left temporomandibular joint/ear, and mandibular molar teeth bilaterally.

All six patients with craniofacial pain as the sole prodromal symptom reported a difference in pain distribution between episodes. During the AMI all these patients again reported craniofacial pain but five of them also developed chest pain and one had back pain in addition to craniofacial pain. Only one of their clinicians had an early suspicion of a possible cardiac source of the prodromal facial pain. Five of these six patients reported that exercise aggravated the symptoms and that rest alleviated the pain. All six patients used the quality descriptors "pressure" and/or "burning" to describe the prodromal pain in the craniofacial areas. Prodromal craniofacial pain in combination with typical anginal locations (chest, arms, shoulders, etc) was experienced by another 12% (n=14) of the AMI patients.

### ***Intra-individual symptom variability between myocardial ischemia episodes***

Of the 150 patients who experienced at least two episodes of myocardial ischemia, four with prodromal angina had not sought medical care for their prodromal symptoms and were discarded from the intra-individual variation analysis of the location of ECG changes. The combination of pain quality descriptors reported by the patients generally changed significantly between the two ischemic episodes ( $p=0.01$ ), although the quality descriptors “pressure” and “burning” remained the same. Pain intensity was higher during the AMI episodes ( $p<0.001$ ). The location of ischemia in the heart did not vary significantly between episodes, according to the ECG findings ( $p=0.32$ ). Women were more likely than men to experience craniofacial pain in both ischemic episodes ( $P<0.01$ ). No additional age or gender associations were found in the intra-individual analyses.

### ***AMI with prodromal symptoms vs.***

### ***AMI with abrupt onset.***

No age or gender difference was found between groups. Patients in the prodromal angina group were more likely to be obese ( $p=0.026$ ) and tended to have a family history of cardiovascular disease ( $p=0.05$ ).

## **DISCUSSION**

Prodromal angina and ischemic preconditioning has been shown to protect the myocardium, slow the process of cell death, limit the infarct size, and improve patient prognosis<sup>18,22,29</sup>. Prodromal angina pectoris, specifically, is a strong predictor of a smaller size infarct and lower mortality<sup>17</sup>; a meta-analysis showed that the occurrence of prodromal angina during the 24 hours before the onset of an AMI was associated with a 39% reduction of in-hospital mortality<sup>30</sup>. Studies of prodromal angina and its protective role have been largely limited to patients with chest pain symptoms and have not considered patients with prodromal craniofacial pain as the only symptom of cardiac ischemia<sup>12,16,17,21-23</sup>.

Our study revealed that one in 20 AMI patients reported pain in the craniofacial structures as the only prodromal symptom. Considering that patients without chest pain during an AMI run a greater risk of death<sup>7,31</sup> and some of them therefore never reach the emergency room, it is highly plausible that our

findings on prevalence constitute an underestimation. It was noticeable that patients with craniofacial pain as the only pre-infarction alert for the patient and the clinician consistently developed additional typical anginal symptoms during the AMI. It may be that the pathological changes occurring during the AMI episode released several chemical mediators and activated more nerve afferent fibers, inducing changes in the pain pattern.

It is generally believed that atypical presentation of an AMI is a well-known problem but clinical awareness appears low. Ninety percent of nurses and 100% of physicians from an Emergency Care Unit in North America stated that they had experience with atypical presentations of an AMI but 75% of nurses and 80% of clinicians reported that they do not look for atypical symptomatology in female patients<sup>32</sup>. Furthermore, during this same interview, pain in the craniofacial region was not cited by physicians or nurses as a symptom that would point to an AMI. In line with these findings, neither the patients with craniofacial pain as the sole prodromal symptom nor their clinicians recognized the cardiac origin of the pain in five out of the six patients included in our study. These findings point to the need for educational initiatives both for the general public and clinicians regarding this atypical AMI prodromal presentation.

In a previous study we showed that the quality descriptors “pressure” and “burning” were statistically associated with craniofacial pain induced by myocardial ischemia, with or without an AMI<sup>25</sup>. This study confirms that these pain descriptors were used also by patients in their reporting of pre-infarction craniofacial symptoms. The location of the prodromal craniofacial pain is also in line with our previous report regarding craniofacial pain locations during myocardial ischemia<sup>2</sup>.

Orofacial location of pain is likely to cause the patient to seek a dentist, a general physician or an otorhinolaryngologist. The clinical information presented in this study regarding pain quality, aggravation by exercise and pain relief during rest, combined with the prevalence of these symptoms, should alert the clinician to consider the possibility of an AMI prodromal condition; failure to consider this possibility could have potentially fatal results.

This study is to our knowledge the first to report that craniofacial pain can be the only symptom precursor of an AMI. Its prevalence points to a

significant number of patients per year being at risk of misdiagnosis. The risk of overestimation of the prevalence is considered negligible since the sample size is well over the statistical calculations for minimum size. On the contrary, there is a risk of underestimation because a significant number of patients with atypical prodromals, i.e. those with craniofacial pain alone, never sought medical care and/or died before reaching the hospital.

In conclusion, craniofacial pain constituted the sole prodromal AMI symptom in one out of 20 AMI

patients in our study. The explanation for the low recognition of this atypical symptom presentation is most likely that research on prodromal AMI symptoms has to date focused only on patients with chest pain as an inclusion criterion, regardless of other symptoms. To avoid a potentially fatal misdiagnosis, awareness of this clinical presentation of prodromal craniofacial pain alone needs to be conveyed to clinicians and the general public. Future research on prodromal AMI symptoms should also include patients without chest pain.

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## ASSOCIATION BETWEEN PERIODONTAL DISEASE AND ENDOTHELIAL DYSFUNCTION IN SMOKING PATIENTS

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### ABSTRACT

Over the past two decades, there has been increasing interest in the impact of oral health on cardiovascular disease, particularly regarding the effects of chronic infections such as periodontitis on the endothelium. The aim of this study was to evaluate in healthy smokers whether there are any significant differences in the frequency of endothelial dysfunction between subjects with chronic moderate to severe periodontal disease and periodontally healthy subjects. An observational cross-sectional study was conducted. The target population was adults older than 40 years of age. Blood tests were performed to determine values of CBC, glycaemia, total cholesterol, HDL-C, and LDL-C. Periodontal examinations and probing were conducted with

a Florida Probe<sup>®</sup>, and standardized procedures were used to measure flow-mediated dilation. Out of 150 subjects [69 male (46%) and 81 female (54%)], 75 (50%) had chronic periodontitis. The mean value for baseline flow-mediated dilation was 4.04% and the mean value for final flow-mediated dilation was 4.66%, with a 0.62% mean difference showing a statistically significant increase ( $p < 0.001$ ). This study found no significant difference in the flow-mediated dilation values between periodontally healthy subjects and those with periodontitis, in contrast to the literature, which suggests a negative impact of periodontal disease on endothelial function.

**Key words:** Atherosclerosis, endothelium, vasodilation.

## ASOCIACIÓN ENTRE ENFERMEDAD PERIODONTAL Y DISFUNCIÓN ENDOTELIAL EN PACIENTES FUMADORES

### RESUMEN

Durante las últimas dos décadas ha venido incrementándose el interés acerca del impacto de la salud oral, sobre las enfermedades cardiovasculares. El objetivo del estudio fue evaluar si en pacientes sistémicamente sanos y con tabaquismo, se encontraban diferencias significativas en la frecuencia de disfunción endotelial entre personas con enfermedad periodontal crónica de moderada a severa y personas con salud periodontal. El diseño empleado fue un estudio observacional de corte transversal. La población blanco pacientes adultos mayores de 40 años de edad. Se tomaron muestras de sangre para obtener los valores de cuadro hemático, glucemia, colesterol total, cHDL y cLDL. Se realizó el examen periodontal diligenciando el anexo de periodoncia de la Facultad de Odontología y de acuerdo con el instructivo. El sondaje se realizó en todos los dientes presentes en boca

utilizando la sonda electrónica periodontal (Florida Probe<sup>®</sup>). La técnica y procedimientos para la vasodilatación mediada por flujo utilizados fueron los estandarizados internacionalmente. De los 150 participantes, 69 eran hombres (46%) y 81 mujeres (54%) con promedio de edad de 50.2 años. De estos, 75 pacientes (50%) correspondían al grupo de periodontitis crónica. Al evaluar la vasodilatación mediada por flujo el promedio inicial encontrado fue de 4.04% y el final fue de 4.66%, con un 0.62 % de aumento en promedio ( $p < 0.001$ ). Se puede concluir que a pesar de la evidencia en la literatura que sugiere que la enfermedad periodontal impacta negativamente la función endotelial, medida por vasodilatación mediada por flujo, el presente estudio no encontró una diferencia significativa.

**Palabras clave:** aterosclerosis, endotelio, vasodilatación.

### INTRODUCTION

Over the past two decades there has been increasing interest in the impact of oral diseases, (especially periodontitis) on cardiovascular diseases. Cardio-

vascular disease appears today as the main cause of premature death in both developed and developing countries, and as a common disease in adult populations<sup>1</sup>.

The pathophysiological basis of coronary disease is atherosclerosis, which has been defined as a progressive disease that consists of the thickening of the inner and medial layers of arteries, which may be triggered by a cellular reaction as a response to an endothelial injury.

The pathophysiological basis of all proatherogenic changes thus lies in the endothelium. Endothelium physiology, capacity to dilate, response to injury, and protection from coagulation and rupture are all significantly deteriorated by the process of atherosclerosis. This is known as endothelial dysfunction.

Chronic infectious processes such as periodontal disease may exert a proatherogenic effect by acting at a systemic level or at a local level on the vascular wall. The presence of multiple positive serologies (infectious burden) would significantly increase the risk of vascular disease. There is a significant relationship between the number of infectious pathogens to which an individual has been exposed and the extent of the atherosclerotic lesion. Several studies have found a positive association between infectious burden and prevalence of cardiovascular events. Moreover, the risk of vascular death increases with the number of infectious pathogens, especially in patients with advanced atherosclerosis<sup>2,3</sup>.

The literature is still very controversial regarding diseases such as periodontal and cardiovascular diseases, which share some risk factors such as smoking. For example, Beck et al. conducted a study in which they analyzed patients with cardiovascular risk who were smokers or non-smokers. A complete periodontal evaluation was performed and it was found that periodontal disease was not significantly related to cardiovascular disease, regardless of smoker status<sup>4</sup>.

Based on the aforementioned context, the goal of this study was to evaluate in healthy smokers whether there are any significant differences in the frequency of endothelial dysfunction between subjects with chronic to moderate periodontal disease and subjects with normal periodontal health.

## MATERIALS AND METHODS

An observational, cross-sectional study was conducted. The population was made up of adult patients, 40 years of age or older, living in Bogotá, who were receiving healthcare services at the Hospital Universitario de San Ignacio and the

Dental clinics at the Dental School in the Pontificia Universidad Javeriana.

Comparisons were made between subjects older than 40, smokers (5 to 15 cigarettes a day), who had a mild – severe chronic periodontitis diagnosis (PD) and smokers (5-15 cig/day) older than 40, who were not diagnosed with PD. Subjects with diabetes, high blood pressure or who took any drugs that modify endothelial function (aspirin, statins, ACE inhibitors, ARBs, calcium antagonists, sildenafil, L-arginine and antioxidants, corticosteroids or estrogens) were excluded from the study. For both groups, PD was diagnosed according to the Armitage classification (1999).

Sample size was calculated using data from the CANDEV study (López PJ, 2001), with the software TAMAMU 1.1<sup>®</sup>. The calculated sample was 150 patients: 75 with periodontitis and 75 in periodontal health.

## METHOD

Each patient that met the inclusion criteria was asked to sign a written Consent Form. Blood samples were taken to determine values of CBC, glycaemia, total cholesterol, HDL-C, and LDL-C, which were processed at the Hospital Universitario de San Ignacio. Patients were required to fast for at least 12 hours before sample collection.

Periodontal examination was conducted according to the Dental school formats, and each tooth in the mouth was probed using a periodontal electronic probe (Florida Probe<sup>®</sup>). Once the subject was identified and assigned to one of the two groups, the FMD was performed after measuring blood pressure and calculating arterial index. Internationally standardized techniques and procedures for FMD were used and the results are provided in millimeters. Since smoking is a significant risk factor for endothelial dysfunction, smokers were selected in order to increase the probability of finding dysfunction; otherwise the sample size needed would have increased significantly. There was an accepted risk that the strong effect of smoking could minimize the impact of periodontal disease, but the main comparison between patients with and without periodontitis, within the moderate risk produced by smoking, can show differences if they are significant enough to be detected.

The endothelial function was evaluated by means of the non-invasive Flow Mediated Dilation (FMD)

test of the brachial artery; ultrasound images were obtained from the brachial artery in the cubital fossa using a 14 MHz transducer. Initial images were obtained after 10 minutes' rest in supine position. FMD was determined as the change in arterial diameter responding to a reactive hyperemia (final image vs. initial image). Reactive hyperemia was induced by inflating a pneumatic cuff around the arm (beside the segment where the image was to be taken) up to a pressure of 200 mmHg for 5 minutes. Images were taken 2 minutes after deflating the cuff (final image). Percentage of FMD through the brachial artery was calculated according to the following formula:

$$\%VMF = ((\text{Average Final score} - \text{Average Initial score}) / \text{Average Initial score}) * 100\%$$

The reference value in the literature is 4%. Any result having a dilation percentage equal to or less than 4 was considered as endothelial dysfunction. According to established regulations (Resolution 8430, Colombian Ministry of Health) and the CIOMS, the study was classified as minimal risk research. Approval was obtained from the IRBs at Schools of Dentistry and Medicine at Javeriana University.

### STATISTICAL ANALYSIS

Descriptive data were obtained for demographics, periodontal evaluation results and FMD, through means, medians, ranges, standard deviations and 95% confidence intervals. FMD was reported in absolute values, percentage of change, and in categorical terms as normal or abnormal, using international standards. In the analysis of outcomes, odds ratios were used for the analysis of likelihood of having dysfunction between both groups and controls. Adjustments were made for age and sex. T-Student or chi-square tests were used for comparisons between groups, when appropriate. A difference that had a value of  $p < 0.05$  (two tails) was considered significant.

### RESULTS

Out of the 150 participants, 69 were male (46%) and 81 female (54%), with mean age 50.2 years. Of these, 75 patients (50%) had chronic periodontitis. They smoked an average 10.28 cigarettes/day, with a minimum of 5 and a maximum of 40. The average laboratory values found for these patients were:

glucose 95.8 mg/dL, total cholesterol 202 mg/dL, triglycerides 147.4 mg/dL, HDL-C 42.8 mg/dL and LDL-C 129.5 mg/dL. Regarding exercise, 5.3% exercised at least 30 minutes, 5 times a week; 22% exercised between 1 and 4 times a week, 5.3% exercised less than once a week, 24% did not exercise but had physical activity and 43.3% neither exercised nor engaged in physical activity.

Average initial flow-mediated dilation (Initial FMD) was 4.04 and average final flow-mediated dilation (final FMD) was 4.66, with a difference of 0.62 average increase, which was found to be statistically significant ( $p < 0.001$ ). The comparison of the initial and final percentage changes showed 16.07% at the beginning and 16.97% at the end, with a difference of 0.89%, which was not statistically significant ( $p = 0.284$ ).

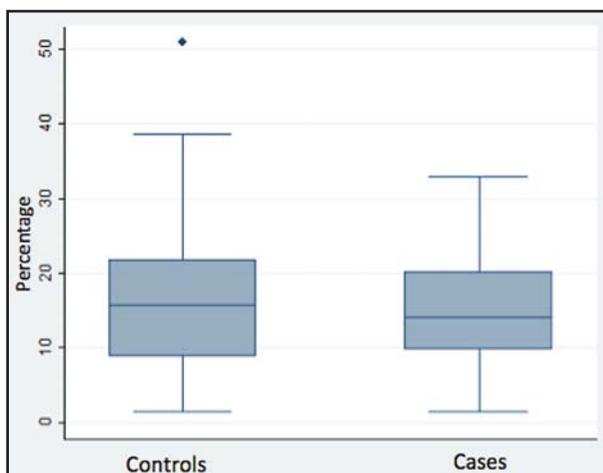
Analysis of variables according to sex showed a statistically significant difference for glucose, triglycerides, HDL-C, and Initial FMD and final FMD. With the exception of HDL-C, all values were higher in males (Table 1). A statistically significant difference according to gender, with less vasodilation response in women, was also observed. The analysis according to periodontitis showed that the mean age for the group with periodontitis was 49.85 years (S.D. 7.9) and for the periodontally healthy group 50.60 years (S.D. 7.09), with no statistically significant difference ( $p = 0.545$ ). No statistically significant difference was found in smokers between groups ( $p = 0.128$ ). Significant differences were found in exercise done less than once a week, favoring the periodontally healthy group, and in non-exercise but with physical activity, favoring the periodontal disease group (Table 2).

In the periodontally healthy group, mean initial flow-mediated dilation (Initial FMD) was 3.97%, and mean final flow-mediated dilation (Final FMD) was 4.57%, with 0.60% difference in average increase, which was statistically significant ( $p < 0.0001$ ). In the group with periodontitis, mean initial flow-mediated dilation (Initial FMD) was 4.10% and mean final flow-mediated dilation (Final FMD) was 4.75%, with 0.65% difference in average increase, which was also statistically significant ( $p < 0.001$ ). This shows that there was a significant increase in FMD in both groups, with an apparent greater change in the group with periodontitis.

Mean initial FMD was 4.10% for the group with periodontitis and 3.97% for the periodontally healthy

**Table 1: Variable distribution by gender.**

Variable		Female	Male	P value
		81	69	
Age ( $\bar{x}$ )		50.95	49.38	0.202
Number of cigarettes/day ( $\bar{x}$ )		10.28	10.28	0.991
Group	Periodontitis	37 (45.6%)	38 (55%)	0.326
	Periodontally Healthy	44 (54.3%)	31 (44.9%)	
Glycemia ( $\bar{x}$ )		91.14	101.4	0.000*
Cholesterol ( $\bar{x}$ )		206.14	197.2	0.111
Triglycerides ( $\bar{x}$ )		124.3	174.6	0.000*
cHDL ( $\bar{x}$ )		46.8	38.15	0.000*
cLDL ( $\bar{x}$ )		133	125.3	0.090
Initial FMD ( $\bar{x}$ )		3.69	4.44	0.000*
Final FMD ( $\bar{x}$ )		4.23	5.17	0.000*
% FMD ( $\bar{x}$ )		15.19	17.69	0.191
Endothelial Dysfunction	Yes	9 (11.1%)	1 (1.4%)	0.021*
	No	72 (88.8%)	68 (98.5%)	
Exercise 30 minutes 5 times a week (n)		3 (3.7%)	5 (7.2%)	0.471
Exercise 1 to 4 times a week (n)		11 (13.5%)	22 (31.8%)	0.010*
Exercise less than 1 time a week (n)		5 (6.17%)	3 (4.3%)	0.726
No exercise, but with physical activity		20 (24.7%)	16 (23.1%)	0.850
No exercise or physical activity		43 (51.8%)	23 (33.3%)	0.031*

*Fig. 1: Percentage of Vessel Dilation.*

group, with a difference of 0.13%, which was not statistically significant ( $p = 0.309$ ). This shows that the groups were comparable. Mean final FMD was 4.75% for the group with periodontitis and 4.57% for the periodontally healthy group, with a difference of 0.182%, which was not statistically significant

( $p = 0.209$ ), meaning that the responses in the groups were similar (Fig. 1).

The risk of developing endothelial dysfunction according to gender showed an OR=8.5 CI 95% (1.04 – 68.8) ( $p=0.0450$ ) for women.

## DISCUSSION

Endothelial dysfunction is the vascular event preceding atherogenesis and is caused by an early lesion in the endothelium. This deterioration in endothelium physiology constitutes the basic mechanism of the onset of atherosclerosis and is the pathophysiological basis of cardiovascular disease.

In addition to the traditional risk factors for cardiovascular disease, which are also related to endothelial dysfunction, such as smoking, age, hypercholesterolemia, hypertension and hyperglycemia, there are others such as excess weight, insulin resistance, inflammation and chronic infections, which can be detected by tests such as C-reactive protein and have been added as risk

**Table 2: Variable distribution by group.**

Variable		Periodontitis group	Periodontally healthy group	P value
		75	75	0.545
Age ( $\bar{x}$ )		50.6	49.85	0.128
Number of cigarettes/day ( $\bar{x}$ )		10.85	9.71	0.679
Glycemia ( $\bar{x}$ )		95.29	96.48	0.915
Cholesterol ( $\bar{x}$ )		202.3	201.7	0.530
Tryglicerides ( $\bar{x}$ )		151.8	143.0	0.200
cHDL ( $\bar{x}$ )		41.6	44.0	0.539
cLDL ( $\bar{x}$ )		130.9	128.1	0.310
VMF Inicial ( $\bar{x}$ )		4.10	3.97	0.209
VMF Final ( $\bar{x}$ )		4.75	4.57	0.721
% VMF ( $\bar{x}$ )		16.68	16.0	1.0
Endothelial Dysfunction	Yes	5 (6.6%)	5 (6.6%)	1.0
	No	70 (93.3%)	70 (93.3%)	
Exercise 30 minutes 5 times a week (n)		4 (5.3%)	4 (5.3%)	0.431
Exercise 1 to 4 times a week (n)		14 (18.6%)	19 (25.3%)	0.006*
Exercise less than 1 time a week (n)		0 (0%)	8 (10.6%)	0.000*
No exercise, but with physical activity		30 (40%)	6 (5%)	0.099
No exercise or physical activity		27 (36%)	38 (50.6%)	

factors<sup>5</sup>. Although men in this study showed more risk factors as glycaemia, cholesterol, tryglicerides and cLDL, women showed less initial and final FMD than men. It is important to take into account that exercise and smoking may have influence, either positive or negative, on this finding. Moreover, 9 women, but only 1 man was found to have endothelial dysfunction. These women had other potentially related factors such as smoking and periodontal status; however, conclusions cannot be drawn for any association because the sample is too small. A new study is currently underway to enlarge the sample and find more conclusive results.

This study analyzed 150 patients, 75 with periodontitis and 75 periodontally healthy, all smokers. The patients were healthy and comparable in age, smoking, blood sugar and cholesterol levels. Mean age was 49.85 years for the group with periodontitis and 50.60 years ( $p = 0.545$ ) for the periodontally healthy group. When smoking status was compared between groups, no statistically significant difference ( $p = 0.128$ ) was found. Smokers were selected on the basis that they are assumed to have some degree of basal endothelial dysfunction that might

be increased by adding another risk factor such as periodontitis. Since smoking is a strong predictor of endothelial dysfunction, only smokers were selected, in order to increase the chances of finding dysfunction. Smoking status may have masked a few of the possible differences, but the groups with and without periodontitis differed significantly in the factor to be evaluated.

Nevertheless, our results showed that of the 150 patients analyzed, all smokers, only 10 presented endothelial dysfunction (6.6%).

These results contrast with those of several studies, which have been emphatic in stating that smoking causes peripheral vascular endothelial dysfunction and that smokers have a greater impairment of endothelium-dependent vasodilation. Heffernan et al.<sup>6</sup> examined the association between endothelial function and exercise capability in chronic smokers versus non-smokers, concluding that FMD was significantly lower in smokers than in nonsmokers ( $8.9 \pm 0.9$  vs.  $12.6 \pm 0.7\%$ ,  $p < 0.05$ ).

Similarly, Wiesmann et al.<sup>7</sup> observed a reduction in brachial artery VMF in smokers compared to non smokers ( $7.5 \pm 2.7\%$  vs.  $15.5 \pm 2.0\%$ ,  $p = 0.03$ ),

which was related to impaired endothelium-dependent dilation. Barua et al.<sup>8</sup> reported that smoking was associated with decreased endothelium-dependent vasodilation and reduction in oxide production.

This study only found statistically significant differences in subjects who exercised less than once a week, in favor of the periodontally healthy group, and no exercise but physical activity favoring the group with periodontitis. Nevertheless, the two groups had identical endothelial dysfunction frequencies of 6.6% (5 periodontitis patients and 5 periodontally healthy patients). This contrasts with results reported in human studies in which strict exercise regimes under supervision improved endothelial function in coronary and brachial function. The low number of periodontal groups found with endothelial dysfunction may be responsible for the lack of differences, mainly due to lack of statistical power.

Heffernan et al.<sup>6</sup> reported a positive association for vasodilation and exercise for both non-smokers and smokers. Braga et al.<sup>9</sup> reported that exercise, even in estrogen deficiency conditions, can improve endothelial dependent vasodilation in rat aorta via enhanced nitric oxide (NO) bioavailability and reduced reactive oxygen (ROS) species levels. De Souza et al.<sup>10</sup> reported that endothelial dysfunction was greater in sedentary groups when comparing FMD of the brachial artery in sedentary young men, sedentary old men, young and old men in physical training.

This study found that when patients with periodontitis were compared to periodontally healthy patients, periodontitis and chronic infections did not impact or add a risk factor to endothelial dysfunction with OR = 1.0 95% CI (0.277 - 3.6) (p = 1.0).

These results are consistent with those reported by Aristizabal, Gomez et al.<sup>11</sup>, who in a systematic review of the literature found that there is little evidence for the direct relation of the periodontal disease with endothelial dysfunction in patients aged between forty and eighty. The relationship between periodontal disease and endothelial dysfunction was supported by the effect of periodontal treatment on endothelial dysfunction. The previous systematic review also reported that endothelial dysfunction was analyzed differently in studies by clinical, subclinical, and through biomarkers of systemic inflammation. They noted that it cannot be concluded that periodontal disease

is a risk factor for endothelial dysfunction. However, based on the findings reported in the selected articles it was concluded that intensive periodontal treatment in the long term (over six months) improved endothelial function; but the degree of improvement was not directly correlated with the change in inflammatory biomarkers.

Regarding the effect of periodontal treatment on endothelial dysfunction, the literature is controversial. There is evidence as reported by D' Aiuto et al.<sup>12</sup>; Blum et al.<sup>13</sup>; Tonetti et al.<sup>14</sup>, and Piconi et al.<sup>15</sup> in which the treatment of periodontal disease significantly improves endothelial function, and it was reported that better results were obtained with intensive periodontal therapy (antibiotic) than with basic periodontal therapy<sup>12-15</sup>. However, in contrast to the above, Li et al.<sup>16</sup> noted that periodontal treatment had a neutral effect on peripheral endothelial function. Aristizabal Gomez et al.<sup>11</sup> justified these controversial findings by the strict criteria for patient selection and the method for measuring endothelial function. Moreover, they considered that if patients had an underlying disease that predisposed to endothelial dysfunction, it was unlikely that they could achieve an immediate benefit on endothelial function related only to periodontal treatment. The analysis of the controversial results of this research could therefore be aligned with the issues raised by Aristizabal Gomez et al.<sup>11</sup> because in this study, patients were strictly selected, excluding other systemic diseases such as diabetes, hypertension or dyslipidemia, which may have been the reason why the results showed neither the presence of endothelial dysfunction even though patients were smokers, nor a difference in dysfunction between patients with periodontitis and periodontally healthy subjects.

## CONCLUSIONS

Despite the evidence in the literature suggesting that periodontal disease has a negative impact on endothelial dysfunction, as measured by FMD, this study found no significant difference between periodontitis and periodontally healthy subjects. The pathophysiology of the disease, however, suggests the rationale for further studies to show that chronic infection and inflammation caused by periodontal disease may disrupt endothelial function and become athero thrombotic risk factors for disease, and show that controlling periodontal disease may reduce the risk of atherosclerosis.

The findings may be explained by lack of statistical power, or they may point to racial or local differences in the weight of the influence of periodontal disease on the endothelium, or even that the effect exists but is so small that the results were difficult to detect in the study population.

Some other interesting explanations, to be further explored in future studies, include protective factors

related to demographic characteristics, or even that the international standards for endothelial dysfunction do not correctly validate in our population.

The results are a contribution to the understanding of the complex relationship between local chronic inflammation and endothelium function. Further studies are needed in the region, exploring the different risk and protective factors.

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## POST-BLEACHING SENSITIVITY IN PATIENTS WITH SICKLE CELL DISEASE

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### ABSTRACT

Sickle cell disease (SCD) is a monogenic disease that affects millions of people worldwide. This study analyzed the effectiveness of bleaching and tooth sensitivity after in-office bleaching in patients with SCD. Forty volunteers were randomly assigned to four groups of 10 patients each (five with the SCD and five healthy controls) and treated using in-office bleaching with 35% hydrogen peroxide and different light activation protocols. No statistically significant difference was

observed with relation to presence of tooth sensitivity, with or without use of a source of light for peroxide activation, and all bleaching therapies were effective, regardless of the technique employed and the presence/absence of sickle cell disease. The data showed that in-office dental bleaching is a viable alternative for improvement of oral esthetics for patients with SCD.

**Key words:** Esthetics, hydrogen peroxide, tooth bleaching.

## SENSIBILIDADE DENTÁRIA PÓS-CLAREAMENTO EM PACIENTES COM DOENÇA FALCIFORME

### RESUMO

A doença falciforme (DF) é a doença monogênica e afeta milhões de pessoas em todo o mundo. Este estudo analisou a eficácia do clareamento e a presença de sensibilidade dentinária após clareamento dental em portadores de DF. Quarenta voluntários distribuídos aleatoriamente em quatro grupos com diferentes fontes de luz foram submetidos à técnica de clareamento em consultório, utilizando peróxido de hidrogênio a 35%. Não foram encontradas diferenças estatisticamente significativas em relação à presença de sensibilidade,

com ou sem a utilização de uma fonte de luz para acelerar a ativação do peróxido e todos os clareamentos foram efetivos, independentemente da técnica utilizada e da presença/ausência de DF. Os dados mostraram que o clareamento dental em consultório, utilizando peróxido de hidrogênio a 35% é uma alternativa viável para melhoria da estética em pacientes com DF.

**Palavras-chave:** Clareamento dental, Estética, Peróxido de hidrogênio.

### INTRODUCTION

Sickle cell disease (SCD) is the most common monogenic disease in the world<sup>1</sup>, affecting millions of people. In Brazil, 20,000 to 30,000 people are estimated to have sickle cell disease, which tends to increase as a result of the high degree of miscegenation<sup>2,3</sup>.

The disease is caused by the replacement of glutamic acid with valine in the sixth position of the  $\beta$ -globin chain, resulting in abnormal cells referred to as hemoglobins S (HbS, as in sickle). When oxygen is absent or low, HbS molecules are polymerized and

lose their normal round morphology, shortening the mean life of red blood cells, causing vessel occlusion and infarction, and consequently leading to ischemia, pain, necrosis, dysfunction, permanent damage to tissues and organs, and chronic hemolysis<sup>4,5</sup>.

In 1973, life expectancy of patients with sickle cell disease was 14 years; at present, in patients under strict medical monitoring, life expectancy can reach approximately 70 years<sup>6</sup>.

As a result of the peculiarities of sickle cell disease, these patients require special attention during treatment and clinical management, requiring pre-

established protocols for procedures considered routine in dental practice<sup>7</sup>.

Tooth bleaching is a conservative method for dental structures and is therefore widely sought for the improvement of oral esthetics. Tooth sensitivity after bleaching is the side-effect most frequently reported by patients who have undergone bleaching<sup>8</sup>.

This study analyzed the effectiveness of bleaching, and tooth sensitivity after in-office bleaching, in patients with SCD. It was motivated by the growing interest in dental bleaching among patients with sickle cell disease and the scarcity of studies on bleaching efficacy and the behavior in this specific population regarding the procedure.

## MATERIALS AND METHODS

### *Ethical considerations*

This study was approved by the Research Ethics Committee of Universidade Estadual de Ciências da Saúde de Alagoas (protocol no. 949/08) and written informed consent was obtained from all study participants.

### *Selection of subjects*

This study enrolled forty volunteers aged 18 to 45 years, of both genders, with no caries, with vital teeth in the gap between first premolars of the maxilla and mandible and good periodontal health, who agreed to participate in the study and signed informed consent.

Exclusion criteria were patients who had used any bleaching agents within the past year, had deficient restorations, reported any tooth sensitivity, spontaneous or triggered by air spray, had taken any kind of analgesic or anti-inflammatory drugs within one month before bleaching, smokers and pregnant or lactating women.

Volunteers were randomly assigned to four groups of 10 patients each: five with sickle cell disease and five healthy controls.

### *Data collection*

All participants underwent in-office bleaching with 35% hydrogen peroxide (Whiteness HP Maxx, FGM Dental Products, Joinville, Brazil). The bleaching agent was applied three times, each session lasting 15 minutes. Before the bleaching procedures, they received prophylaxis with pumice and water in a rubber cup.

Group 1 was treated with a halogen light unit (photopolymerizer CL-K220, Kondortech, São Carlos, Brazil), and the bleaching agent was activated on each individual tooth for 30 seconds per tooth. In Group 2, a light emitting diode (LED) unit was used (Easy Bleach, Clean Line, Taubaté, Brazil), and activation also took place on each individual tooth, for 30 seconds per tooth. In Group 3, a LED/laser unit was used (Easy Bleach, Clean Line, Taubaté, Brazil), and peroxide was activated simultaneously on both arches, for 3 minutes. In Group 4, no light source was used to activate the bleaching agent.

Bleaching treatment efficacy was assessed using the Vitapan Classical shade guide (Vita-Zahnfabrik, Bad Säckingen, Germany). In this guide the colors are organized from B1 (lighter) to C4 (darker) and numbered from 1 (B1) to 16 (C4)<sup>9</sup>. The area for color matching was the middle third of the buccal surface of the central upper left incisor and it was evaluated by a duly calibrated examiner. Color was determined before and after bleaching.

Absence of tooth sensitivity was evaluated at the following times: during the first 5 hours, at 6-12 hours, 13-24 hours, 25-72 hours (2-3 days), 73-192 hours (4-8 days), and 193-240 hours (8-10 days). Each patient was also asked to describe whether tooth sensitivity was spontaneous or provoked. If provoked, patients were asked to report the factor that triggered sensitivity: heat, cold, speaking or other.

### *Statistical analysis*

Statistical analysis was performed using the BioEstat software, version 5.0 (Optical Digital Technology, Belém, Brazil). Tooth sensitivity data were analyzed by Fisher's exact test and effectiveness of bleaching data was analyzed by paired t test. Significance level was set at 5% ( $p < 0.05$ ).

## RESULTS

All bleaching therapies were effective, regardless of the technique employed and the presence/absence of sickle cell disease (Table 1, Fig. 1). Provoked and/or spontaneous sensitivity were experienced by 72.5% (29 out of 40) of the volunteers. However, it was absent in the following periods: 73-192 hours (4-8 days) and 193-240 hours (8-10 days).

**Table 1: Analytical results for color determined before and after in-office dental bleaching with 35% hydrogen peroxide.**

Technique	Halogen unit		LED		LED/laser		No light	
	SCD	No SCD	SCD	No SCD	SCD	No SCD	SCD	No SCD
Volunteers								
Mean Differences	3.0000	3.2000	3.2000	2.6000	3.8000	3.6000	3.4000	2.6000
Standard Deviation (SD)	1.2247	1.3038	1.3038	1.1402	1.3038	0.5477	1.6733	0.8944
t- value	5.4772	5.4880	5.4880	5.0990	6.5169	14.6969	4.5354	6.5000
P- value (2-tailed)	0.0054*	0.0054*	0.0054*	0.0070*	0.0029*	0.0001*	0.0105*	0.0029*

\*Significance (P &lt; 0.05)

**Table 2: How sensitivity developed in 40 volunteers (20 with and 20 without sickle cell disease) after in-office dental bleaching, according to technique employed.**

	Halogen		LED		LED/laser		No light	
	SCD	No SCD	SCD	No SCD	SCD	No SCD	SCD	No SCD
SENSITIVITY								
Heat	-	-	-	-	-	-	-	-
Cold	-	-	-	1	-	1	2	-
Speaking	-	3	-	1	-	-	-	-
Heat + Cold	-	-	-	-	-	-	-	-
Heat + Speaking	1	1	-	-	-	-	-	-
Cold + Speaking	-	-	3	-	-	-	-	-
Heat + Cold +Speaking	1	1	2	1	3	2	-	1
Spontaneous	1	-	-	-	1	-	2	1
No sensitivity	2	-	-	2	1	2	1	3

- no occurrence

The way in which sensitivity developed varied among volunteers. Five volunteers reported only spontaneous sensitivity; twenty-four reported provoked sensitivity, of whom seventeen also reported spontaneous sensitivity.

The factor that triggered sensitivity also differed among volunteers, with the factor most often mentioned being SPEAKING, which was cited by 50% of volunteers (Table 2).

Only two participants mentioned others factors, both associated with SPEAKING that triggered tooth sensitivity: when brushing teeth and when smiling.

Whether or not a light source was used to activate

hydrogen peroxide did not produce statistically significant differences in terms of tooth sensitivity, regardless of the presence or absence of sickle cell disease (Table 3).



*Fig. 1: Tooth color before and after in-office bleaching with 35% hydrogen peroxide, (A) volunteer with SCD (B) volunteer without SCD.*

**Table 3: P values obtained for presence of sensitivity in 40 volunteers (20 with and 20 without sickle cell disease) after in-office dental bleaching according to technique employed.**

Technique compared	Sickle cell disease	No sickle cell disease	Significance
	P	P	
Halogen vs. LED	0.4444	0.4444	NS
Halogen vs. LED/laser	1.0000	0.4444	NS
Halogen vs. no light	1.0000	0.1667	NS
LED vs. LED/laser	1.0000	1.0000	NS
LED vs. no light	1.0000	1.0000	NS
LED/laser vs. no light	1.0000	1.0000	NS

*P* = critical value, NS = not significant.

## DISCUSSION

Pain is the most frequent symptom in patients with sickle cell disease. The frequency and severity of painful episodes vary among patients. Some patients feel pain on a daily basis; others only occasionally<sup>10,11</sup>. Dehydration, infection, stress and cold temperatures are some of the factors that may trigger painful episodes<sup>12</sup>. Painful crisis duration may range from some hours to weeks<sup>10</sup>.

An esthetically pleasing appearance contributes to increasing self-esteem and wellbeing. In this scenario, tooth bleaching has become a popular treatment, because it preserves tooth structure and improves appearance without any major adverse effects<sup>13</sup>.

Approximately 67.5% of patients with sickle cell disease exhibit intrinsic opacity related to organic matrix hypocalcification during the mineralization phase<sup>14,15</sup>. Because of the increased levels of opacity observed in these patients, Okafor et al.<sup>15</sup> suggested that they should undergo treatment by bleaching or capping of the teeth, depending on the severity of the problem or esthetic compromise.

Almeida et al.<sup>16</sup> and Mondelli et al.<sup>17</sup> evaluated 40 and 48 patients respectively. Both demonstrated that all bleaching techniques tested were equally effective with or without light. They concluded that light sources were unnecessary to bleach teeth. These results are compatible with the findings of our study, where bleaching was effective in all groups, regardless of whether or not a light source was used to activate the hydrogen peroxide.

For sensitivity triggers, the data show that HEAT alone did not trigger sensitivity, being mentioned only when associated with SPEAKING, which in this case might have been a confounding factor.

Presence of sensitivity and whether or not a light source was used to accelerate the hydrogen peroxide treatment showed no statistically signi-

ficant difference in patients with or without sickle cell disease. Almeida et al.<sup>18</sup> and Bernardon et al.<sup>19</sup> found similar results in their studies. However, these findings differ from those reported by Kossatz et al.<sup>20</sup>, who observed more persistent and higher sensitivity levels 24 hours after treatment in the group treated with light activation. Similarly, He et al.<sup>21</sup> observed that the light-activated system produced a higher percentage of tooth sensitivity than the non-light-activated system during in-office bleaching.

Tooth sensitivity was reported by 72.5% of the patients and lasted 3 days or less. Tang and Millar<sup>22</sup>, also reported that 85.2% of the patients experienced tooth sensitivity at some point following the bleaching procedures.

Despite the fact that in-office bleaching is a lengthy procedure, where the patient spends about an hour motionless, in a situation that can easily cause stress, none of the volunteers with SCD reported a painful crisis after the procedure, suggesting that there is no need for specific protocols to perform in-office dental bleaching with 35% hydrogen peroxide in these patients. However, due to the peculiarities of SCD, the most appropriate time to perform the procedure should be selected in conjunction with the medical staff in charge of the patient.

No statistically significant difference was observed in relation to presence of tooth sensitivity among patients with and without sickle cell disease, regardless of whether or not a source of light was used for peroxide activation. On the other hand, our data showed a significant statistical difference for bleaching effectiveness, proving that the purpose of the procedure was achieved. Teeth were bleached in both groups assessed, showing that in-office dental bleaching is a viable alternative for improvement of oral esthetics for patients with SCD.

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## NICKEL ALLERGY: BLOOD AND PERIODONTAL EVALUATION AFTER ORTHODONTIC TREATMENT

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### ABSTRACT

The aim of this study was to assess periodontal status and blood parameters in orthodontic patients with nickel allergy one month after removal of brackets. Ninety-six randomly selected patients were initially evaluated. Allergy to nickel was diagnosed using a patch test. After determining the prevalence of subjects allergic to nickel, two groups were formed: 16 allergic (experimental) and 16 non-allergic (control) patients. Their periodontal status was determined regularly by a single, blinded, duly calibrated examiner using the Löe Index (GI) and their blood was tested (complete blood test, including nickel and IgE levels) after nine months of orthodontic treatment and again one month after removing the orthodontic appliances. Statistical analyses included paired and non-paired t-tests, Mann-Whitney, Wilcoxon, McNemar and linear

trend chi-square tests ( $p \leq 0.05$ ). Comparison of the values recorded during orthodontic treatment and one month after removing the appliances showed that in the allergic group there was significant increase in eosinophils ( $p=0.046$ ), basophils ( $p=0.001$ ) and monocytes ( $p=0.002$ ), and decrease in number of bands ( $p=0.000$ ), while in the control group, there was increase in lymphocytes ( $p=0.039$ ) and decrease in segmented neutrophils ( $p=0.030$ ) and IgE levels ( $p=0.001$ ). In both groups, plasma nickel levels increased ( $p=0.010$ ;  $p=0.039$ ) and GI scores decreased. One month after removing the brackets, blood and periodontal parameters from patients with and without nickel allergy were similar.

**Key words:** Allergy and immunology, blood, hypersensitivity, nickel, orthodontics.

## ALERGIA AO NÍQUEL: AVALIAÇÃO PERIODONTAL E SANGUÍNEA APÓS O TRATAMENTO ORTODÔNTICO

### RESUMO

O objetivo do presente estudo foi avaliar a condição periodontal e os parâmetros sanguíneos em pacientes alérgicos ao níquel, um mês após remoção dos aparelhos. Noventa e seis pacientes selecionados aleatoriamente foram inicialmente avaliadas. Alergia ao níquel foi diagnosticada usando um teste de contato. Após a determinação da prevalência de alergia ao níquel, formaram-se dois grupos: 16 pacientes alérgicos (experimental) e 16 não alérgicos (controle). Condição periodontal foi diagnosticada através do Índice de Löe (IG). Parâmetros sanguíneos foram determinados por meio de um exame de sangue completo, incluindo a quantificação de níquel e níveis de IgE. Avaliações do estado periodontal foram realizados por um único examinador de forma cega, devidamente calibrado e amostras de sangue foram tomadas depois de nove meses de tratamento e um mês após a remoção dos aparelhos ortodônticos. Análise estatística utilizada foi testes t pareado e não pareado, Mann-Whitney, Wilcoxon,

McNemar e qui-quadrado de tendência linear ( $p \leq 0,05$ ). Em comparação com os valores observados durante o tratamento, o número de eosinófilos ( $p=0,046$ ), basófilos ( $p=0,001$ ) e monócitos ( $p=0,002$ ) aumentou significativamente depois da remoção dos aparelhos ortodônticos, ao passo que o número de bastões ( $p=0,000$ ) diminuiu entre os períodos no grupo alérgico. O número de linfócitos ( $p=0,039$ ) aumentou no grupo controle e o número de segmentados ( $p=0,030$ ) diminuiu. A diminuição dos níveis de IgE ( $p=0,001$ ) entre os períodos ocorreu no grupo de controle. Níveis de níquel no plasma aumentou após a remoção de aparelhos ortodônticos em ambos os grupos ( $p=0,010$ ;  $p=0,039$ ). O IG diminuiu em ambos os grupos. Parâmetros periodontais e sanguíneos de pacientes com alergia ao níquel foram semelhantes aos não alérgicos um mês após a remoção dos aparelhos.

**Palavras chave:** Alergia e Imunologia, Sangue, Hipersensibilidade, Níquel, Ortodontia.

### INTRODUCTION

Industrialization and modern life have contributed to an increase in dermal exposure to metals, increasing the incidence of allergies, especially to

nickel<sup>1</sup>, the so-called allergic contact dermatitis<sup>2-4</sup>. The prevalence of nickel allergy in the general population ranges from 8% to 17% in females and 1% to 5% in males<sup>5</sup>. Nickel is potentially allergenic

and capable of causing a late-phase, type IV hypersensitivity reaction characterized by signs such as gingival overgrowth, burning sensation in the mouth, metallic taste, angular cheilitis and labial desquamation in the oral cavity.<sup>6</sup>

Chemicals of small molecular weight (i.e., haptens) can irritate tissues by inducing the production of various pro-inflammatory and chemotactic molecules which are potentially allergenic when able to bind to proteins, such as immune response molecules.<sup>7,8</sup> Nickel can induce T lymphocytes to produce cytokines, such as INF- $\gamma$ , IL-2, IL-5 and IL-10, thereby stimulating tissue proliferation, which may favor gingival hyperplasia. It is assumed that the continuous release of small amounts of nickel into the epithelium could constitute an initiating factor of gingival overgrowth induced by orthodontic brackets<sup>9</sup>. The increasing incidence of periodontal diseases and Ni allergy on the one hand and the high need for orthodontic treatment on the other highlight the importance of improving the knowledge of Ni(II)-induced mechanisms<sup>10</sup> while maintaining strict control of hygiene during orthodontic treatment<sup>11</sup>.

A recent systematic review<sup>10</sup> on hypersensitivity to nickel and orthodontic treatment pointed to serious methodological limitations such as inadequate description of the use and composition of braces, contact test standardization, lack of control groups and cross-sectional studies. An *in vitro* study suggested that nickel has various modifying effects on IL-1 $\beta$ -induced inflammatory processes, depending on the concentration, although the authors acknowledge that there are limitations in transferring their findings to an *in vivo* situation of the oral cavity<sup>11</sup>.

A previous study by our group suggested that nickel is potentially capable of affecting periodontal status and blood cells in allergic patients during orthodontic treatment<sup>12,13</sup>. However, those results seemed to be more related to a local inflammatory response than to a systemic allergic reaction. Moreover, after conducting those studies, another question arose of whether the cumulative effect of nickel during orthodontic treatment is reversed after brackets are removed or whether there are significant lasting effects on periodontal status at the end of orthodontic treatment.

Thus, the aim of this study was to evaluate periodontal status and blood parameters one month after the removal of brackets in patients with nickel allergy.

## MATERIAL AND METHODS

### *Sample characteristics and study design*

Procedures involving evaluation of this sample have been published elsewhere<sup>12</sup>. Briefly, ninety-six randomly selected orthodontic patients were initially evaluated and a case-control study was conducted. All subjects were white; 58 (60%) were female and 32 (40%) male; their ages ranged from 10 to 43 years. Allergy to nickel was diagnosed using a patch test. After determining the prevalence of patients allergic to nickel, two groups were formed: 16 allergic and 16 non-allergic patients.

### *Data collection*

All 32 participants underwent full blood tests nine months after beginning orthodontic treatment<sup>12</sup> and again one month after the removal of the brackets, to determine leukogram, total immunoglobulin E (IgE) and circulating blood levels of nickel. Six mL of blood were vacuum collected (vacuo-time system) from each patient after fasting for 8 hours. For the blood count, 3mL of blood in a vacuum tube with the EDTA anticoagulant were analyzed on an automated hematology analyzer, using the ABX Micros CRP device (OT-CT-OS-CS, France). Blood cell differential count was performed using a blood smear without anticoagulant, which was stained with Single Prov stain (NewProv – 1% solution of eosin methylene blue in cyclohexadiene), viewed under a microscope immersion objective for platelet count. For the evaluation of total IgE, 1 mL of serum without anticoagulant was analyzed using the chemiluminescence method on Immulite equipment. Two mL of blood were used to assess the amount of circulating nickel, collected in a trace tube for atomic absorption spectrophotometry (graphite Kiln with Zeeman corrector). The blood count determined number of leukocytes, basophils, eosinophils, myelocytes, metamyelocytes, bands, neutrophils, lymphocytes and monocytes.

Feces were examined to determine parasitic infestations (helminth eggs and larvae, protazoon cysts) which might affect the white blood cell count, especially the number of eosinophils, in order to control for confounding variables. Any subject with this type of alteration would be excluded from the study to prevent any camouflaging of the results. None of the subjects needed to be excluded.

The skin patch test was used for diagnosing nickel allergy. It is the most efficient method for

confirming the etiologic diagnosis of allergic-contact eczema. It consists of a 2 X 2 cm patch (Finn Chambers) which is attached to the patient's back at 2 points 10 cm apart after cleansing of the skin with cotton soaked in alcohol. Because of the extensive area involved, a suitable amount of the gel (standardized by the manufacturer) containing a 5% nickel sulfate antigen (solid petroleum jelly) (Epitest Ltd Oy, Tuusula, Finland) is left in contact for 48 hours. Patients were instructed to remove the patches if they experienced any reaction beyond what was expected, and to call the researchers in charge and seek care at the municipal medical emergency room. After 48 hours, the patches were removed, and only 1 reading was made, following the standards of the International Contact Dermatitis Research Group<sup>14</sup>, as follows: (-) negative; (+) discrete erythema with some papules; (++) erythema, papules and vesicles; (+++) intense erythema, papules, and vesicles. All patients considered negative had no clinical condition visible to the naked eye, and all patients considered positive had erythema, edema, papules, and blisters (+++).

Periodontal status was assessed by a single, blinded, duly calibrated ( $Kappa > 0.90$ ) examiner at regular three-month intervals over a period of 12 months (four evaluations altogether) during treatment, as described elsewhere<sup>12</sup>. Since each patient finished treatment at a different time, the final evaluation was standardized as one month after the treatment had been completed. Prophylaxis with bicarbonate spray was performed at each session (following periodontal evaluation). All patients were monitored monthly for biofilm (plaque) control and hygiene guidance. Clinical gingival characteristics (color and volume) were assessed using the Löe gingival index<sup>15</sup> with a standardized millimeter probe, which takes into account qualitative changes in the gingival tissue. The Löe index used the following classification: 0, normal gingiva; 1, mild inflammation, slight change in color, with no bleeding on probing; 2, moderate inflammation, reddish appearance, mild edema, bleeding on probing; and 3, severe inflammation, reddish appearance, clear edema, ulceration, tendency toward spontaneous bleeding. This index was chosen because we have used it previously in this sample and thus maintain the same standard of evaluation, it is easy to perform, provides good reproducibility and its use is well established in the literature<sup>16-18</sup>. Morelli<sup>®</sup> brackets (Sorocaba, São Paulo, Brazil) were attached. Fig. 1 provides a flowchart illustrating the study design and sequence of procedures.

### Statistical analysis

Statistical analysis involved t-tests, paired t-tests, Mann-Whitney and Wilcoxon tests for the intergroup/intragroup comparisons of blood components recorded after nine months of orthodontic treatment and again one month after the removal of the orthodontic appliances. The linear trend chi-square test was used to compare periodontal status between groups in the same periods. The McNemar test was used to compare gingival index (dichotomized as absence/presence) within each group between the two evaluation times. Differences were considered significant when  $p \leq 0.05$ .

### Ethical considerations

All aspects of this study, including methods for obtaining informed consent and agreement from participants (parents/caregivers and adolescents), were independently reviewed and approved by the Human Research Ethics Board of the *Centro*

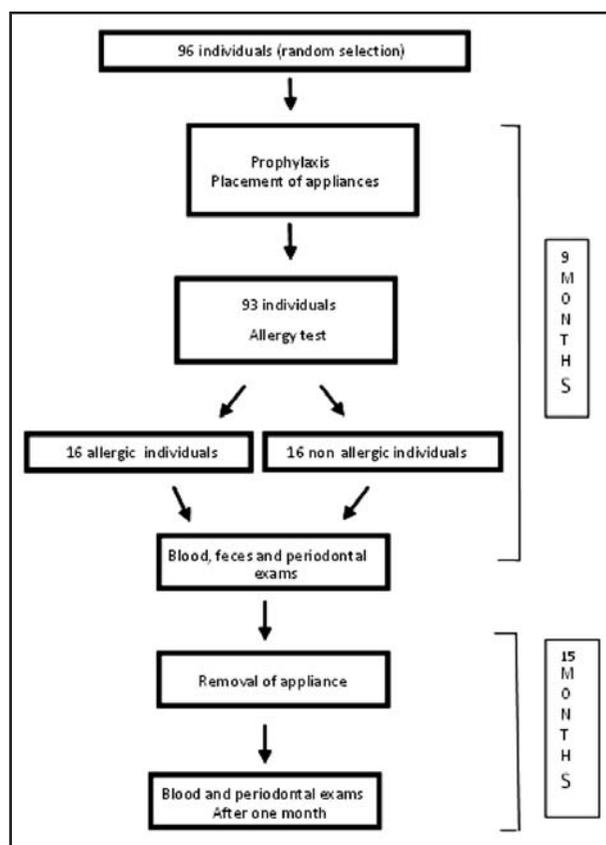


Fig. 1: Study Flowchart.

*Universitário de Lavras* (Brazil) under process number 0001.0.380.000-07. This study was conducted in accordance with the principles for medical research involving human subjects set forth in the Helsinki Declaration. Collected data remained anonymous and confidential.

## RESULTS

The following changes occurred between month nine of orthodontic treatment and one month after removal of the orthodontic appliances. In the allergic group, the number of eosinophils, basophils and monocytes increased significantly, whereas the number of bands decreased ( $p < 0.05$ ) (Table 1). In the control group,

the number of lymphocytes increased, while IgE levels and number of segmented neutrophils and neutrophils decreased ( $p < 0.05$ ) (Table 1). In both groups, plasma nickel levels increased ( $p < 0.05$ ) (Table 1), while Gingival Index (GI) scores decreased ( $p < 0.05$ ) (Table 2).

Figs 2 and 3 show the periodontal condition of patients with and without nickel allergy, respectively.

## DISCUSSION

After conducting the first experiment<sup>12</sup>, one question remained: Would the periodontal and blood conditions be similar between allergic and non-allergic patients after removing the brackets despite

**Table 1: Comparative analysis of blood components in experimental (allergic) and control (non-allergic) groups during and after orthodontic treatment.**

	Experimental Group (AL)			Control Group (NAL)				
	During mean±sd	After mean±sd	D-A p-value	During mean±sd	After mean±sd	D-A p-value	During (AL) x During (NAL)	After (AL) x After (NAL)
Leukocytes (mil/mm <sup>3</sup> )	6411.75±1759.20	6052.90±1872.85	0.412 <sup>c</sup>	5657.14±1264.73	5862.50±1142.60	0.957 <sup>c</sup>	0.821 <sup>a</sup>	0.299 <sup>a</sup>
Eosinophils (mil/mm <sup>3</sup> )	132.70±60.38	259.35±187.83	<b>0.046<sup>c</sup></b>	136.00±104.00	197.80±195.77	0.967 <sup>c</sup>	0.204 <sup>a</sup>	0.322 <sup>b</sup>
Basophils (mil/mm <sup>3</sup> )	6.25±17.95	33.50±32.00	<b>0.001<sup>c</sup></b>	11.95±22.42	23.75±35.75	0.150 <sup>c</sup>	0.392 <sup>b</sup>	0.168 <sup>b</sup>
Lymphocytes (mil/mm <sup>3</sup> )	2115.58±633.35	2115.58±633.35	0.226 <sup>c</sup>	1874.37±461.98	2170.00±461.05	<b>0.039<sup>c</sup></b>	0.264 <sup>a</sup>	0.345 <sup>a</sup>
Segmented (mil/mm <sup>3</sup> )	3702.47±1116.30	3066.95±1303.25	0.073 <sup>c</sup>	3400.25±1010.65	3254.75±947.85	<b>0.030<sup>c</sup></b>	0.991 <sup>a</sup>	0.264 <sup>a</sup>
Bands (mil/mm <sup>3</sup> )	127.47±48.90	24.35±47.50	<b>0.000<sup>c</sup></b>	67.45±48.27	53.20±80.80	0.240 <sup>c</sup>	0.982 <sup>a</sup>	0.277 <sup>b</sup>
Monocytes (mil/mm <sup>3</sup> )	327.30±87.20	442.95±111.10	<b>0.002<sup>c</sup></b>	355.25±137.35	374.25±138.38	0.295 <sup>c</sup>	0.900 <sup>b</sup>	0.917 <sup>a</sup>
IgE (UI/mL) <sup>1</sup>	631.30±821.11	597.90±675.10	0.637 <sup>c</sup>	446.67±425.00	392.90±353.07	<b>0.001<sup>c</sup></b>	0.061 <sup>a</sup>	0.078 <sup>a</sup>
Nickel (mcg/L)	1.68±3.4	3.81±1.05	<b>0.010<sup>d</sup></b>	0.68±2.95	2.82±1.65	<b>0.039<sup>d</sup></b>	0.212 <sup>b</sup>	0.282 <sup>b</sup>

<sup>a</sup>T test; <sup>b</sup>Mann Whitney test; <sup>c</sup>Paired t test; <sup>d</sup>Wilcoxon. \* ( $p < .05$ ); AL= allergic; NAL= non-allergic.

All variables were tested with Kolmogorov-Smirnov normality. <sup>1</sup>IgE was transformed by a square root.

D-A: Comparison of mean values observed "during treatment" and "after brackets removal"

**Table 2: Comparative analysis of gingival index in the experimental (AL) and Control (NAL) groups during orthodontic treatment and one month after removing orthodontic appliances.**

TESTS	Groups	Results GI				AL x NAL <sup>a</sup>
		0	1	2	3	P Value
During	Experimental <sup>A</sup>	1 (5.9%)	7(41.2%)	7(41.2%)	2(11.8%)	<b>0.026*</b>
	Control	5(31.3%)	7(43.8%)	4(25%)	0(0%)	
After	Experimental <sup>B</sup>	8(47.1%)	8(47.1%)	1(5.9%)	0(0%)	0.160
	Control	11(68.8%)	5(31.3%)	0(0%)	0(0%)	

<sup>a</sup>Linear trend Chi-square Test comparing gingival index between groups during and after orthodontic treatment.

<sup>A,B</sup> McNemar test for gingival index dichotomized into absence (0) and presence of periodontal inflammation (1, 2 or 3) withineach group comparing times: during and after orthodontic treatment. (Experimental  $p = 0.016^*$ ; Control  $p = 0.070$ ).

\* ( $p < .05$ ); AL= allergic; NAL= Non allergic.



Fig. 2: Periodontal condition of allergic patient.



Fig. 3: Periodontal condition of non-allergic patient.

the possible cumulative effects of orthodontic treatment? We collected data again to answer this question.

Current data indicate a significant reduction in GI and band counts after the removal of orthodontic appliances, showing that periodontal and blood alterations tend to disappear. As orthodontic appliances hamper oral hygiene, dental biofilm accumulates more easily on tooth surfaces and appliances in most patients and the consequent periodontal disease leads to an increase in neutrophils<sup>19</sup>. Once the appliance is removed, the conditions favoring the formation of biofilm are no longer present and there is a consequent reduction in gingival index scores and amount of bands. Thus, the reduction in the number of bands may be explained by the decrease in bacterial accumulation and consequent decreased inflammatory reaction<sup>20</sup>.

Gingival index scores were higher in the experimental group at both times. In addition to being a direct sensitizing agent of skin and mucosa, nickel appears to alter periodontal status, acting as a modifying factor of periodontal disease in sensitive patients<sup>21</sup>. The gingival epithelium is the first barrier which comes into contact with corrosive materials such as Ni (II) and bacteria<sup>22</sup>. This suggests a cumulative effect of nickel throughout orthodontic treatment, with nickel potentially influencing periodontal status of allergic orthodontic patients<sup>23</sup>. IgE levels decreased between the evaluations performed during treatment and after the removal of the orthodontic appliances in the control group. Circulating levels of nickel increased between evaluations in both groups, although within normal limits. Other studies have reported serum nickel levels to increase up to 5-fold during the 6-week post-closure period, and mean concentrations of nickel in serum to have returned to baseline levels within 4-6 months<sup>24</sup>. An analysis of white blood cells in allergic and non-allergic patients during and after treatment (Table 1) showed an increase in eosinophils, monocytes and basophils. We hypothesized that these results were not related to the removal of the appliances, but rather to the extensive exposure to nickel during treatment, as there was an increase in plasma nickel concentration in both groups. The continuous low-level stimulus of antigens such as nickel raises the level of IL-4 produced by T cells, regardless of whether or not an individual is allergic, which favors a polarized immune response for a  $T_H2$  profile, with a characteristic cell and molecule population through a pathway dependent on STAT-6 and GATA-3<sup>25</sup>. In our study, lymphocytes, eosinophils and IgE increased in both allergic and non-allergic patients, indicating the onset of a  $T_H2$  immune response. These results corroborate findings in other studies, which observed the systemic response to nickel<sup>26</sup>. A number of studies also report a nickel-produced response with a predominance of  $T_H1$   $CD4^+$  T cells due to the presence of interferon- $\gamma$ , but the balance generally tends to favor the expression of  $T_H2$  cells and inhibit other subpopulations<sup>27-29</sup>. Analyses of cytokine production by Ni-specific T cells have demonstrated a mixed  $T_H1$  and  $T_H2$  cytokine profile in both T-cell clones and peripheral blood mononuclear cells<sup>30</sup>. However, in our study, allergic patients exhibited a greater response than non-allergic patients because they were more

sensitive to the allergen (nickel). Only the allergic group exhibited a significant increase in eosinophils, monocytes and basophils (Table 1). Similarly, the number of lymphocytes also increased after braces were removed. This may be explained by the fact that persons who are allergic to nickel have few or no specific suppressor T cells, which regulate the number of leukocyte populations<sup>31</sup>.

Studies on hapten-induced contact hypersensitivity, which represents the classic model of a T cell-mediated hypersensitivity reaction, show that a strong inflammation response on the skin is elicited well before the activation of nickel-specific T cells<sup>32</sup>. A broad spectrum of chemokines is released, including Regulated on Activation, Normal T cell Expressed and Secreted (RANTES), which can play a fundamental role in histamine and serotonin generation and trigger human mast cell degranulation<sup>33</sup>.

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The results of our study analyzing the period of direct contact with the allergenic agent and after its removal indicate that orthodontic treatment with conventional stainless steel appliances does not initiate or aggravate a hypersensitive reaction to nickel. However, as periodontal alterations may be associated to nickel, it is important for orthodontists to seek alternatives to treat patients who exhibit compromised periodontal health. Moreover, the results of our study demonstrate that the allergic effect of conventional braces is reversed after the removal of the appliances, and metal ions released from appliances should not be cause for concern in utilizing the appliance<sup>34</sup>.

In conclusion, no difference was found in blood or periodontal parameters between orthodontic patients with and without allergy to nickel one month after removing brackets.

#### CORRESPONDENCE

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## CREATINE METABOLISM: DETECTION OF CREATINE AND GUANIDINOACETATE IN SALIVA OF HEALTHY SUBJECTS

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### ABSTRACT

Creatine (Cr) plays an important role in storage and transmission of phosphate-bound energy. Cerebral creatine deficiency syndromes comprise three inherited defects in Cr biosynthesis and transport. The aim of this study was to investigate whether Cr and Guanidinoacetate (GAA) can be detected in saliva of healthy subjects and to establish the relationship between salivary and plasma levels of these molecules. An adapted gas chromatography (GC) method is described for the quantification of Cr and GAA biomarkers in saliva. Reference values were established for GAA

and Cr in saliva. These values were age dependent ( $p=0.001$ ). No difference between genders was observed. We detected a difference between GAA and Cr concentrations in saliva and in plasma. The GC method for simultaneous determination of GAA and Cr in human saliva is fast, reliable, sensitive, non-invasive and precise to use as a biochemical approach in early detection of cerebral creatine deficiency syndromes.

**Key words:** Creatine deficiency, biomarkers, saliva, brain, gas chromatography.

## METABOLISMO DE CREATINA: DETECCIÓN DE CREATINA Y GUANIDINOACETATO EN SALIVA DE SUJETOS SANOS

### RESUMEN

La creatina (Cr) juega un importante rol en el almacenamiento y el transporte de energía unida al fosfato. Los síndromes de deficiencia de creatina cerebral comprenden tres defectos genéticos en la biosíntesis y transporte de creatina. Es propósito de este estudio investigar si el guanidinoacetato (GAA) y la Cr pueden ser detectados en saliva de sujetos sanos e investigar la relación entre los valores de GAA y Cr en saliva con los niveles en plasma de estas moléculas. Se describe un método modificado de cromatografía gaseosa para la cuantificación de los biomarcadores, Cr y GAA en este biofluido. Se establecieron valores de

referencia para GAA y Cr. Estos valores dependen de la edad ( $P=0.001$ ). No se observaron diferencias entre género. Se detectó una diferencia entre la concentración de GAA y Cr en saliva con respecto al plasma. El método adaptado de cromatografía gaseosa para la determinación simultánea de GAA y Cr en saliva humana es fácil, seguro, sensible, no invasivo y preciso para utilizar como aproximación bioquímica en la detección temprana de los síndromes de deficiencia de creatina cerebral.

**Palabras clave:** deficiencia de creatina, biomarcadores, saliva, cerebro, cromatografía gaseosa.

### INTRODUCTION

The creatine (Cr)/phosphocreatine (PCr) creatine kinase (CK) system plays essential roles, maintaining the high energy levels necessary for the development and functions of the central nervous system by regenerating ATP and buffering ATP levels<sup>1,2</sup>. In humans, the Cr/PCr pool is maintained by endogenous biosynthesis and nutritional intake of Cr. Cr biosynthesis is a two-step process occurring mainly in kidney, pancreas and liver<sup>3</sup>. In the first step, L-arginine: glycine amidinotransferase synthesizes guanidinoacetate (GAA) from arginine and glycine. In the second step, guanidinoacetate methyltransferase methylates GAA to form Cr. The

methyl group donor is S-adenosylmethionine, which is subsequently converted to S-adenosyl homocysteine. Finally, Cr is distributed and actively taken up by different via the Cr transporter (CRT, encoded by *SLC6A8*)<sup>4</sup> (Fig.1).

Of the three cerebral creatine deficiency syndromes (CCDS), L-arginine: glycine amidinotransferase (AGAT) deficiency and guanidinoacetate N-methyltransferase (GAMT) deficiency are caused by defects in the enzymes, whereas CRT deficiency results from the defect in CRT caused by *SLC6A8* defect. Cr is found in blood and cerebrospinal fluid, but cannot enter brain cells, as the cell membranes are an effective barrier to Cr transport<sup>5</sup>. The clinical

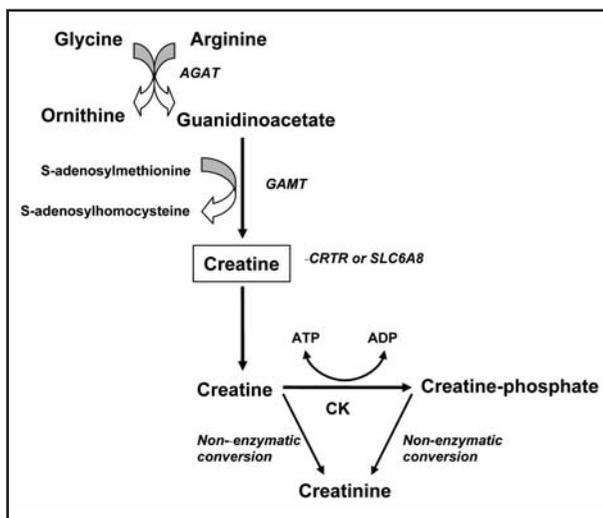


Fig. 1: Metabolic pathway of creatine/creatine-phosphate. AGAT: arginine:glycine amidinotransferase; GAMT: guanidinoacetate methyltransferase; CRTR: creatine transporter; CK: creatine kinase.

presentation of all CCDS patients is characterized by developmental delay/arrest, mental retardation, language delay and autism spectrum disorders<sup>6</sup>. Proton magnetic resonance spectroscopy (MRS) of affected patients shows absence or decrease of the Cr peak<sup>7,8</sup>.

Saliva is a biofluid that is useful for novel approaches to prognosis, laboratory or clinical diagnosis, and monitoring and management of patients with oral disorders such as caries and periodontal disorders, and systemic diseases of different etiologies, including AIDS, systemic lupus erythematosus, Sjogren's syndrome, hepatitis A, B and C, rheumatoid arthritis, Type II diabetes, cystic fibrosis, hormonal dysfunctions, oral cancer, neurological and cardiovascular disorders<sup>9</sup> and in some lysosomal storage disorders diagnosed at the Center for the Study of Inherited of Metabolic Diseases, Córdoba, Argentina (Cemeco)<sup>10</sup>. It is easily collected and stored and ideal for early detection of disease because it contains specific soluble biomarkers. Saliva contains multiple biomarkers which make it useful for multiplexed assays that are being developed such as point-of-care devices, rapid tests, or in more standardized formats for centralized clinical laboratory operations. Recent studies indicate coexistence of total salivary proteins and their mRNAs, which can be used as proteomic and genomic biomarkers of specific disorders, such as oral cancer<sup>11</sup>. Progress in

the use of saliva as a diagnostic biofluid has been remarkably influenced by biotechnological progress, the analysis of small samples (microfluidic), its high sensitivity and specificity, ease of use and low cost<sup>12</sup>. In addition, the values of serum components can be measured and compared through saliva<sup>13</sup>. The aim of this study was to determine whether Cr and GAA can be detected in saliva of healthy subjects, and to investigate the relationship between salivary and plasma levels of these molecules compared to our reference values. We found that saliva can be used as a biological indicator to facilitate the recognition of CCDS in our environment, with the advantages of using a non-invasive, readily available method.

## MATERIALS AND METHODS

### Sample collection

Control values were determined in saliva samples from individuals (age range: male: 2 - 48, female: 3 - 67) with no metabolic, renal or neurologic disorder. A sample of unstimulated whole saliva (+/- 0.3 ml) was collected from the mouth under the tongue with a disposable Pasteur pipette. The saliva was obtained 60 minutes after mouth cleansing and before breakfast from 97 subjects (48 male and 49 female). Subjects were recruited from healthy volunteers at Hospital de Niños Córdoba, Argentina. Following collection, the saliva samples were immediately placed on ice and stored at -20°C. Blood samples were collected by venipuncture into heparin tubes and immediately centrifuged to separate blood components. Plasma supernatants were transferred by cryotubes for storage and frozen at -20°C.

This study was approved by the Institutional Committee of Health Research Ethics, CIES, of Polo Hospitalario (Hospital de Niños de la Santísima Trinidad, Nuevo Hospital San Roque, Hospital Rawson), Argentina. Informed consents were signed by the study population.

### Sample preparation

The method mainly follows Struy et al.<sup>14</sup>, with modification. Fifty µl saturated aqueous sodium bicarbonate, 50 µl hexafluoroacetylacetone and 500 µl toluene were added to 100 µl saliva or plasma. The mixture was heated to 80°C for 12h. From the upper toluene phase, 400 µl were transferred to a clean vial and dried under nitrogen flow at room

temperature. Pentafluorobenzyl (PFB) derivatives were formed by treating the residue with 10  $\mu\text{l}$  triethylamine and 100  $\mu\text{l}$  7% pentafluorobenzyl bromide in acetonitrile (v/v) at room temperature for 30 min. After adding 200  $\mu\text{l}$  0.5 N HCl, the derivatives formed were extracted with 0.5 ml hexane and then analyzed by GC.

### Standard curves

Calibration curves were established with different concentrations of Cr (2.5- 180  $\mu\text{mol/L}$ ) and GAA (0- 35  $\mu\text{mol/L}$ ) and a constant amount of internal standard  $\beta$ -guanidinopropionic acid (254  $\mu\text{mol/L}$ ). The linear regression equations derived from calibration curves were used for calculating Cr and GAA concentrations in saliva and plasma samples.

### Gas chromatography

The samples were analyzed in a Hewlett Packard gas chromatograph 5890 series II. Chromatographic separation was achieved on a DB-35 MS capillary column, 30 mm in length, 0.25 mm in internal diameter and 0.25  $\mu\text{m}$  in film thickness. One microliter of each sample was injected in the splitless mode. Injector temperature was 250  $^{\circ}\text{C}$ . Initial oven temperature was 110  $^{\circ}\text{C}$ , followed by a ramp up to 152 $^{\circ}\text{C}$  at 3 $^{\circ}\text{C}/\text{min}$ , then by a ramp up to 280 $^{\circ}\text{C}$  at 70 $^{\circ}\text{C}/\text{min}$ . Helium was used as the carrier gas at an initial pressure of 20 psi, constant flow.

### Statistical analysis

The population was initially subdivided into two age-related subgroups (*GAA*: 0-15 years (*y*); >15 *y*; *Cr*: 0-10 *y*; > 10 *y*) because the concentrations of Cr and GAA in plasma are age-dependent<sup>15</sup>. The value followed a non-Gaussian distribution. The data for Cr and GAA concentration in saliva were analyzed with t-Student for one parameter in order to compare the average values in saliva vs. standard average values in plasma, obtained from our population; plasma value: (*GAA*, 0-15 *y*: 0.01 to 2.8  $\mu\text{M}$ ; >15 *y*: 0.3 to 3  $\mu\text{M}$ ; *Cr*, 0-10 *y*: 7 to 142  $\mu\text{M}$ ; > 10 *y*: 20 to 96  $\mu\text{M}$ ). The Wilcoxon Test for independent samples was performed to compare salivary values between age ranges. Statistical significance was set at  $p \leq 0.05$ .

## RESULTS

Salivary GAA and Cr concentrations were analyzed in the 97 samples. The method was selective and

no interfering peaks were observed. Cr and GAA were expressed and detected in all saliva samples (Fig. 2 A, B).

In saliva, it was observed that the GAA value ranges for control subjects were for 0-15 years ( $n=48$ ): 0.1 to 13.4  $\mu\text{M}$ , for > 15 years ( $n=49$ ): 0.08 to 6.1  $\mu\text{M}$  (Table 1). Average GAA concentration in saliva was found to be significantly higher ( $p$ -value= 0.000) in individuals aged 0-15 (3.94  $\mu\text{M}$ ) in comparison with subjects over 15 years old (2.55  $\mu\text{M}$ ) with regard to the upper limit value in blood per age range studied (2.8  $\mu\text{M}$  in 0 to 15, and 3  $\mu\text{M}$  in > 15). On the other hand, a remarkable difference was observed between age ranges in males ( $p$ -value = 0.0057) for concentrations of GAA in saliva within the age ranges.

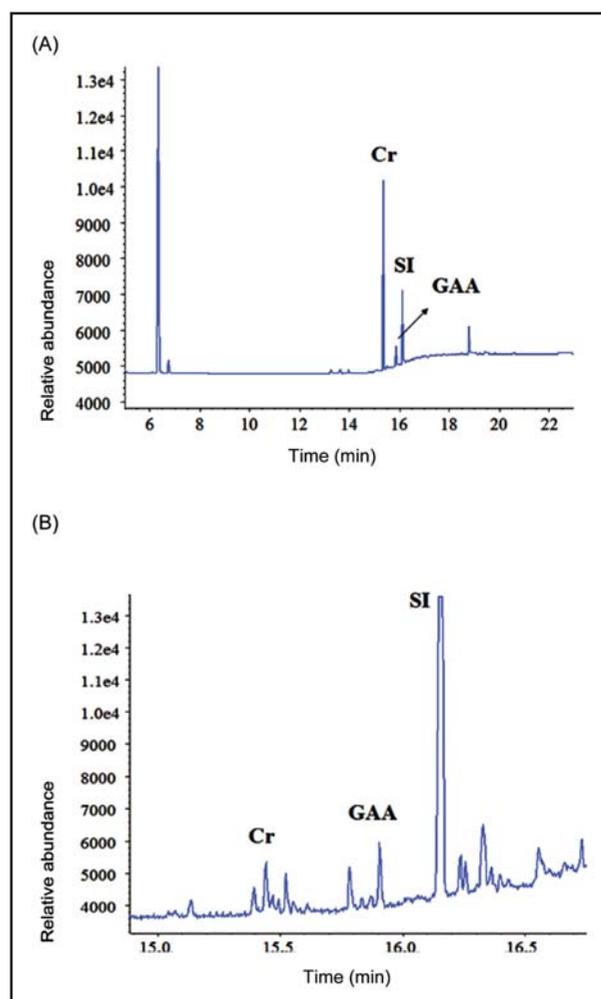


Fig. 2: Representative GC chromatograms. (A) Creatine (Cr), guanidinoacetate (GAA) standards and  $\beta$ -guanidinopropionic acid Internal standard (SI); (B) saliva sample from a healthy individual.

**Table 1: Concentration of GAA (A) and Cr (B) in saliva of healthy adult subjects (n= 97), according to two age ranges and gender.**

	Gender	Agerange (years)	n	Average	SD	Minimum	Maximum	P(25)	P(50)	P(75)
GAA $\mu$ M	Female (n=48)	0-15	22	3.94	2.92	0.11	13.40	1.60	3.70	5.40
		>15	26	2.69	1.60	0.16	6.15	1.60	2.40	3.60
	Male (n=49)	0-15	26	4.02	2.16	0.67	8.70	3.10	3.40	5.20
		>15	23	2.38	1.44	0.08	5.40	1.40	2.10	3.20
Cr $\mu$ M	Female (n=48)	0-10	21	8.07	2.98	1.40	14.30	6.30	8.22	9.50
		>10	27	10.67	5.07	2.44	22.60	7.60	9.40	10.70
	Male (n=49)	0-10	23	7.65	3.12	0.29	12.20	5.90	8.40	9.30
		>10	26	9.24	2.76	5.20	15.20	7.50	8.60	10.60

The range of Cr for control subjects was: for 0-10 (n=44): 0.2 to 14.3  $\mu$ M for > 10 (n= 54): 2.4 to 22.6  $\mu$ M (Table 1). Cr concentrations in saliva were significantly lower than standard blood parameters ( $p$ -value = 0.000) in subjects aged 0-10 (7.85  $\mu$ M) and over 10 (9.98  $\mu$ M) and with regard to the highest values in plasma for each age range studied (0-10: 142  $\mu$ M and 96  $\mu$ M in > 10). On the other hand, there was a remarkable difference between age ranges in females ( $p$ -value= 0.0001) for concentrations of Cr within the age ranges.

Normal GAA and Cr ranges are very wide in different age groups, which are explained by the fact that reference values in the population do not follow normal distribution.

In saliva, GAA and Cr were found to differ according to age ( $p$ =0.001). No gender-related difference was observed for either compound ( $p$  (GAA)= 0.6874;  $p$ (Cr)= 0.2620).

## DISCUSSION

So far, several methods have been developed to measure Cr and GAA in plasma, urine and CSF<sup>16-18</sup>. We have described a modified method to measure GAA and Cr in saliva by gas chromatography<sup>14</sup>. According to our results, this technique may be suitable for analyzing GAA and Cr in saliva, and could therefore be used to study Cr biosynthesis and its relation to diseases associated with altered Cr

metabolism. These findings suggest that the detection of Cr and GAA in saliva may be used as a noninvasive, safe, inexpensive tool for diagnosing Cr genetic disorders. We have not found any recently published data on GAA and Cr concentration or total GAA and Cr content in saliva. In AGAT deficiency, low Cr and GAA levels are found in plasma, urine and CSF whereas in GAMT deficiency, low Cr and high GAA levels are diagnosis hallmarks<sup>19, 20</sup>. In CRTR deficiency, Cr and GAA are normal in plasma but elevated in urine in male patients<sup>21</sup>.

The clinical spectrum of CCDS has widened in recent years: epilepsy, mental retardation and autism have remained the principal neurological signs reported to date<sup>2, 3</sup>. In addition, AGAT and GAMT deficiencies are easily treatable<sup>22</sup>. Consequently, early diagnosis of these inherited conditions is very important.

In conclusion, paper provides reference values for metabolites in Cr metabolism (GAA, Cr) in saliva for different age groups. We also show that salivary GAA and Cr concentration is age-dependent but not under the influence of gender. In our opinion, this biochemical approach should be offered for patients with unexplained mental retardation, dysphasia, epilepsy, and /or autistic behavior. Follow-up studies by molecular analysis of the gene and/or functional tests (AGAT, GAMT activities or Cr uptake) are warranted for definition diagnosis.

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## FREQUENCY OF THE MESIOPALATAL CANAL IN UPPER FIRST PERMANENT MOLARS VIEWED THROUGH COMPUTED TOMOGRAPHY

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### ABSTRACT

The success of any endodontic therapy depends on factors such as correct diagnosis and prognosis. Unawareness or failure to locate additional canals, such as the mesiopalatal canal in the upper first molar, may lead to unsuccessful treatment. Hence, it is valid to consider all the resources available for locating this anatomic structure, e.g. the Cone-Beam Computed Tomography (CBCT). The purpose of this study was to verify the frequency of mesiopalatal canals in upper first permanent molars through computed tomography. Tomography images from a digital archive of a diagnostic imaging center were

analyzed. Eighty (80) upper right first molars were assessed through CBCT in axial cross sections at 6mm and 3mm from the root apex; 40 were females and 40, males, randomly chosen. The results revealed that the mesiopalatal canals were present in 56.25% of the sections at 6mm from the root apex, and in 23.75% at 3mm from the apical limit. CBCT scan has proved to be a valid resource for locating mesiopalatal canal, especially in cases where location was not feasible through clinical means.

**Key words:** Computed tomography, endodontics, root canal.

## FREQUÊNCIA DO CANAL MESIOPALATINO EM PRIMEIROS MOLARES PERMANENTES SUPERIORES VISUALIZADOS EM TOMOGRAFIA COMPUTADORIZADA

### RESUMO

O sucesso do tratamento endodôntico depende de fatores como o correto diagnóstico e prognóstico. O desconhecimento ou a não localização de canais adicionais, como o canal mesiopalatino no primeiro molar superior, pode levar ao insucesso do tratamento. Portanto, é válido considerar todos os recursos para localização desta estrutura anatômica, dentre eles a tomografia computadorizada cone beam (TCCB). O presente trabalho teve como objetivo verificar a frequência do canal mesiopalatino em primeiros molares permanentes superiores através da tomografia computadorizada. Os exames analisados fazem parte do arquivo digital de uma clínica de diagnóstico por imagem particular.

Foram avaliados 80 primeiros molares superiores direitos em cortes axiais a 6 e 3 milímetros do ápice radicular observados nas TCCB, sendo 40 em pacientes do gênero feminino e 40 do gênero masculino escolhidos aleatoriamente. Os resultados revelaram que o canal mesiopalatino está presente em 56,25% dos cortes a 6mm do ápice radicular e em 23,75% a 3mm do limite apical. TCCB é um recurso válido para localização do canal mesiopalatino, sobretudo nos casos em que não foi possível a sua localização clínica.

**Palavras-chave:** Tomografia computadorizada, Endodontia, Canal radicular.

### INTRODUCTION

Success in endodontic therapies depends on factors such as correct diagnosis and prognosis, which in turn, depend on clinical criteria and knowledge of the root canal systems anatomy. Frequently, ignoring the presence of any additional canal may lead to unsuccessful treatment as this may cause secondary infection. Root canal systems can be

complex and difficult to evaluate. Recognizing their multiple anatomic variations, whether under normal conditions or not, reduces the chances of failure during endodontic procedures.<sup>1</sup>

The morphology of upper first permanent molars has been extensively studied. They usually have three roots and three or four canals but there are variations that may include one, four or five roots.

Cases with five or six canals have also been reported.<sup>2,3</sup> Hence, being acquainted with variations in root canal systems is highly relevant for an efficient treatment.<sup>4</sup>

Tools for visual magnification, such as magnifying glasses and surgical microscopes, are resources critical for practitioners to identify extra canals.<sup>2</sup> So much so considering that the difficult location of mesiopalatal canals is worsened due to its proximity to mesiovestibular canals and to a limited ability for viewing it through radiographic exams.<sup>5,6</sup>

Computed Tomography (CT) scans are alternative supplementary techniques, and may be crucial for any correct diagnosis of presence of fourth root canals in mesiovestibular roots of upper first permanent molars. Cone-Beam Computed Tomography (CBCT) scans, are more advantageous as patients are exposed to lower levels of radiation doses. Effective doses of CBCT may be as low as those of conventional panoramic X-Ray and considerably lower than those of medical computed tomography scans (MCT). When prescribed, CBCT 3D images should supplement 2D conventional radiographic techniques as the benefits derived from these two systems need to be exploited.<sup>7</sup>

Failure to locate mesiopalatal canals of upper first molars may cause a significant increase in failure rates in endodontic procedures. Hence, all of the resources available for locating such anatomical details are to be taken into account, including CBCT. Thus, it is considered relevant to assess the frequency of such canals through axial sections in CBCTs.

## MATERIALS AND METHODS

The study included Cone-Beam Computerized Tomography of maxillae in patients with different indications and having their upper first molars in place. CBCTs form part of the digital archive of a diagnostic imaging center in Teresina Piauí, State of Piauí, Brazil. The upper first right molars of subjects were assessed. The sample included 80 tomography scans: 40 in female patients, and 40 in male patients whose ages were chosen at random. Upper left first molars (26) were assessed when upper right first molars (16) were missing in the subjects or when upper right first molars had undergone endodontic treatment.

Images were assessed through a specific software (ICAT CHORAN), in a 32" high resolution display, in an environment with low luminosity levels, and

researchers were allowed 10-minute breaks for every 20 tomography scans being assessed.

Axial sections located at 3mm and 6mm from the root apex were reconstructed and then assessed for identifying presence or absence of fourth root canals in mesiovestibular roots of upper first permanent molars (16). The presence of fourth root canals was detected through two axial sections at 3mm and 6mm from the apex.

Category variables, presence of the fourth root canal in first molars permanent (16 and 26) and gender were analyzed through percentage readings; numerical variables, such as age, were analyzed in terms of average and standard deviation.

The results were exhibited in tables and in tomography images. This research was classified as a minimal risk survey, as per Provision 466/12 of the National Health Council, the regulatory authority of research in human beings. The project was approved by the Ethics and Research Committee of UNINOVAFAPI (Teresina, State of Piauí, Brazil), as per protocol CAAE 10930012.2.0000521.

## Statistical Analysis

A Qui-Square test was performed for verifying the existence of any link between the presence of the fourth canal, gender and age range with an  $\alpha$  significance level of 5%.

## RESULTS

From the 80 upper first permanent molars that were analyzed, tomography images showed that 56.25% had mesiopalatal canals in the axial section at a 6 mm distance from the root apex. (Table 1). In axial sections at a 3 mm distance from the root apex 23.75% have root canals (Table 2).

Tables 3 and 4 show frequency of mesiopalatal canals at 3mm and 6mm from the root apex according to gender and age range. No significant differences were observed ( $p>0,05$ ) between the presence of fourth canals at 3mm and 6mm and age range and gender.

## DISCUSSION

Prior knowledge of root canal anatomy enables an easier and more accurate detection of any kind of canal during endodontic treatment. The recent use of CBCT has enabled performing 3D global analyses that are non-destructive for the external and internal morphology of root canal systems.

**Table 1: Prevalence of Mesiopalatal Canals in Upper First Permanent Molars at 6mm from Root Apex.**

	6mm from Apex						
		Yes		No		Total	
		Nº	%	Nº	%	Nº	%
Tooth	16	39	60,00	26	40,00	65	100,00
	26	6	40,00	26	60,00	15	100,00
	Total	45	56,25	35	43,75	80	100,00

Source: Pesquisa direta p=0,16

**Table 2: Prevalence of Mesiopalatal Canals in Upper First Permanent Molars (16) & (26) at 3mm from Root Apex.**

	3mm from Apex						
		Yes		No		Total	
		Nº	%	Nº	%	Nº	%
Tooth	16	15	23,08	50	76,92	65	100,00
	26	4	26,67	11	73,33	15	100,00
	Total	19	23,75	61	76,25	80	100,00

Source: Pesquisa direta p=0,77

**Table 3: Prevalence of Fourth Canals at 3mm from Root Apex, as per Gender and Age Range. Teresina (PI), 2013.**

				3mm from Apex											
				Yes				No				Total			
				Nº	%	Avrge.	Strd. Dev.	Nº	%	Avrge.	Strd. Dev.	Nº	%	Avrge.	Strd. Dev.
Gender*	Males	Age	- than 30	1	12,50			7	87,50			8	100,0		
		Range	30 to 40	1	12,50			7	87,50			8	100,0		
		(years)	+ than 40	8	32,00			17	68,00			25	100,0		
	Females	Age	- than 30	3	60,00			2	40,00			5	100,0		
		Range	30 to 40	-	-			5	100,0			5	100,0		
		(years)	+ than 40	6	20,69			23	79,31			29	100,0		
	Total *	Age	- than 30	4	30,77			9	69,23			13	100,0		
		Range	30 to 40	1	7,69			12	92,31			13	100,0		
		(years)	+ than 40	14	25,93			40	74,07			54	100,0		
Age						44	12			46	14		45	13	

Source: Pesquisa direta. \* p&gt; 0,05

The morphology of upper first permanent molars has been extensively reviewed. These molars have three roots and three or four canals. Other variations include one, four or five roots. Cases with five, six or seven canals have also been reported.<sup>2-3,8</sup> On the other hand, the second channel of the mesiobuccal

root may be called fourth canal<sup>1,4</sup>, the second canal in the mesiovestibular root - MV2<sup>9</sup>. Yet, it is considered that the most adequate name for it is “mesiopalatal” as the correct position of the entry of the canal in the upper first molar is in fact mesiopalatal<sup>8</sup>.

**Table 4: Prevalence of Fourth Canals at 6mm from Root Apex, as per Gender and Age Range. Teresina (PI), 2013.**

				6mm from Apex											
				Yes				No				Total			
				Nº	%	Avrge.	Strd. Dev.	Nº	%	Avrge.	Strd. Dev.	Nº	%	Avrge.	Strd. Dev.
Gender*	Males	Age	- than 30	7	87,50			1	12,50			8	100,0		
		Range (years)	30 to 40	5	62,50			3	37,50			8	100,0		
			+ than 40	16	64,00			9	36,00			25	100,0		
	Females	Age	- than 30	3	60,00			2	40,00			5	100,0		
		Range (years)	30 to 40	2	40,00			3	60,00			5	100,0		
			+ than 40	12	41,38			17	58,62			29	100,0		
	Total *	Age	- than 30	10	76,92			3	23,08			13	100,0		
		Range (years)	30 to 40	7	53,85			6	46,15			13	100,0		
			+ than 40	28	51,85			26	48,15			54	100,0		
Age						43	13			48	13			45	13

Source: Pesquisa direta. \* p> 0,05

Unsuccessful endodontic procedures are associated with different factors. Among such factors, there is failure to detect all the root canals for lack of knowledge of the internal molar configuration or for difficulty in viewing the entry level of root canals, due to anatomic variations or calcification in the pulp cavity. Recognizing anatomical variations decreases the rate of failure and of unsuccessfulness in endodontic procedures, thus assuring a good prognosis for treatment.<sup>9,10</sup>

In lateral and accessory canals, or in a delta morphology, where instruments have no access the use of chemical substances may be necessary. If this is a significantly important factor during preparation stage of root canals, it is even more important to locate any root canal. Failure to locate and treat a fourth root canal may be the link between coronal leakage and the presence of microorganisms in the apical area.<sup>11</sup>

The results from this survey are consistent with research done with CBCT. Blattner *et al*<sup>12</sup>, identified mesiopalatal canals in 57.9% of the cases; Kim *et al*<sup>13</sup> identified fourth canals in 63.59% of the cases in a Korean population and Baratto Filho *et al*.<sup>14</sup>, 67.14%, while Somma *et al*<sup>10</sup>, detected mesiopalatal canals in 80% of the cases by using micro-tomography. Blattner *et al*<sup>12</sup> reported higher prevalence levels in root slices (*ex-vivo*), where mesiopalatal canals were positively identified in 68.4% of the samples. Notwithstanding, CBCT has

proved to be a relevant auxiliary tool for locating mesiopalatal canals (*in vivo*).

Research studies have reported that more than 50% of mesiopalatal canals are united in the apical area and end in a single foramen<sup>8,15</sup>. Domark *et al*<sup>16</sup> reported that 69% of the cases with mesiopalatal canals ended in two or more different foramina. This research reported that 56% of mesiovestibular roots that had two canals at 6 mm from the apex presented only one single canal at 3 mm from the apex in CBCT scans. This may be due to the convergence of canals, and this may clinically justify the high success rate of endodontic treatment practices in upper first molars where mesiopalatal canals were not located. CBCT has the unique ability of rendering high resolution images of different views, and of avoiding any overlapping of adjacent structures, and of identifying canal unions.

As regards age ranges, the higher prevalence of presence of mesiopalatal canals at 6 mm from the root apex was in patients of age less than 30 years (76.92% accounted for females and 60%, for males), and that this rate decreased with age increase. In patients between 30 and 40 years of age, prevalence of mesiopalatal canals was 53.85% for both males and females; in patients of more than 40 years old, both male and female, the prevalence rate was 51.85%. Even though literature states that the shape, size and number of root

canals depend on age due to dentine deposition, which turns canals smaller and thinner may be total obliterated<sup>13, 16,17</sup>, we did not find any statistically significant difference.

CBCT should not be used as routine practice in endodontic treatments since it exposes patients to a significant dose of radiation, and has a high cost<sup>18,19</sup>. Yet, this resource may be an alternative when, despite a correct procedure, successful treatment is

not achieved and mesiopalatal canals may not viewed with conventional X-Ray.

It is concluded that mesiopalatal canals were found in 56.25% of upper first molars with the methodology used. There was no statistical difference when gender was considered and CBCT scan has proved to be a valid resource for locating mesiopalatal canal, especially in cases where location was not feasible through clinical means.

## CORRESPONDENCE

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## EFFECT OF ANGIOTENSIN-CONVERTING ENZYME INHIBITORS ON VASCULAR ENDOTHELIAL FUNCTION IN HYPERTENSIVE PATIENTS AFTER INTENSIVE PERIODONTAL TREATMENT

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### ABSTRACT

There is a relation between vascular endothelial function, atherosclerotic disease, and inflammation. Deterioration of endothelial function has been observed twenty-four hours after intensive periodontal treatment. This effect may be counteracted by the action of angiotensin-converting enzyme inhibitors, which improve endothelial function. The aim of the present study was to evaluate vascular endothelial function after intensive periodontal treatment, in hypertensive patients treated with angiotensin-converting enzyme inhibitors.

A prospective, longitudinal, comparative study involving repeated measurements was conducted. Fifty-two consecutive patients with severe periodontal disease were divided into two groups, one comprising hypertensive patients treated with converting enzyme inhibitors and the other comprising patients with no clinical signs of pathology and not receiving angiotensin-converting enzyme inhibitors. Endothelial function was assessed by measuring post-ischemic dilation of the humeral artery (baseline echocardiography Doppler), and intensive periodontal treatment was performed 24h later. Endothelial function was re-assessed 24h and 15 days after

periodontal treatment. Statistical analysis: Results were analyzed using the SPSS 20 statistical software package. Student's *t* test and MANOVA were calculated and linear regression analysis with 95% confidence intervals and  $\alpha < 0.05$  was performed. Arterial dilation at 24 hours was lower compared to baseline in both groups; values corresponding to the groups receiving angiotensin-converting enzyme inhibitors were  $11.89 \pm 4.87$  vs.  $7.30 \pm 2.90\%$  ( $p < 0.01$ ) and those corresponding to the group not receiving ACE inhibitors were  $12.72 \pm 4.62$  vs.  $3.56 \pm 2.39$  ( $p < 0.001$ ). The differences between groups were statistically significant ( $p < 0.001$ ). Conclusion: The increase in endothelial dysfunction after intensive periodontal treatment was significantly lower in hypertensive patients treated with angiotensin-converting enzyme inhibitors. Endothelial function improved 15 days after periodontal treatment, reaching baseline values. These results support the protective effect of angiotensin converting enzyme inhibitors on the endothelial function after intensive periodontal treatment.

**Key words:** Hypertension, angiotensin-converting enzyme inhibitors, periodontal diseases.

## EFFECTO DE LOS INHIBIDORES DE LA ENZIMA CONVERTIDORA SOBRE LA FUNCIÓN DEL ENDOTELIO VASCULAR EN PACIENTES HIPERTENSOS QUE RECIBIERON TRATAMIENTO PERIODONTAL INTENSIVO

### RESUMEN

Existe relación entre la disfunción del endotelio vascular; la enfermedad aterosclerótica y la inflamación. A las 24 h del tratamiento intensivo de la enfermedad periodontal se produce un deterioro de la función endotelial. Este efecto podría ser balanceado por la acción de los inhibidores de la enzima convertidora de la angiotensina que mejoran la función endotelial. El objetivo del presente estudio fue evaluar la función endotelial vascular después del tratamiento periodontal intensivo, en pacientes hipertensos tratados con inhibidores de la enzima convertidora de la angiotensina. Se realizó un estudio prospectivo, longitudinal, comparativo, con mediciones repetidas. Se incorporaron 52 pacientes consecutivos, con enfermedad periodontal severa divididos en dos grupos, uno con hipertensión arterial tratados con inhibidores de la enzima convertidora y el otro sin inhibidores ni patología clínicamente evidente. Se determinó la función endotelial cuantificando la dilatación de la arterial humeral post isquemia ecocardiografía Doppler basal. A las 24 h se efectuó el tratamiento periodontal intensivo; a 24 h y 15 días posteriores se reevaluó la función

endotelial. Análisis estadístico: se empleó el paquete estadístico SPSS 20. Se realizaron: *t*-test de Student, MANOVA y análisis de regresión lineal con intervalos de confianza del 95% y  $\alpha < 0.05$ . Resultados: a las 24 h post tratamiento periodontal se observó una menor dilatación arterial en ambos grupos en relación a la dilatación arterial basal, siendo para el grupo con inhibidores  $11.89 \pm 4.87$  vs.  $7.30 \pm 2.90\%$ ,  $p < 0.01$  y para el grupo sin inhibidores  $12.72 \pm 4.62$  vs.  $3.56 \pm 2.39$ ,  $p < 0.001$ , con diferencias significativas entre ambos  $p < 0.001$ . En conclusión el aumento de la disfunción endotelial post tratamiento intensivo periodontal fue significativamente menor en hipertensos que recibieron inhibidores de la enzima convertidora de la angiotensina. La función endotelial mejoró a los 15 días de efectuado el tratamiento, alcanzando los valores iniciales. Estos resultados permitirían relacionar a los inhibidores de la enzima convertidora con un efecto protector del endotelio posterior al tratamiento intensivo de la enfermedad periodontal.

**Palabras Clave:** Hipertensión arterial, inhibidores de la enzima convertidora, enfermedad periodontal.

## INTRODUCTION

Vascular endothelial function (VEF) plays a fundamental role in the pathogenesis of atherosclerosis<sup>1</sup>. The endothelium plays an important part in maintaining vascular homeostasis, and is involved in hemodynamics and antithrombotic activity<sup>2</sup>. It consists of a single layer of cells that separates the circulating blood from the smooth vascular muscle, and produces signaling molecules that regulate vascular tone, monocyte and neutrophil adhesion, and platelet aggregation. The endothelium produces nitric oxide (NO)<sup>3</sup> from the amino acid L-arginine, through activation of NO synthase. NO plays a key role because of its potent vasodilator action. It inhibits leukocyte adhesion and platelet aggregation, and inhibits smooth muscle cell proliferation. Because NO acts as a free radical scavenger, it has antioxidant properties, and it is involved in the release of plasminogen activator, which has a fibrinolytic effect and antithrombotic properties.<sup>4-6</sup> There are certain substances that act on the vascular endothelium exerting a proinflammatory effect, and have the capacity to generate reactive oxygen species (ROS). The latter are responsible for the degradation of NO and modulate vascular tone. One of such substances with the strongest vasoconstrictor effect is angiotensin II.<sup>2-7</sup>

A disturbance in the balance between vasodilators and vasoconstrictors causes vascular endothelial dysfunction, with decreased production and increased degradation of NO. This alteration in VEF causes vasoconstriction, platelet aggregation, leukocyte adhesion, and proliferation of smooth muscle cells<sup>8-11</sup>. VEF is assessed by measuring brachial artery flow-mediated dilation following ischemia<sup>12</sup>. The latter is associated with NO released by the arterial endothelium in response to the shear stress in the blood flow. The decrease in NO release from dysfunctional endothelium generates a paradoxical vasoconstrictive response to acetylcholine<sup>4</sup>.

Other works reported in the literature have shown vascular endothelial dysfunction to be associated with a variety of diseases and risk factors for atherosclerosis, ranging from hypertension (HTN), aging<sup>13</sup>, dyslipidemia<sup>14</sup>, diabetes<sup>15</sup> and smoking<sup>16-17</sup>, to specific cardiovascular diseases (CVD), including coronary, cerebral, and peripheral arteriopathy<sup>14-16</sup> as well chronic infection and inflammation, such as periodontal disease.<sup>18-22</sup> Case-control-cohort studies have shown that periodontitis is associated with

endothelial dysfunction<sup>23</sup>, atherosclerosis<sup>24-26</sup>, and increased risk of myocardial infarction and stroke<sup>27-29</sup>. Despite these findings, the statement of the American Heart Association indicates that there is no causative relationship between periodontal and atherosclerotic vascular disease. It is of note, however, that both pathologies share risk factors, such as diabetes and smoking<sup>30</sup>. Tonetti M et al.<sup>31</sup> found that intensive periodontal treatment caused endothelial dysfunction during the first 24 h, though dysfunction improved 60 days after treatment. Deterioration of VEF leads to an increase in pro-inflammatory and thrombogenic potential, an increase in the likelihood of ischemic episodes, and a higher incidence of cardiovascular events in periods immediately after invasive periodontal treatment.

Hypertensive patients have vascular endothelial dysfunction<sup>32-34</sup> and administration of angiotensin-converting enzyme inhibitors (ACEI)<sup>35-38</sup> is a therapeutic pillar in the treatment of HTN. In addition to lowering blood pressure, renin-angiotensin system blockade with ACEI provides a rational approach to reverse endothelial dysfunction.

The beneficial effects of ACEI on VEF in hypertensive patients may go beyond HTN. The aim of the present study was to evaluate vascular endothelial function after intensive periodontal treatment, in hypertensive patients treated with ACEI.

## MATERIALS AND METHODS

A prospective longitudinal study involving repeated measures was conducted at the Departments of Periodontics and of Buccodental Pathology of the School of Dentistry, University of Buenos Aires, and the Department of Cardiology of the Spanish Hospital of Buenos Aires, between September 2010 and August 2014. The study design was similar to that used in controlled clinical trials. The study protocol was approved by the ethics committees of both institutions. Consecutive patients with severe periodontal disease (SPD) were divided into two groups: one group comprised patients with HTN treated with enalapril (ACEI) in doses according to blood pressure (cases), and the other group included non hypertensive patients (controls). The subjects were male and female outpatients over the age of 18 years. Patients receiving vasodilators, transplant patients, renal failure patients on dialysis, patients with concomitant infection or inflammation, pregnant and lactating patients, patients receiving

antibiotics within three months and/or periodontal treatment within 6 months prior to the study, patients with fewer

than 10 teeth, inadequately controlled hypertensive patients or with blood pressure values above 140/90 mmHg at the onset of the study, and patients who failed to sign the informed consent form, were excluded from the study.

The following covariables were recorded: sex, age, CVD, smoking, HTN, dyslipidemia and diabetes (DBT). The patients who met the inclusion criteria underwent VEF assessment (baseline), and received intensive periodontal treatment 24 h later. VEF assessment was repeated twenty-four hours and 15 days after periodontal treatment. The study design is shown in Fig. 1.

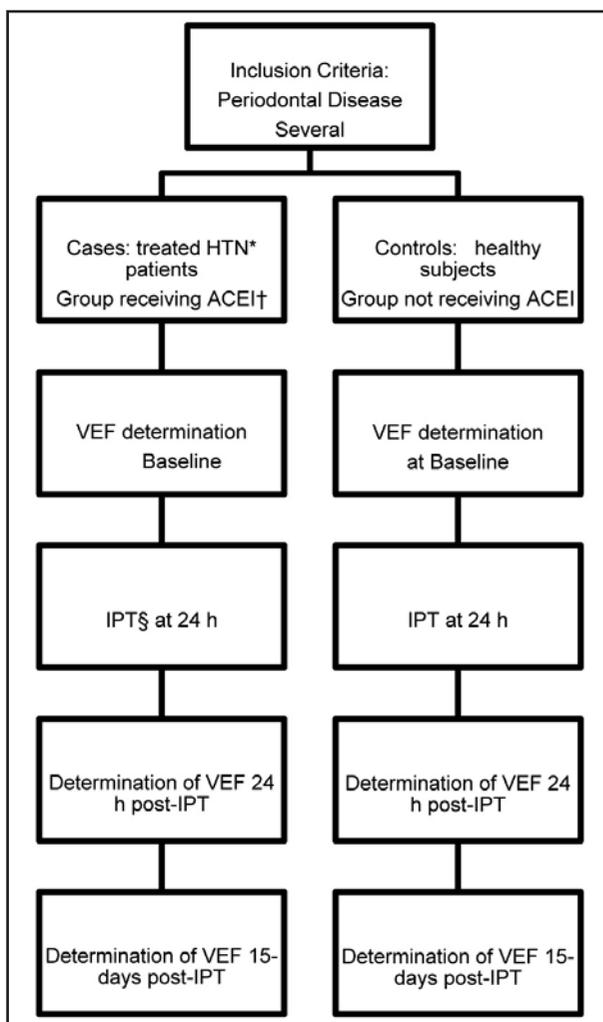


Fig. 1: Study Design. \*HTN: Arterial hypertension; †ACEI: Angiotensin-converting enzyme inhibitors; ‡VEF: Endothelial Vascular Function; §IPT: Intensive Periodontal Treatment.

All enrolled patients underwent clinical and radiographic dental examination. The radiographic study included a standardized periapical radiographs using a CS2200 - intraoral x-ray system (Carestream Health, Inc). All permanent teeth, except for the third molars, were evaluated. Patients with fewer than 10 teeth were excluded from the study in order to ensure that periodontal diagnosis was representative of the clinical dental status of the patient and avoid overestimation of diagnosis of periodontal disease.

Clinical periodontal examination was performed by a single calibrated operator. Periodontal measurements were performed at six sites per tooth (mesio-buccal, mid-buccal, disto-buccal, mesio-lingual/palatal, mid-lingual/palatal and disto-lingual/palatal) on all present teeth, using a manual Marquis Type periodontal probe (Hu-Friedy®). The studied periodontal parameters included Probing depth (PD), Clinical attachment level (CAL), Bleeding on probing (BOP), and radiographic alveolar bone loss (ABL), which were assessed following a standardized protocol.

Intra-examiner reproducibility of PD and CAL measurements was assessed before the study. The weighted  $K$  coefficients for PD and CAL were 0.96 and 0.91 respectively.

Diagnosis of periodontal disease was performed based on CAL values, in keeping with the 1999 International Workshop for a Classification of Periodontal Disease accepted by the American Academy of Periodontology (AAP)<sup>39</sup>.

Severe generalized periodontal disease (SPD) was established when:

- Average CAL values were  $\geq 5$  mm. This value was obtained by averaging all six determinations performed on each of the teeth. This method allows avoiding overestimation of periodontal disease diagnosis, since many patients have a high CAL value at only one periodontal site as a result of trauma (for example, trauma associated with tooth brushing, ill-fitting removable dentures, or harmful oral habits, among other causes).
- Average CAL values were  $< 5$  mm but with values  $\geq 5$  mm at more than 30% of studied sites, in addition to horizontal alveolar bone loss  $> 1/3$  of the root length in at least 30% of teeth, as measured on the radiographic images.

### Periodontal treatment

One hour before intensive periodontal treatment, all patients underwent a protocol of antibiotic prophylaxis, and intra and extra oral antiseptics was carried out. Intensive periodontal treatment was performed in a single session<sup>40</sup>. Under local infiltration anesthesia (lidocaine), root scaling and planing was performed using rigid Gracey-type curettes (Hu-Friedy®), numbers 5/6, 7/8, 11/12 and 13/14 according to the surface, and an ultrasound cavitation machine (cavitron plus scaler, Dentsply®).

### Endothelial function assessment

Endothelial vascular function was assessed in the morning after 12 h fasting, before smoking and after 15 minutes rest under controlled temperature conditions (22 - 26° C). An ECG lead and a blood pressure cuff were placed on the patient's forearm. Endothelial-dependent dilation was assessed with the FMD (Flow mediated dilation) of the brachial artery using Doppler ultrasonography, according to the Guidelines of the International Brachial Artery Reactivity Task Force<sup>41</sup>. The forearm was occluded by cuff inflation to at least 50 mmHg above systolic pressure for 5 min, resulting in a reactive hyperemia after the release of the cuff, and the increased shear stress led to endothelial-mediated vasodilatation. FMD was measured with an ultrasound scanner with a 7.5 MHz linear transducer (ATL HDI 3000); five consecutive discrete measurements were obtained and averaged into the final value at each time point. The coefficient of variation of brachial artery hyperemic flow values was calculated by dividing peak hyperemic flow by peak baseline flow. After 15 minutes, smooth muscle response to an exogenous stimulus was measured. Brachial artery

diameter was determined at baseline, and three times consecutively 3 minutes after sublingual administration of 0.4mg; the obtained values were averaged<sup>41</sup>.

### Statistical Analysis

The data were entered on an Excel spreadsheet and analyzed using the SPSS 20 software package. Frequency distribution and percentages of categorical variables were calculated. Number of cases, minimum value, maximum value, arithmetic mean, standard deviation of each variable measured on ordinal scale were calculated. Data were analyzed using Student's *t* test, MANOVA, and linear regression analysis, with  $\alpha < 0.05$  and 95% confidence intervals (CI). Sample size calculation: Because the statistical analysis was based on regression procedures, given an effect size of 0.35, a power of 0.8, and an alpha value of 0.05, and considering a maximum of 5 predictors, minimum sample size was calculated to be 43 patients.

### RESULTS

Out of a total of 86 patients studied initially, 52 patients with SPD were included in the study. The ACEI group comprised 51.9% (n=27) of the study population, and the remaining 48.14% (n=25) were included in the control group. The patients in the ACEI group received 14.20  $\pm$  4.25 mg of enalapril twice daily (IC 95% 12.51-15.88) for HTN treatment. Baseline values of both groups are shown in Table 1.

Severity of periodontal disease was similar in ACEI - treated patients and controls, as shown by the following results: present teeth 19.11  $\pm$  5.6 vs. 16.14  $\pm$  6.18 (p=0.258), CAL 3.49  $\pm$  1.49 vs. 4.39  $\pm$  1.72

**Table 1: Baseline characteristics of the population.**

CHARACTERISTICS	HTN* WITH ACEI (n27)	WITHOUT ACEI (n25)
Age in years	64.85 $\pm$ 9.7	62 $\pm$ 6.7
Female gender	11 (40.74%)	11 (44%)
Active smokers	1 (3.70%)	2 (8%)
Diabetes mellitus	2 (7.40%)	0
Hypercholesterolemia	8 (29.60%)	6 (24 %)
Systolic pressure in mmHg	121.36 $\pm$ 9.43	116.57 $\pm$ 11.14
Diastolic pressure in mmHg	79.48 $\pm$ 4,36	72.51 $\pm$ 6.23
†HR Bpm	64.38 $\pm$ 10.68	62.72 $\pm$ 8.42

\* HTN: Arterial hypertension ; † HR Bpm: Heart rate in beats per minute

mm ( $p=0.21$ ), PD  $3.45 \pm 0.63$  vs.  $3.59 \pm 0.64$  mm ( $p=0.61$ ), sites with BOP  $43.56 \pm 20.58$  vs.  $42.5 \pm 30.34$  ( $p=0.92$ ), and ABL  $36.87 \pm 14.64$  vs.  $38.7 \pm 10.99$  % ( $p=0.76$ ).

Baseline values of end-diastolic humeral artery diameter and nitroglycerin-mediated humeral artery dilation were  $4.3 \pm 0.50$  vs.  $4.7 \pm 0.3$  mm and  $19.32 \pm 8.62$  vs.  $20.64 \pm 6.43$ % respectively.

A significantly lower percentage of humeral artery flow mediated dilation was observed in both groups 24 h after intensive periodontal treatment, as compared to baseline values, showing deterioration of VEF (ACEI  $p<0.01$ ; Controls  $p<0.001$ ) (Table 2).

Deterioration of VEF was significantly greater 24 after intensive periodontal treatment in patients not

receiving ACEI ( $p<0.001$ ). VEF improved 15 days after treatment, and was not significantly different from baseline values (Fig. 2). ACEI treatment was a significant variable in the differences between groups, having an average impact of 26.7% (95% CI 1-49.2).

## DISCUSSION

A number of studies have demonstrated association between periodontal disease and CVD. The link between both pathologies remains to be elucidated in order to determine the causal, contributing or coincidental role of periodontitis in CVD, irrespective of shared risk factors. Periodontal disease is caused by an array of microorganisms, such as *Porphyromonas gingivalis*, which have the ability to invade endothelial cells<sup>42</sup>. In addition, periodontal pathogens have been identified in atherosclerotic plaque specimens obtained during carotid endarterectomy<sup>43</sup>.

These microorganisms can act directly on the endothelium or via a systemic inflammatory response, thus contributing to arterial damage<sup>44</sup>.

Atherosclerosis is a complex process involving a number of different cell types (monocytes, macrophages, T lymphocytes, smooth muscle cells, endothelial cells) and inflammatory molecules, such as C Reactive Protein (CRP), interleukin 1 $\beta$ , interleukin 6, and tumoral necrosis factor (TNF), among others<sup>19, 45-49</sup>.

Its pathogenesis involves a variety of immune mechanisms which interact with multiple causal factors capable of triggering, developing, perpetuating, and activating arterial lesions<sup>50</sup>.

The aforementioned factors include metabolic disorders such as DBT, hypercholesterolemia,

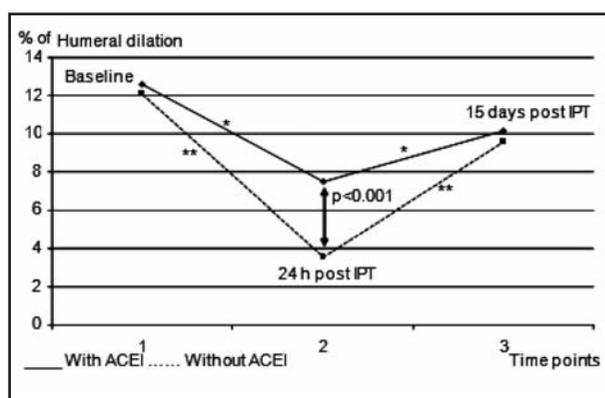


Fig. 2: Endothelial function at the different post-treatment time points. 1) Baseline determination: Humeral artery flow-mediated dilation; 2) Determination 24 h after intensive periodontal treatment; 3) Determination 15 days post-treatment.

\* Level of significance  $p<0.01$

\*\* Level of significance  $p<0.001$

Table 2: Endothelial function in the studied groups at the studied time points.

Time points	Group	VEF*	n	95 % IC
Baseline	Without ACEI	$12.72 \pm 4.62$	25	10.81-14.62
	With ACEI	$11.89 \pm 4.87$	27	9.96 -13.81
	Total	$12.29 \pm 4.72$	52	10.97-13.60
24 hs post treatment	Without ACEI	$3.56 \pm 2.39$	25	2.57-4.54
	With ACEI	$7.30 \pm 2.90$	27	6.15 -8.44
	Total	$5.50 \pm 3.25$	52	4.59 -6.40
15 days post treatment	Without ACEI	$10.12 \pm 3.38$	25	8.72 -11.51
	With ACEI	$12.19 \pm 5.00$	27	10.21-14.16
	Total	$11.19 \pm 4.38$	52	9.97-12.40

\* VEF: Vascular Endothelial Function, assessed by the percentage of humeral artery flow-mediated dilation

obesity, and other independent risk factors like hypertension, smoking, and infection<sup>51-56</sup>.

Elevated inflammatory reactants have been found in patients with periodontitis and vascular endothelial dysfunction<sup>57-58</sup>.

Underlying acute cardiovascular events, there is an inflammatory phenomenon that triggers plaque instability. Endothelial dysfunction is the initial pathophysiological step in a progression of vascular damage, and it is considered an early event in the development of atherosclerosis<sup>59</sup>.

Periodontal disease treatment would lower the inflammatory component and improve endothelial function (6 months after intensive periodontal treatment), as shown by Tonietti et al<sup>31</sup>, providing an additional benefit to VEF. It is well documented that HTN results in endothelial dysfunction<sup>60</sup>. Nevertheless, no significant differences in baseline VEF indices were observed between HTN patients and controls in the present study. This finding is most likely the result of treatment with ACEIs, which have been shown to improve endothelial dysfunction.<sup>61-62</sup>

The decrease in inflammatory reactants and improvement in VEF observed 6 months after intensive periodontal treatment<sup>31</sup> is preceded by a period of endothelial function deterioration and increase in inflammatory response immediately after treatment.

The increase in inflammatory reactants is shown by the increase in serum levels of RCP, IL-6, E-selectin, tissue plasminogen activator (tPA), plasminogen activator inhibitor type 1 (PAI-1), von Willebrand factor, and neutrophils.

As a result, during that period, the patient is at higher risk for a cardiovascular event. Moreover, a higher incidence of stroke and acute myocardial infarction has been observed in the first 4 weeks

after periodontal disease treatment.<sup>32</sup> It must be pointed out that around 40% of the patients studied here had coronary disease and an even higher percentage also had other risk factors for CVD. In agreement with findings reported by Tonetti et al, deterioration of VEF was observed immediately after intensive periodontal treatment (24h) in both groups, though the impact in the group receiving ACEI was lower.

Our results showed no significant differences between VEF determinations obtained at baseline and 15 days after intensive periodontal treatment.

No cardiovascular events occurred during the study, likely due to treatment with ACEI, in view of the beneficial effect of these drugs on the endothelium<sup>63</sup>. One of the limitations of the present study is the small number of patients. However, the differences in VEF observed after intensive periodontal treatment are so significant that they render the sample sufficient to demonstrate the protective effect of ACEI. Follow-up at 15 days does not allow establishing the incidence of cardiovascular events beyond that period. Further studies using longer follow-up periods are necessary to establish the incidence of events beyond the time point established in the protocol used herein.

## CONCLUSIONS

Endothelial dysfunction after intensive treatment of severe periodontal disease was significantly lower in patients receiving angiotensin converting enzyme inhibitors.

Endothelial function improved 15 days after treatment, returning to baseline values.

There are data that would seem to indicate a protective effect of ACEI on vascular endothelium after intensive treatment for severe periodontal disease.

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## DENTAL BLEACHING WITH OZONE: EFFECTS ON COLOR AND ENAMEL MICROHARDNESS

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### ABSTRACT

The aim of this *in vitro* study was to evaluate the effects of dental bleaching with ozone ( $O_3$ ) on color change and enamel microhardness. Enamel blocks (3 x 3 x 3mm) were randomly distributed for treatments ( $n=10$ ). Color change ( $\Delta E$ ) and Knoop microhardness of the enamel blocks were evaluated before and after the following treatments: C – deionized water (control); HP – 37.5% hydrogen peroxide (Pola Office+/SDI); PLA – placebo gel;  $O_3$  – ozone; and  $O_2$  – oxygen. Four 8-minute applications were used for HP and PLA, and one 19-minute application for  $O_3$

and  $O_2$ . One-way ANOVA revealed that  $\Delta E$  was not significantly influenced by the treatment ( $p = 0.112$ ). For the treatments with HP, PLA,  $O_3$  and  $O_2$ ,  $\Delta E$  was greater than 3.3. The paired *t* test showed significant decrease in microhardness after treatments ( $p < 0.001$ ) but no significant difference between treatments (ANOVA;  $p = 0.313$ ). Dental bleaching treatments with  $O_3$ , HP,  $O_2$  and PLA induced enamel color changes that may be clinically discernible, although enamel microhardness decreased.

**Key words:** Hydrogen peroxide, ozone, tooth bleaching.

## CLAREAMENTO DENTAL COM OZÔNIO: EFEITOS NA COR E NA MICRODUREZA DO ESMALTE

### RESUMO

O objetivo deste estudo *in vitro* foi avaliar os efeitos do clareamento dental com ozônio ( $O_3$ ) quanto à alteração de cor e microdureza do esmalte. Blocos de esmalte (3 x 3 x 3mm) foram aleatoriamente distribuídos entre os tratamentos ( $n=10$ ). Alteração de cor ( $\Delta E$ ) e microdureza Knoop foram avaliados antes e após cada um dos seguintes tratamentos: C – água deionizada (controle); PH – peróxido de hidrogênio a 37,5% (Pola Office+/SDI); PLA – gel placebo;  $O_3$  – ozônio;  $O_2$  – oxigênio. Quatro aplicações de PH e PLA foram realizadas por 8 minutos cada e uma aplicação de  $O_3$  e  $O_2$  foram realizadas por 19 minutos em cada bloco de esmalte. ANOVA a um critério mostrou que os valores de  $\Delta E$  não foram

significativamente influenciados pelo tratamento ( $p = 0,112$ ). Para os tratamentos com PH, PLA,  $O_3$  e  $O_2$ , o  $\Delta E$  foi maior que 3,3. O teste *t* pareado mostrou diminuição significativa dos valores de microdureza no final do tratamento quando comparado com o tempo baseline ( $p < 0,001$ ), mas não houve diferença significativa entre os tratamentos (ANOVA;  $p = 0,313$ ). O tratamento com  $O_3$ , PH,  $O_2$  e PLA levou a alteração de cor do esmalte clinicamente perceptível, embora tenha sido observada diminuição da microdureza do esmalte com a realização dos tratamentos.

**Palavras-chave:** Peróxido de hidrogênio, ozônio, clareamento dental.

### INTRODUCTION

With the aim of improving smile esthetics, in-office bleaching treatment or at-home techniques using individual trays have been developed to treat teeth with color changes.<sup>1-4</sup> In addition to hydrogen peroxide and carbamide peroxide - the most commonly used bleaching agents - dentists have used ozone, which may or may not be used together with hydrogen peroxide.<sup>5-8</sup>

Ozone is a naturally occurring gas composed of three oxygen atoms ( $O_3$ ). It is a highly oxidizing agent, able to partake in diverse chemical reactions

with organic and inorganic substances.<sup>9</sup> Its clinical use is via a mixture of oxygen and pure ozone, at a rate of 0.05 - 5% ozone and 95 - 99.95% oxygen. Due to its instability, the ozone molecule must be prepared immediately prior to clinical use, as long-term storage is impossible<sup>10</sup>. Ozone may be used safely in situations where diffusion is an important factor, such as in dental hard tissues, acting on organic substances of the tooth tissues.<sup>11</sup>

The effectiveness of short-term exposure to ozone has been shown in different studies. Tessier et al.<sup>8</sup> used ozone for bleaching stains caused by

tetracycline, finding it effective at three to five minutes at a concentration of 2100ppm  $\pm$  5% and flow rate of 615cc/min<sup>1</sup>. Other studies have used ozone application times of 40<sup>6</sup> or 60 seconds<sup>12</sup> with the same concentration and flow rate. Elhamid and Mosallam<sup>7</sup> reported the use of ozone at a concentration of 175 $\mu$ g/mL to 1mL<sup>3</sup>/s incorporated into a gel, for a period of 30 minutes. Zanjani et al.<sup>13</sup> applied ozone for four minutes, although no information was provided regarding concentration and flow.

Exposure to ozone has been found to reduce enamel stains by 28% after three minutes and by 56% after five minutes.<sup>8</sup> Other studies have compared the bleaching efficacy of ozone to other techniques. Elhamid and Mosallam<sup>7</sup> reported that an ozonized gel provided better dental bleaching than carbamide peroxide. In contrast, Manton et al.<sup>5</sup> applied ozone for 40 seconds every four hours over a layer of carbamide peroxide solution for 52 hours of bleaching treatment, finding no significant increase in bleaching effectiveness, and less bleaching effect when it was used prior to bleaching with peroxide. Zanjani et al.<sup>13</sup> demonstrated that hydrogen peroxide produced more color change than ozone applied for four minutes.

Regarding the use of peroxides, due to the long period of close contact between the bleaching agent and the tooth and the generally inherent acidic characteristics of the product components, porosity and surface erosion may be observed,<sup>3,14-18</sup> as well as a decrease in surface microhardness of the enamel and dentin.<sup>19-23</sup> Using ozone for bleaching purposes may produce a lesser side-effect on microhardness, as no residual bleaching agent remains in contact with the tooth.

Thus, the aim of this *in vitro* study was to evaluate the effects of ozone bleaching treatment in terms of color change and microhardness of the enamel surface. The null hypotheses were: a) ozone does not promote color changes on teeth; b) ozone does not cause changes to enamel microhardness.

## MATERIALS AND METHODS

Specimens were 100 blocks of human dental enamel, with 50 blocks assigned to color assessment and 50 to microhardness. For both experiments, the 50 blocks were randomly distributed and subject to five different procedures (n=10). The study factors were:

- A) Five treatments: control (C) – deionized water; ozone (O<sub>3</sub>), oxygen (O<sub>2</sub>), 37.5% hydrogen peroxide (HP) and a placebo gel without 37.5% hydrogen peroxide (PLA).
- B) Two evaluation times: before treatment (baseline) and after treatment.

The response variables were color change ( $\Delta E$ ) evaluated using the CIELab system, and microhardness measured quantitatively in Knoop hardness numbers (KHN) after staining and application of the treatment agents.

### *Obtaining and preparing the enamel blocks*

Following approval from the Research Ethics Committee (number 838.553), twenty-five human third molars with clinically sound crowns and without cracks or stains were used. The remains of the periodontal ligament were removed and the teeth stored in 0.1% thymol solution for one to six months.<sup>24</sup>

The dental crowns were cut using a doubled-sided diamond disc (Microdont Micro Usinagem de Precisão Ltda, São Paulo, SP, Brazil) and low-speed handpiece (Kavo do Brasil, Joinville, SC, Brazil). Enamel blocks 3 x 3 x 3 mm were then cut starting from the palatal and buccal aspects of each crown, under deionized water cooling to prevent enamel cracks. An average four enamel blocks were obtained from each crown and stored in relative humidity at a temperature of 37<sup>o</sup>  $\pm$  1<sup>o</sup> C throughout the experiment. They were randomly distributed among groups to evaluate color change and microhardness.

### *Preparation of the enamel blocks for staining and color analysis*

In order to create stains prior to treatment,<sup>25-26</sup> fifty enamel blocks were coated with wax (DentBras Ind. Com. Imp. Exp. de Prod. Odont. Ltda, Pirassununga, SP, Brazil) to limit the staining to surface enamel only and immersed individually in 2 ml Methyl Orange II solution (0.15 mM) for 15 days, with the solution being changed after 7 days.<sup>25-26</sup>

The 50 blocks were randomly assigned to the experimental groups (n=10) according to treatment: control (C) – deionized water; ozone (O<sub>3</sub>), oxygen (O<sub>2</sub>), 37.5% hydrogen peroxide (HP) and a placebo gel without the 37.5% hydrogen peroxide (PLA). Color was analyzed following Jaime et al.<sup>26</sup> The blocks were photographed before and after

bleaching procedures using a digital camera (Nikon D70, Nikon Factory, Ayuthaya, Thailand) and a macro lens with a built-in circular flash (Medical-NIKKOR, Tokyo, Japan). The digital camera was standardized to obtain photographs at a release speed of 1/125, diaphragm aperture F16, sensor sensitivity ISO 200, manual function with activated flash. The blocks were placed on a flat surface against a black background, with their outermost surface as parallel as possible to the horizontal plane. Images were assessed using image editing software (Photoshop CS6, Adobe Systems Ireland Ltd., San Jose, CA, USA) to generate numerical values, according to the CIELab system (Commission Internationale de l'Eclairage" - CIE). The CIELAB system was used with the parameters: L\* indicating lightness, ranging from 0 (black) to 100 (white); a\* representing the saturation of the red (+) and green axes (-); and yellow (+) and blue (-) represented by the b\* axis.

For the microhardness experiments, 50 enamel blocks were embedded in polyester resin (Maxi Rubber, Diadema, SP, Brazil). The enamel surface was planed using a pneumatic polisher (Ecomet 250, Buehler Ltd., Lake Bluff, IL, USA) with an attached head (Buehler Automet 250, Buehler Ltd., Lake Bluff, IL, USA) containing 600 and 1200 grit aluminum oxide sandpaper (Arotec S/A Ind. e Com., Cotia, SP, Brazil). Final polishing was performed with a synthetic velvet polishing cloth (Supra, Arotec S/A Ind. e Com., Cotia, SP, Brazil)

moistened with 3 µm diamond paste for metallography (Arotec S/A Ind. e Com., Cotia, SP, Brazil).

Enamel microhardness analysis was performed using a digital durometer (Pantec HVS 1000, Panambra, São Paulo, SP, Brazil) and Knoop-type penetrator, with a static load of 25 g for five seconds. Three microhardness indentations were performed at randomly selected sites on each enamel surface at each time (before and after treatment), with each indentation separated by a distance of 200 µm.

To avoid differences in the initial mean values (baseline) of enamel microhardness between groups, test specimens that presented similar microhardness values were selected. Fifty test specimens were randomly allocated to each experimental group (n=10): control (C) – deionized water; ozone (O<sub>3</sub>), oxygen (O<sub>2</sub>), 37.5% hydrogen peroxide (HP) and a placebo gel without 37.5% hydrogen peroxide (PLA).

#### Experimental treatments

Table 1 provides the specifications of the agents used. The pH of the agents (deionized water, HP and PLA) was measured with a pH-meter (W3B, Bel Equipamentos Analíticos Ltda, Piracicaba, SP, Brazil), using the mean of three values. The pH values were measured shortly after handling HP and PLA at baseline and eight minutes.

For the control group (C), 0.01 ml deionized water was applied for 8 minutes, after which it was

**Table 1: Treatment agents, composition and manufacturer.**

Treatment agent/ Commercial name/ Lot number	Composition	Manufacturer (city, state, country)	Mode of use	pH value	
				Baseline	8 minutes
Distilled water (C)	H <sub>2</sub> O	Campinas, SP, Brazil	Four 8-minute applications	6.73	7.29
Ozone (O <sub>3</sub> )	O <sub>3</sub> + O <sub>2</sub>	Ozone & Life Ind. Com. and Sis. Ltda, São José dos Campos, SP, Brazil	One 19-minute application	-	-
Oxygen (O <sub>2</sub> )	O <sub>2</sub>	São José dos Campos, SP, Brazil	One 19-minute application	-	-
Hydrogen peroxide (HP)/ Pola Office+/ P1307011; P1310121	37.5% hydrogen peroxide; water; glycol polyethylene; blue coloring; potassium nitrate	SDI Bayswater, Victoria, Australia	Four 8-minute applications	6.53	6.96
Placebo (PLA)	Carbopol gel without coloring and carbopol gel with blue coloring	Farmö Manipulação e Cosméticos, Aracaju/SE, Brazil	Four 8-minute applications	6.76	6.34

removed from the surface with gauze, and the procedure repeated a further three times (four applications altogether). Then the enamel surface was washed with deionized water for 10 seconds and dried with gauze.

For the O<sub>3</sub> group, an ozone gas generator was used (Ozone & Life Ind. Com. and Sis. Ltda, O&L 1.5M, São José dos Campos, SP, Brazil) with an output of 80 W, connected to an oxygen cylinder via a flow control valve.<sup>27</sup> In order to apply the gas directly to the enamel blocks, a 1L volume rectangular stainless steel box with a side opening for entrance and exit was created, so that the entire volume of the box could be filled with gas. The enamel blocks were lined up in the box, allowing simultaneous exposure of the enamel to the gas. The box was then sealed with adhesive tape and the ozonization performed in a fume cupboard. Dose was adjusted in terms of ozone concentration, in ppm, and application time in minutes. An ozone concentration of 4mg/L was used, at an oxygen flow of ¼ l/min, as per the manufacturing guidelines. This concentration and flow-rate were based on the premise that 1mg/L corresponds to 667 ppm, and since an approximate value of 2100 ppm was desired,<sup>8,27</sup> the closest value (4 mg/L), as per the table provided by the manufacturer was used. Total application time was 19 minutes, with the first four minutes corresponding to total filling time of the 1L box with ozone, plus 15 minutes of concentrated application. This protocol was adapted from Tessier et al.<sup>8</sup> and Zárata et al.<sup>28</sup> in order to maximize the effectiveness of the ozone treatment.

For the O<sub>2</sub> group, an oxygen cylinder with flow control valve was used. The gas was applied using the same stainless steel box. Dose was adjusted in terms of oxygen concentration applied, in ppm, and exposure time in minutes, with the oxygen flow rate used being ¼ l/min (approximately 2100 ppm). Total application time was 19 minutes, with the first 4 minutes being the time taken to fill the box to a maximum capacity (1L) plus 15 minutes of oxygen application.

For the HP group, a bleaching gel containing 37.5% hydrogen peroxide (Pola Office Plus, SDI Limited, Bayswater, Victoria, Australia) was used. A thin layer (0.01 ml) of gel was applied to each surface and left for eight minutes. The gel was then removed from the surface enamel and reapplied as described above for group C.

For the PLA group, the gel was similar to the commercial product (HP) but lacking hydrogen peroxide. A thin layer (0.01 ml) of gel was applied to the enamel surface and left for eight minutes. The gel was removed from the surface with gauze and the procedure repeated as described above for the C and HP groups.

Following treatment application, the enamel blocks were stored in a humid environment for 12 hours before color and microhardness evaluation.

Prior to statistical analysis, the data for color and microhardness were evaluated for the assumptions of normality (Shapiro-Wilk test) and homogeneity of variance (Levene test), both of which were confirmed. The initial color (parameters L\*, a\* and b\*) and microhardness values were compared to the final values using the paired t test. Color changes ( $\Delta E$ ) were obtained via the CIELab system using the formulas:  $\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$ , where  $\Delta E$  is color change;  $\Delta L = L_{\text{final}} - L_{\text{initial}}$ ;  $\Delta a = a_{\text{final}} - a_{\text{initial}}$ ; and  $\Delta b = b_{\text{final}} - b_{\text{initial}}$ . An  $\Delta E$  value of <sup>3</sup> 3.3 is considered discernible clinically.<sup>29</sup>

To ascertain the presence of a significant difference between the treatments applied to the enamel blocks in terms of  $\Delta L$ ,  $\Delta a$ ,  $\Delta b$ ,  $\Delta E$  and  $\Delta KHN$ , one-way analysis of variance (ANOVA) was used. The Tukey test was applied for multiple comparisons. Confirmation of correlation between the enamel color and microhardness values following different treatments was performed using the Pearson test. All statistical tests adopted a significance level of 5%, using SPSS 20 (SPSS Inc., Chicago, IL, USA).

## RESULTS

One-way analysis of variance revealed no significant difference for  $\Delta E$  values for the treatments applied to the enamel blocks ( $p = 0.112$ ) (Table 2). However, with the exception of the control group (immersed in deionized water), all the other groups had  $\Delta E$  values higher than 3.3.

The paired t test was applied to compare initial and final Knoop microhardness values, independently of treatment. Knoop microhardness decreased significantly compared to baseline ( $p < 0.001$ ) for all groups, but there was significant difference in  $\Delta KHN$  values among groups ( $p = 0.313$ ) (Table 2).

Pearson's test did not reveal significant correlation between  $\Delta E$  values and Knoop microhardness changes ( $\Delta KHN$ ) ( $p = 0.415$ ;  $r^2 = -0.118$ ) (Fig. 1).

**Table 2: Means and standard deviations for the parameters shade ( $\Delta E$ ) and Knoop microhardness ( $\Delta KHN$ ) of the enamel, according to treatment.**

Group	C	O <sub>3</sub>	O <sub>2</sub>	HP	PLA	ANOVA
$\Delta E$	3 (2)	6 (3)	5 (3)	4 (3)	4 (2)	p = 0.112
$\Delta KHN$	-15 (110)	-83 (84)	-31 (44)	-67 (72)	-70 (87)	p = 0.313

$\Delta$ : Final – initial value; C: control (distilled water); O<sub>3</sub>: ozone; O<sub>2</sub>: oxygen; HP: 37.5% hydrogen peroxide; PLA: placebo.

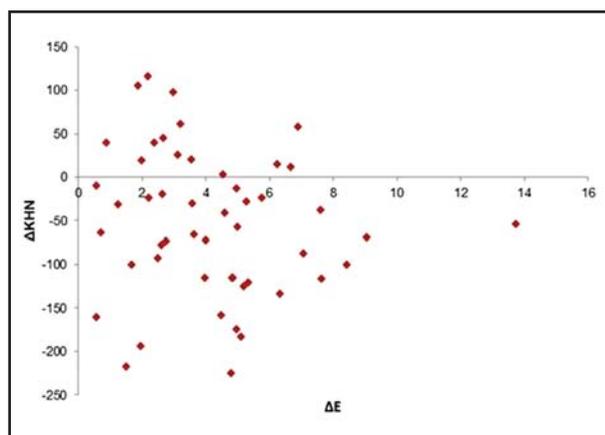


Fig. 1: Dispersion diagram of  $\Delta E$  in terms of  $\Delta KHN$  values.

## DISCUSSION

Treatment effectiveness and safety are among the primary factors to consider when selecting a technique or bleaching agent.<sup>1</sup> The effectiveness of a bleaching agent is evaluated by objective methods based on the  $L^*$ ,  $a^*$ ,  $e b^*$  (CIELab) system, which is appropriate for scientific research and provides definitions of parameters that can be evaluated quantitatively.<sup>30-31</sup>  $\Delta E$  describes color change by including these three dimensions of color, and values above 3.3 are considered clinically discernible color changes proving the effectiveness of bleaching procedures.<sup>32-33</sup> With the exception of the control group, all treatments in this study produced  $\Delta E$  values above 3.3, confirming that they were effective in promoting color change.

The teeth used to explore color change were stained prior to treatment in order to enhance the results of the treatment agents, following methods validated in previous studies.<sup>25-26</sup> The greatest mean  $\Delta E$  value was observed for ozone, although there was no significant difference with the other treatments. Other studies have also demonstrated the effectiveness of bleaching following application of ozone.<sup>7-8</sup> Ozone is an unstable gas that rapidly

releases nascent oxygen molecules to form oxygen.<sup>11</sup> It is a highly oxidizing agent, capable of participating in diverse chemical reactions with both organic and inorganic substances.<sup>9</sup> The ozone molecule and, in particular, the radical hydroxyl ( $OH^\cdot$ ), a non-selective oxidant formed by ozone decomposition, play an important role in the oxidation process.<sup>7,34</sup> Ozone decomposes in aqueous solution, which is the starting point in the formation of the peroxide radical ( $HO_2^\cdot$ ) and, subsequently, hydroxyl radicals ( $OH^\cdot$ ). These reactions occur simultaneously, however, chromophore groups may be broken by ozone, forming smaller molecules, resulting in a tooth bleaching effect by one of three of the following fundamental mechanisms: a) bonding mechanism, which occurs via the addition of ozone by a double bond to form ozonide in the presence of non-aqueous solvents yet, once water is present, ozone is hydrolyzed to other products via cleavage of the double bond that would have occurred; b) substitution mechanism, where one atom or functional group is replaced by another one; c) cleavage mechanism, in which a carbon-carbon bond is separated to produce composite organic fragments.<sup>9</sup>

Nevertheless, ozone might be incapable of such intense penetration when compared to other peroxide-based bleaching agents, since a greater number of applications are required to achieve satisfactory results.<sup>28,35</sup> However, in our study, the color change produced by ozone was similar to that of hydrogen peroxide, probably due to the ozone protocol used. Zárate et al.<sup>28</sup> used a low concentration (0.5 ppm) for 20 minutes daily, which is different from our study, which used 2100 ppm. Manton et al.<sup>5</sup> analyzed the use of ozone prior to and simultaneously with bleaching with carbamide peroxide and were unable to verify an increase in bleaching effectiveness with the combined treatment. However, they used an application time of only 40 seconds, confirming that there is variability among ozone application methods and therefore divergent

results would be expected. Tessier et al.<sup>8</sup> demonstrated the possibility of effective bleaching with ozone on even more severely stained teeth, such as in situations of staining secondary to tetracycline, using a short exposure time (3 to 5 min). Longer bleaching sessions may increase bleaching efficacy, since chromophore saturation of the dental tissues occurs gradually.<sup>36</sup>

In our study,  $\Delta E$  values greater than 3.3 were also observed for the placebo and oxygen groups. Although we did not expect this color change for the oxygen group, it may be explained by oxygen being a potential oxidant, which is the basis of bleaching treatments. In accordance with Abdelaziz et al.<sup>34</sup> although the oxidation potential of oxygen is five times lower than that of ozone, it may explain the bleaching capacity of the two gases used in this study. Additionally, ozone is 10 times more soluble than oxygen<sup>13,37</sup> and since the samples were submitted to ozone and oxygen treatments in a dry environment, ozone may not have had the diffusion expected on the substrate and, consequently, may not have produced a difference in enamel color change.

Other studies have also shown that hydrogen peroxide bleaching leads to noticeable color change.<sup>1,2,4,15,32-33,36</sup> Its mechanism of action is based in its ability to generate active oxygen and free radicals such as perhydroxyl, which are highly reactive, extremely electrophilic and unstable, degrading organic molecules to acquire stability and creating other radicals. The radicals may easily react with most unsaturated bonds, resulting in mono- or di-hydroxylation of the bonds. These agents react with highly coupled organic molecules, which can break the coupled electrons and change the energy absorption of the molecule. Hydrogen ions ( $H^+$ ) are produced in the process of breaking down hydrogen peroxide, which may produce a relatively acidic environment and may affect tooth surface and subsurface integrity.<sup>38</sup>

Some bleaching products are made with low pH values in order to guarantee the stability of hydrogen peroxide, an important factor in the bleaching reaction process.<sup>16</sup> However, consecutive bleaching sessions at high concentrations of hydrogen peroxide may lead to micromorphological and chemical changes to the enamel structure.<sup>14,33,36</sup> Therefore, evaluation of surface microhardness is frequently used to assess the effects of bleaching agents in terms of changes to the

mineral content that may occur to the tooth.<sup>18,20,23</sup> This study found that regardless of treatment, microhardness decreased significantly, with no difference between treatments. This decrease in surface microhardness resulting from bleaching is a concern, with other studies corroborating these results when using hydrogen peroxide at high concentrations.<sup>3,18-21,23,32,39</sup>

In contrast, other studies have not observed significant changes in enamel microhardness,<sup>4,15</sup> a difference which may be explained by the methods used, exposure time, composition, pH and concentration of bleaching agent, as well as by differences in the evaluation time intervals of the treatment. It should be noted that although in our study the action time for the bleaching gel was similar (four 8-minute applications) to the study by Borges et al.<sup>4</sup> (three 10-minute applications), there was a difference in the composition of the bleaching agent.

We used a bleaching gel and placebo agent containing the thickening agent cabopol, which has acidic properties,<sup>40</sup> whereas Borges et al.<sup>4</sup> used a bleaching agent containing an acrylic thickener. In addition, in the studies by Borges et al.<sup>4</sup> and Lia Mondelli et al.<sup>23</sup> the specimens were stored in artificial saliva, providing the potential for remineralization from the solution to the substrate, whereas we used deionized water, which is sub-saturated in terms of the mineral content of the enamel and may have led to decreased hardness.<sup>41</sup> Tahmassebi et al.<sup>24</sup> found no decrease in enamel microhardness using ozone, suggesting that it does not alter the mineral content of the enamel, and Duggal et al.<sup>42</sup> observed no reduction in microhardness when using ozone with or without a fluoride product. However, our study revealed a decrease in microhardness for the group treated with ozone, probably due to the different methods used, with longer application time (19 minutes compared to a 60 seconds),<sup>24,42</sup> and storage of the test specimens in a humid environment with deionized water, which may have reduced enamel surface hardness,<sup>41</sup> whereas in other studies, the enamel was immersed in artificial<sup>24</sup> or natural saliva, in which the demineralization process may be interrupted or reversed by dislocation of ionic changes on the tooth surface in favor of remineralization.<sup>42</sup>

It is well known that dental enamel becomes demineralized at a critical pH value (lower than 5.5).

Bleaching agents with low pH levels may lead to significant enamel erosion,<sup>16</sup> which, interestingly, is also seen when pH is above the critical level.<sup>43</sup> Our study used a bleaching agent with mean pH 6.81. Bleaching agents with high concentrations of hydrogen peroxide, even at neutral pH values, are capable of demineralizing dental enamel<sup>3</sup> while producing effective bleaching.<sup>18</sup>

Our study also found a decrease in microhardness in relation to baseline values for the control, placebo and oxygen groups. This was to be expected for the control group, where the tooth surface remained in contact with deionized water, since this solution is subsaturated with minerals in comparison to the enamel.<sup>41</sup> For the placebo group, the results are in agreement with the findings of Basting et al.,<sup>40</sup> in which the thickening agent in the bleaching gel and placebo was carbopol, showing a continuing demineralization of the enamel and dentin with application time, even at neutral pH, causing significant changes to dental microhardness. For the oxygen group, this decrease in microhardness may be explained by the similar mechanism of action as ozone, and although oxygen is five times less oxidant than ozone, it still has high oxidation potential.<sup>34</sup> Bocci et al.<sup>37</sup> described how ozone can promptly react with the substrate, while oxygen generally requires a catalyzer to start its reaction.

The remineralization produced by saliva and/or its substitutes containing calcium and phosphate

allow maintenance of the baseline microhardness values<sup>23,41,44</sup>, which might have led to increased enamel microhardness for all groups, had artificial saliva been used to store the test specimens in this study. Moreover, although the post-bleaching period was not studied, an increase in microhardness might be expected as a result of the constant contact with saliva and/or re-mineralizing agents used in the treatments with high concentration hydrogen peroxide.<sup>21</sup>

Pearson's test did not reveal a significant correlation between color change ( $\Delta E$ ) and Knoop microhardness ( $\Delta KHN$ ). Therefore, the bleaching agent might act on the chromophores in the tooth structure to promote bleaching effectiveness, as well as altering the mineral content in an isolated manner.

The limitations of this study include short exposure time and evaluation to confirm stability of the bleaching treatments and lack of an artificial saliva immersion step, which may have the potential to remineralize the enamel. Further studies are needed to evaluate the possible structural changes to the enamel following the application of ozone for dental bleaching. In conclusion, the data obtained indicate that treatment with ozone, hydrogen peroxide, oxygen and placebo cause color change to the dental enamel that may be discernible clinically and decrease in dental enamel microhardness, regardless of bleaching treatment type.

## CORRESPONDENCE

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## EFFICACY OF THREE THERMOPLASTIC OBTURATION TECHNIQUES IN FILLING OVAL-SHAPED ROOT CANALS

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### ABSTRACT

The purpose of this study was to assess the efficacy of the thermoplastic filling techniques, Touch'n Heat<sup>®</sup>, TC<sup>®</sup> System and Tagger's Hybrid Technique, in oval-shaped canals at the apical third. Thirty-three human uniradicular lower pre-molar teeth were treated by the reciprocating movement technique and were subsequently split into 3 groups, according to the filling technique performed: Touch'n Heat (TH), TC System (TC) and the Tagger's Hybrid Technique (TG). In the sequence, the teeth were sectioned at 2mm and 4mm from the foramen and images were taken to measure the percentage of canal area filled with the obturation

materials as well as void spaces. Data were submitted for Kruskal-Wallis statistical analysis. Irrespective of levels, data showed that the TC System delivered the best results. ( $p < 0,001$ ). At 2mm and 4mm levels, there was no difference between the TG technique and the TH technique ( $p < 0,001$ ). With all the techniques and at all levels, no differences were observed regarding the void area variable. ( $p > 0,001$ ). The techniques evaluated showed an adequate filling with obturation materials and the TC has reached the highest filling with the gutta-percha material.

**Key words:** Endodontics; root canal therapy; dental materials.

## EFETIVIDADE DE TRÊS TÉCNICAS DE OBTURAÇÃO TERMOPLÁSTICAS NO PREENCHIMENTO DE CANAIS OVAIS

### RESUMO

O objetivo desse trabalho foi avaliar a eficiência de preenchimento das técnicas termoplásticas Touch'nHeat<sup>®</sup>, Sistema TC<sup>®</sup> e Híbrida de Tagger em canais de anatomia oval no terço apical. Trinta e três dentes pré-molares inferiores humanos unirradiculares foram instrumentados pela técnica do movimento reciprocante e posteriormente separados em 3 grupos segundo a técnica de obturação: Touch'nHeat, Sistema TC e Híbrida de Tagger. Em sequência os dentes foram seccionados nos níveis de 2 mm e 4 mm a partir do forame e imagens foram capturadas para mensurar a porcentagem da área do canal preenchida com os materiais obturadores e os espaços vazios. Os dados foram

tabulados e submetidos ao teste estatístico de Kruskal-Wallis. Independente dos níveis, os dados mostraram que a TC apresentou os melhores resultados. ( $p < 0,001$ ). Nos níveis de 2 e 4mm não houve diferença entre as técnicas TG e TH ( $p < 0,001$ ). Em todas as técnicas e níveis não foram observadas diferenças com relação a variável áreas vazias. ( $p > 0,001$ ). As técnicas avaliadas demonstraram um adequado preenchimento pelos materiais obturadores, com destaque para o preenchimento superior com gutta-percha pela técnica TC.

**Palavras chave:** Endodontia; obturação do canal radicular; materiais.

### INTRODUCTION

The main strategy followed for removing microorganisms from root canal systems is chemo-mechanical debridement. Yet, due to the acknowledged anatomical complexity of pulp cavities, microorganisms still remain inside them after the chemical and mechanical preparation stages. Thus, through obturation, periradicular tissues are sealed to prevent the presence of such residual microorganisms<sup>1</sup>.

Filling materials frequently used for obturation practices are gutta-percha and endodontic sealer. There are several different obturation techniques. The major difference among them is the way in which

gutta-percha is used for filling in combination with sealer. Lateral Condensation (LC) is a very popular technique as it is quite safe in order to control of the root canal filling<sup>2</sup>, yet, it has been proved that LC does not effectively fill root canal systems and frequently leaves void spaces and a not too homogeneous distribution of sealers<sup>3,4</sup>. Thermoplastic gutta-percha techniques have been performed in most of the treatments due to its advantages for not being too time-consuming and for delivering better filling levels, compared with the LC technique<sup>4-7</sup>.

There are several different systems for plasticizing gutta-percha. The main advantage of Touch'n Heat<sup>®</sup>

(Analytic Endodontics, Orange, CA) is that it is a simple technique<sup>8</sup>.

Tagger's Hybrid technique uses stainless steel or nickel-titanium rotary instruments, the gutta condensers for mechanically plasticizing gutta-percha cones<sup>9</sup>. This technique is quite widespread, but not easily accepted, mainly by students, because its execution is not easy and because of possible accidents and complications when performing this technique. The TC<sup>®</sup> System (Tanaka de Castro & Minatel Ltda., Cascavel, PR, Brazil) consists in filling canals with previously plasticized gutta-percha in a specific heating device<sup>10</sup>. This is a Brazilian system, not very popular, and its execution requires some degree of training, but it has showed relevant in vitro results in relation to other thermoplastic techniques due to the optimal filling of root canals<sup>6,7,11</sup>.

Even if filling of canals through thermoplasticized gutta-percha gives excellent results, with oval-shaped canals this is still a difficult task<sup>12,13</sup>. The main thermoplastic techniques are now widely used due to their better filling levels, yet, their quality depends on the shape of root canals<sup>11</sup>.

Considering the growing acceptance and use of thermoplastic obturation techniques, the present study was designed for assessing the filling efficacy of thermoplastic techniques such as Touch'n Heat<sup>®</sup>, the TC<sup>®</sup> System and the Tagger's Hybrid in oval-shaped canals.

## MATERIALS AND METHODS

This study was submitted to the Ethics Committee for Research in Human Beings of the Federal University of Mato Grosso do Sul (UFMS) and was approved with Approval # CAAE 42313214.9.0000.0021.

Thirty-three human uniradicular pre-molar lower teeth, extracted for different reasons, were used in the study. The initial length and the verification of presence of a single canal, the absence of root fractures and the complete formation of the apex, were analyzed, both by visual inspection and through X-Rays. The oval-shaped anatomy of these teeth was evaluated by Cone Beam Tomography (I-cat, Kavo Kerr, Germany). Thus, through axial sections, rounded canals were checked for and rejected, and only oval-shaped canals in the third apical of root canals (5mm apical of root) were considered for standard samples.

The root canals were prepared with the Reciproc<sup>®</sup> System (VDW, Munich, Germany), up to the R40

file size, as the initial apical instrument has traditionally been a hand-held ST K#20 file (VDW, Munich, Germany). Sodium hypochlorite (2.5%) was used as auxiliary chemical irrigating solution during the procedure, with an approximate solution volume of 20 ml. per canal. Before obturation, passive ultrasonic irrigation (PUI) was performed, the solution was shaken by a specific ultrasonic tip - *irrisonic* (Helse, Ind. & Com., Santa Rosa de Viterbo-SP, Brazil) coupled to a Jet Sonic ultrasonic device (Gnatus, Ribeirão Preto, Brazil) with the following sequence: irrigation with 2,5% sodium hypochlorite and ultrasonic 20-second activation, flooding with EDTA Trisodium (Biodinâmica, Ibiporã-PR, Brazil); again, a 20-second activation and, once again, irrigation with 2,5% sodium hypochlorite and ultrasonic 20-second activation, totaling a 1-minute activation of the irrigating solution. In the sequence, the canals were thoroughly irrigated with 10 ml of saline solution.

After preparation, as described above, the specimens were randomly distributed into 3 groups, according to the obturation technique being tested: Group 1 (TC<sup>®</sup> System), Group 2 (Touch'n Heat<sup>®</sup> System), and Group 3 (Tagger's Hybrid Technique), each group having 11 specimens. The endodontic sealer used was AH Plus<sup>®</sup> (Dentsply Maillefer<sup>®</sup>, Ballaigues, Switzerland).

### Group 1: TC<sup>®</sup> System (TC)

After drying the canal, the endodontic sealer (AH plus<sup>®</sup>) was introduced with a paper cone having the same diameter of R40 file within the canal, in the working length. The TC<sup>®</sup> System syringe was placed in the heating device. After indication of plasticizing of the gutta-percha (Average: 2.5 minutes), a ST #25 gutta-condensor (Dentsply Maillefer<sup>®</sup>, Ballaigues, Switzerland) was coated, in its active part, with the plasticized gutta-percha and was immediately introduced and activated at a 10,000 rpm speed with short in/out movements (piston movements), turning in a clockwise motion until reaching approximately 1mm shorter than working length. It was then immediately withdrawn against one of the canal walls, also using "piston movements". This procedure took approximately 5 seconds. After removing the gutta-condensor from the canal, a vertical condensation was performed with a cold hand plugger until the gutta-percha was entirely cooled down (hardening of the material).

**Group 2:** Electrical heat source Touch'n Heat® (TH) Prior to obturation, the tip of the device was selected so that it would reach up to 5mm shorter than working length. The power level that was used was 10 (200°C). Working in a sequence, the master M gutta-percha cone (Odous De Deus®, Belo Horizonte-MG, Brazil), tip #40, corresponding to the shape left by the R40 Reciproc file, was introduced, coated with the sealer (AH Plus®), with in/out motions so as to contact all the canal walls and reach the working length. The following step was activating the tip that was advanced through the gutta-percha cone in a single motion until reaching the 5mm limit shorter than the working length. At this moment, the heating process was interrupted and pressure was kept stable until completing the 10-second lapse, which lapse was comprehended from the onset of the procedure. After this, the device pen was tapped again for resuming heating and for withdrawing the tip from the root canal. In the sequence, cold hand pluggers were used for vertical condensation.

**Group 3:** Tagger's Hybrid Technique (TG)

The adaptation of the master M gutta-percha cone (Odous De Deus®, Belo Horizonte-MG, Brazil) was

performed by adjusting its gauge at #40; the cone was coated with sealer (AH Plus®), and both the gutta-percha and the sealer were introduced into the root canal, as described with Group 2. By using digital spacers, some secondary XF gutta-percha cones (Dentsply Maillefer®, Ballaigues, Switzerland) were added. The number of these cones varied according to the gauge of the root canal subject to obturation, thus offering a supply of gutta-percha enough for filling the apical third.

Immediately after this, a gutta-condensor was selected, its gauge two numbers above the gauge of the master gutta-percha cone, that is, # 50 (Dentsply Maillefer®, Ballaigues, Switzerland). Then, the compactor was inserted until reaching resistance, and turned on at a clockwise rotation at 10,000 rpm, with to-and-fro movements until reaching a 3mm depth shorter than the working length. After withdrawing the compactor from the root canal, a vertical condensation procedure was performed with cold hand pluggers.

After obturation, the specimens were kept in a heating device at 37°C, with 100% humidity levels for seven days. After that period, the roots were measured with a digital caliper (Black Bull, USA) and marked at 2mm and 4mm from the apical foramen. The sections were made with a double face diamond disk # 7020, width: 0.10mm ((KG Sorensen, Cotia-SP, Brazil), assembled on a handpiece at 10,000 RPM and under constant cooling conditions derived from the handpiece and an additional water jet directed towards the section area. The resulting sections were fixed on the microscope stage (Carvalhoes, Gravataí-RS, Brazil) with *blu tac* (Bostik, UK). This helped correcting any tilting of the specimens for getting a better focus of the Stereoscopic Magnifying Glass (Coleman, Santo André-SP, Brazil). The sections were examined with a 40-times magnification, and images were taken with a digital camera (Cyber-Shot DSC-WX10, Sony, Japan).

Images were measured with software Axion Vision LE (Carl Zeiss, Thornwood-NY, USA), where the total area of the canal was gauged, as well as the voids and areas filled with gutta-percha and sealer. Measures were taken at a 1:1 scale on the inserted images, and pixels were estimated. Such measures were transformed into percentages of void areas, and sealer and gutta-percha filled areas. (Fig. 1)

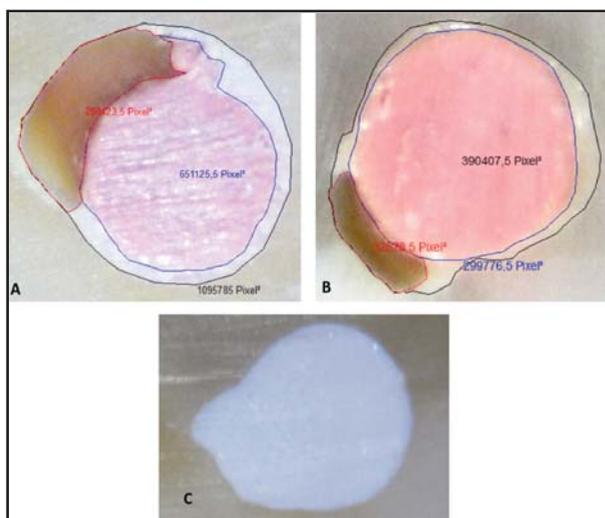


Fig. 1: Void area contoured by the red line; the blue line shows the limit of the gutta-percha, and the black line shows the total canal area. The endodontic sealer was calculated based on the remaining area (Sealer Area = [Total Area – (gutta percha + void area)]. 2mm Level, “A” Tagger’s Hybrid Technique, and in “B”, the Touch’n Heat, and in “C”, the TC System with the entire canal area filled with gutta-percha. The measurements was made in pixels.

In the statistical analysis done for comparing the different techniques, the Kruskal-Wallis test with a significance level of 5% ( $\alpha = 0,05$ ) was used.

**RESULTS**

When the different techniques were evaluated, irrespective of levels, data showed that the TC System delivered the best results. ( $p < 0,001$ ). At 2mm and 4mm levels, there was no difference between the TG technique and the TH technique ( $p < 0,001$ ). With all the techniques and at all levels, no differences were observed regarding the void area variable. ( $p > 0,001$ ).

Data of the filling percentages of gutta-percha, sealer and void areas, obtained from the two assessed levels, groups TC, TG and TH are shown in Fig. 2, 3 and 4.

**DISCUSSION**

One of the factors that may have an impact on the filling supplied by obturation was related to root canal anatomy. Oval-shaped canals are difficult to clean and fill<sup>14</sup>. In this regard, three obturation techniques were evaluated, that used thermoplasti-

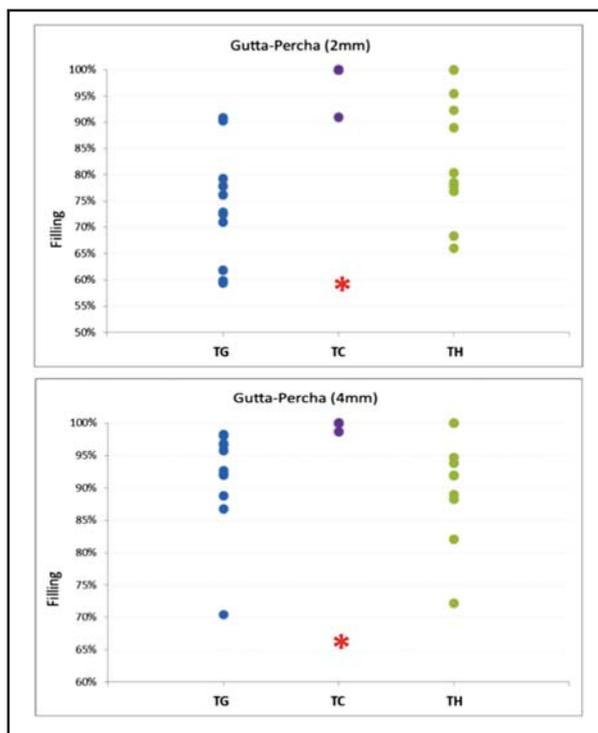


Fig. 2: Graphs show the filling percentages with gutta-percha in both levels (2mm and 4mm). \* represents the technique that was statistically different from the other techniques.

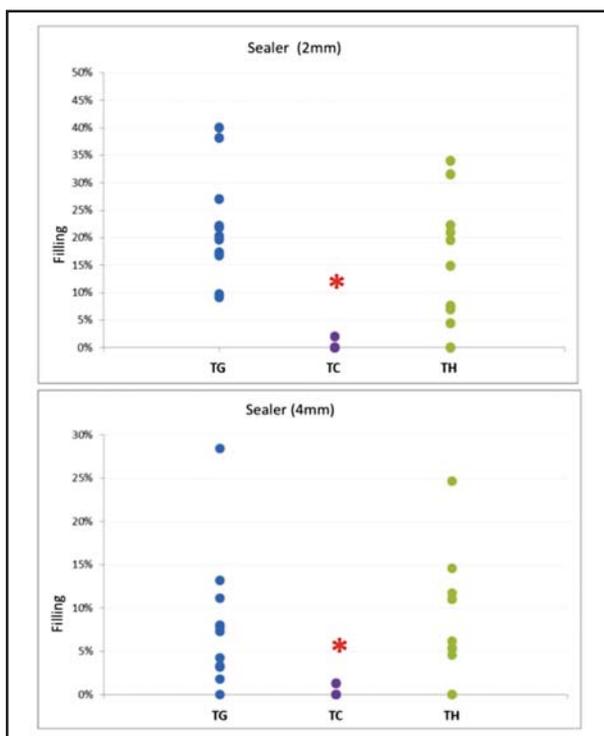


Fig. 3: Graphs show the filling percentages with sealer in both levels (2mm and 4mm). \* represents the technique that was statistically different from the other techniques.

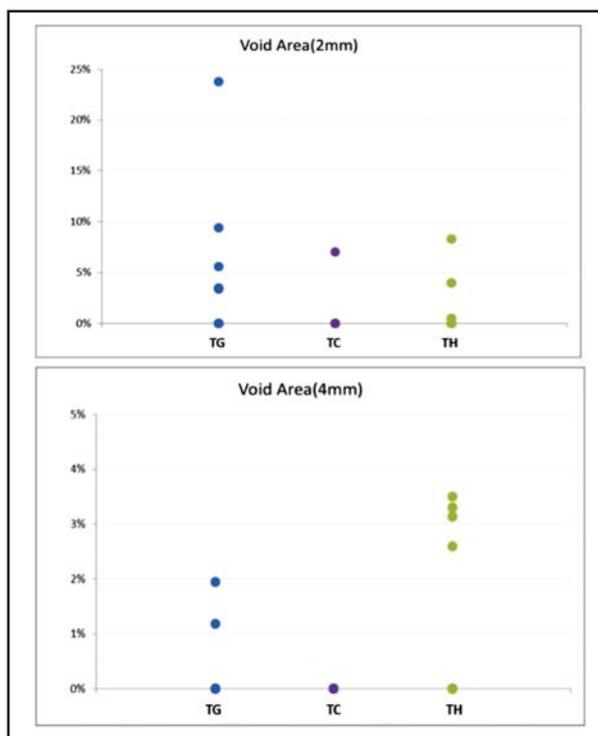


Fig. 4: Graphs show the percentages with void areas in both levels (2mm and 4mm).

cized gutta-percha in human lower pre-molar teeth with an oval cross-section at the apical third.

For obtaining the sections to be analyzed, a highly thin diamond disk (100 µm) was used, which minimizes loss of material during procedures<sup>11</sup>. In order to avoid heat development, besides the irrigation from the handpiece a continuous water jet was applied directly on the section and the lowest possible rotation speed of the handpiece was used<sup>7,11-15</sup>.

Passive Ultrasonic Irrigation (PUI) was applied after chemical and mechanical preparations of the canal so as to improve cleanliness levels of the areas that could not be reached by endodontic instruments. It is important to point out that this may enable cleaning oval-shaped areas and the subsequent filling with the filling materials being studied<sup>15,16</sup>.

As to the experimental design of this research, the TC<sup>®</sup> System showed that gutta-percha had a better filling quality. Such results are consistent with studies undertaken by Ribeiro *et al.*<sup>11</sup>, Pereira *et al.*<sup>6</sup> and Piati *et al.*<sup>7</sup>. Filling of this system seems to be related with the type of gutta-percha being used, the "Alpha" form. This type of gutta-percha has different features that enable this performance, such as viscosity, fluidity and better adhesion<sup>10</sup>. According to Ribeiro *et al.*<sup>11</sup>, the aim of obturation is total filling, with a homogeneous mass of gutta-percha and a perfect adaptation to the canal walls by techniques that reduce the endodontic sealer and void spaces. Hence, under the conditions of this research, the TC<sup>®</sup> System seems to have shown a very good performance in this regard.

Tagger's Hybrid and Touch'n Heat<sup>®</sup> techniques showed similar filling values in the obturation materials, but also showed statistical differences when compared to the TC<sup>®</sup> System. Namely in the apical third, there was a higher amount of endodontic sealer in the TH and TG techniques. In this research, the lower filling ability of gutta-percha in the apical third may be due to the fact that this material has not undergone any plasticizing process in this area, as the devices used do not reach

the apical area. On the other hand, the TC<sup>®</sup> System introduces already plasticized gutta-percha throughout all the root canal. It is recommended that gutta-percha solid cones be used only in rounded canals, because, when used in irregular-shaped canals, it results in void areas or in large volumes of endodontic sealer<sup>17,18</sup>. Due to solubility, to contraction and to lack of dimensional stability, only a thin layer of cement should be applied on the inner walls of root canals<sup>19</sup>.

Even though it is recommended that a small amount of cement be added to the obturation mass, nowadays, endodontic sealers have undergone improvements in their properties, which in the past made their questionable properties to be used in filling large areas of root canal, especially in oval-shaped areas where, as described above, solid gutta-percha is not an effective material. This is the case of the Ah Plus<sup>®</sup> sealer, used in this experiment. This sealer presented adequate standards of radiopacity, solubility, flow, adhesion and setting time when it was tested according to the requirements of the American Dental Association (ADA).<sup>20</sup>

Void areas were scarce and there were no statistical differences between the techniques. This showed that the main purpose of obturation, the sealing of all the root canal, was obtained with all the techniques under study<sup>21,22</sup>.

Even if filling materials have offered good filling levels in all these techniques, endodontic sealer must be selected having a good knowledge of its properties as this sealer cannot go through contractions and cannot be solubilized in the root canal, especially in irregular-shaped canals.

Even though the TC<sup>®</sup> System has been unanimously considered as the most satisfactory filling (with plasticized gutta-percha being used for filling root canals), other studies are still needed for evaluating its performance in terms of technical skill, risk of over-obturation and possible root heating, as there are very few studies available concerning its use.

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## TOOTHBRUSHING PROCEDURE IN SCHOOLCHILDREN WITH NO PREVIOUS FORMAL INSTRUCTION: VARIABLES ASSOCIATED TO DENTAL BIOFILM REMOVAL

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### ABSTRACT

The aim of this study was to establish the association between features regarding brushing procedure performed by schoolchildren without previous formal training and the effectiveness of biofilm removal.

Out of a population of 8900 6- and 7-year-old schoolchildren in Buenos Aires City, 600 children were selected from schools located in homogeneous risk areas. Informed consent was requested from parents or guardians and formal assent was obtained from children themselves. The final sample consisted of 316 subjects. The following tooth brushing variables were analyzed: toothbrush-gripping, orientation of active part of bristles with respect to the tooth, type of movement applied, brushing both jaws together or separately, including all 6 sextants and duration of brushing. The level of dental biofilm after brushing was determined by O'Leary's index, acceptable cut-off point = 20%. Four calibrated dentists performed observations and clinical examinations. Frequency distribution, central tendency and dispersion measures were calculated.

Cluster analyses were performed; proportions of variables for each cluster were compared with Bonferroni's correction and OR was obtained.

The most frequent categories were: palm gripping (71.51%); perpendicular orientation (85.8%); horizontal movement (95.6%); separate addressing of jaws (68%) and inclusion of all 6 sextants (50.6%). Mean duration of brushing was  $48.78 \pm 27.36$  seconds. 42.7% of the children achieved an acceptable biofilm level. The cluster with the highest proportion of subjects with acceptable post-brushing biofilm levels ( $p < 0.05$ ) differed significantly from the rest for the variable "inclusion of all 6 sextants in brushing procedure". OR was 2.538 (CI 95% 1.603 – 4.017).

Inclusion of all six sextants could be a determinant variable for the removal of biofilm by brushing in schoolchildren, and should be systematized as a component in oral hygiene education.

**Key words:** Oral hygiene, toothbrushing, dental plaque prevention and control.

## PROCEDIMIENTO DE CEPILLADO EN ESCOLARES SIN PREVIO ENTRENAMIENTO FORMAL: VARIABLES ASOCIADAS AL BARRIDO DEL BIOFILM DENTAL

### RESUMEN

El objetivo del trabajo fue establecer la asociación entre las características del procedimiento de cepillado en escolares sin previo entrenamiento formal con la efectividad para el barrido del biofilm dental.

Sobre una población de 8900 escolares de Ciudad Autónoma de Buenos Aires de 6 y 7 años de edad, se seleccionaron 600 niños concurrentes a escuelas situadas en áreas de riesgo homogéneo. Sobre esta muestra se solicitó consentimiento informado a los responsables legales y el asentimiento formal, conformando una muestra final de 316 individuos. Se analizaron las siguientes variables del procedimiento de cepillado: tipo de toma del cepillo dental, orientación de la parte activa respecto del diente, tipo de movimiento aplicado, abordaje simultáneo o no de ambos maxilares, inclusión de los 6 sextantes y duración de cepillado. El nivel de biofilm dental posterior al cepillado se determinó con el índice de O'Leary-punto de corte aceptable=20%. Cuatro odontólogos calibrados realizaron las observaciones y exámenes clínicos. Se calculó: distribución de frecuencias, medidas de tendencia central y su dispersión. Se realizó un análisis de

clúster y comparación de proporciones de las variables de cada conglomerado con corrección de Bonferroni y OR.

Las categorías más frecuentes fueron: toma palmar (71,51%); orientación perpendicular (85,8%); movimiento horizontal (95,6%); cepillado de ambos maxilares por separado (68%) e inclusión de los 6 sextantes (50,6%). La media de duración del cepillado fue de  $48.78 \pm 27.36$  segundos. El 42,7 % de los escolares alcanzaron un nivel de biofilm aceptable. El clúster que mostró la mayor proporción de individuos con niveles de biofilm postcepillado aceptables ( $p < 0,05$ ) mostró diferencias estadísticamente significativas con los demás respecto de la variable "inclusión de 6 sextantes en el cepillado". El OR fue igual a 2,538 (IC 95% 1,603 – 4,017).

La inclusión de los seis sextantes podría ser una variable determinante de la efectividad del barrido de biofilm mediante el cepillado en escolares debiendo enfatizarse la sistematización del mismo como componente de la enseñanza de higiene bucal.

**Palabras Clave:** Higiene bucal, cepillado dental, prevención y control de placa dental.

## INTRODUCTION

Oral health is an essential component of general health and wellbeing. Everyday activities such as communicating, eating and tasting food, as well as aspects of social interaction associated to self-esteem and self-confidence may all be affected by alterations in oral health status. On the other hand, everyday decisions and actions can contribute to wellbeing and health<sup>1</sup>.

The most frequent oral diseases –caries and gingivitis– currently affect a large number of children, in particular those living in poverty, causing pain, discomfort, sleepless nights and limited masticatory function, producing nutritional problems, absenteeism from school and impact on families in terms of time and expense<sup>2</sup>. Children with deficient oral health are 12 times more likely to suffer restrictions in their daily activities than children with acceptable oral health status<sup>3</sup>. Despite scientific progress over the past 30 years, large population groups still suffer from preventable diseases. Based on the analysis of the strongest factors influencing children's and youngster's health in Latin American countries, the Pan American Health Organization (2005) has identified some areas which can clearly be defined as priorities for health interventions: healthy environment promotion; life skills development; active lifestyle encouragement and personal hygiene habits development<sup>4</sup>. Oral hygiene is a universal preventive measure, because it helps remove and control dental biofilm. The toothbrush is the most often used tool in daily oral hygiene<sup>5</sup>. People living in high-income countries have incorporated regular toothbrushing habits. However, there are substantial variations within countries, mostly related to educational level. Toothbrushing is less frequent in low-income countries<sup>6</sup>. Regular toothbrushing using an effective technique is a main, critical aim for individual and collective oral health preventive programs.

Effective removal of dental biofilm has been associated with lower prevalence of gingivitis, and in the long term, can prevent or reduce the progression of periodontitis, helping to preserve teeth over time<sup>7</sup>. Researchers such as Zenkner and Nyvad have considered effective biofilm removal as a potential factor regarding caries reduction<sup>8-9</sup>. In a study on 298 schoolchildren, Zenkner showed

that teeth with a high level of biofilm accumulation were 14.5 times (95% CI = 6.5–32.4) more susceptible to caries activity than those without visible biofilm accumulation (data adjusted for molar type and eruption stage) and that molars partially exposed to the oral cavity were 63.6 times (CI 95 % = 22.0 to 183.7) more susceptible to caries activity than molars in full occlusion (data adjusted for biofilm accumulation and molar type)<sup>8</sup>.

There is consensus that the efficacy of brushing techniques lies in the ability to remove dental biofilm<sup>10</sup>. However, none of the brushing techniques proposed to date has proven to be significantly more effective than other. Recommendations of one specific technique over another are largely based on professionals' personal preference, outcomes of personal experiences and empirical evidence. Neither is there clear evidence of which brushing variables have a greater influence on biofilm removal and should be emphasized. Wilson TG Jr. has reported that teaching a hygiene technique that involves major changes for the subject produces short-term results, but these results are not sustained over time. Adherence rate to new brushing techniques is lower than 50%<sup>10</sup>.

This study analyzed one aspect involved in oral hygiene education, namely, toothbrushing technique in children from socially deprived populations, within the framework of school programs conducted during the first years of schooling.

In the understanding that it is important to learn about people's practices and knowledge of daily life in order to design health interventions that will be effective and relevant to the context, and require the lowest level of change in behaviors with the highest efficacy, the team intended:

- To characterize toothbrushing patterns in children without prior systematic instruction,
- To explore observable patterns in children who achieve satisfactory biofilm removal, and
- To identify variables associated to better dental biofilm removal.

The aim of this investigation was, thus, to establish association between features of the toothbrushing procedure in schoolchildren without prior formal instruction and the effectiveness of dental biofilm removal.

## MATERIALS AND METHODS

This was an observational analytical study.

### Sample

The population consisted of 8900 children from schools in Buenos Aires City (CABA).

Eligibility criteria were:

- Age: 6-7 years,
- Geographical residential area: living and attending schools in areas with high density of socially deprived households.

A non-probability sampling technique was used.

Exclusion criteria were:

- having received formal instruction in oral hygiene techniques
- presence of motor disorders.

Steps for sample selection were:

In the first place, a geographic area with critical levels of deprived households, according to Golovanevsky's (2007)<sup>11</sup> criteria for measuring social deprivation was selected. Territorial distribution of deprived households in Buenos Aires City was established following Con et al. (2009)<sup>12</sup> and INDEC (2010)<sup>13</sup> criteria. School District number 19, in the south-west of the city was chosen.

Secondly, schools to participate in the study were selected: opinion of local experts (Secretariat of Educational Inclusion of Buenos Aires City Ministry

of Education) was taken into consideration to select 6 of the 9 schools in School District 19.

The final sample consisted of 316 6- to 7-year-old first-graders.

In order to accomplish ethical aspects of the investigation, informed consent to participate in the study was obtained from parents or legal guardians of the schoolchildren (n=600), as well as formal acceptance from the schoolchildren themselves.

### Variable selection and categories

The following variables were selected to characterize the brushing procedure used by schoolchildren without prior formal training or instruction:

- a) toothbrush gripping,
- b) orientation of active part of the brush with respect to the longitudinal axis of teeth,
- c) type of movement performed,
- d) separate addressing of jaws in toothbrushing procedure,
- e) including all 6 sextants during brushing,
- f) duration of brushing.

Table 1 shows the selected variables and categorization.

Efficacy of biofilm removal was evaluated using O'Leary's plaque index<sup>14</sup> post-brushing procedure, which uses disclosure solution to provide information on presence of biofilm on tooth surfaces.

### Researcher calibration

Four dentists took part in the study as researchers. Two meetings were held in order to standardize observation and recording of toothbrushing variables. These meetings included description of analyzed variables and operationalization description; watching videos to practice recording; and calibration to establish the level of consistency among researcher observations. A reference investigator trained the researchers in detecting biofilm and recording O'Leary's Index. The dentists who achieved inter-rater Kappa agreement statistic higher than 0.75 were included in the study team.

### Study procedure

Observations and clinical examinations were conducted at schools. Researchers provided children with toothbrushes with a straight-handle and 1.5 cm head with three rows of straight nylon bristles (Colgate® Plan Escolar). Children were asked to

**Table 1: Brushing procedure: study variables and categories.**

Variables	
Toothbrush grip	Palm
	Pen
Orientation of the active part of the toothbrush with respect to tooth axis	Perpendicular
	Other
Predominant movement	Horizontal
	Other
Simultaneous addressing of both jaws	Yes
	No
Inclusion of all 6 sextants	No
	Yes
Biofilm level	0% to 20%
	20% to 100%
Brushing duration	In seconds

brush their teeth as usual. Each child brushed his/her teeth individually, out of sight from other children, to prevent them from influencing each other.

Children were examined under standardized conditions using technology with simplified equipment<sup>15</sup>. Presence of biofilm post-brushing procedure was determined using disclosure solution. O'Leary's index<sup>14</sup> was used to quantify biofilm.

Both procedures were recorded on an *ad-hoc* chart (Fig. 1).

### Statistical processing

Frequency distribution was used to describe categorical variables, and central tendency and dispersion measures were used for quantitative variables.

Euclidian distances were used to establish the criterion for clusters similarity. Fifteen clusters were obtained, of which those with highest number of cases ( $n \geq 30$  cases) were selected for analysis: clusters 1, 3, 10, 11 and 13. The remaining ten clusters were considered non-classifiable because they included few cases (Table 2).

The proportions of variables in each cluster were compared with Bonferroni's correction coefficient. The Odds Ratio (OR) was calculated to assess the risk level of the variable that showed statistically significant differences in the cluster that achieved acceptable biofilm removal.

### Compliance with legal aspects

The study was conducted within the framework of an agreement between Buenos Aires City Government's

Ministry of Education and Buenos Aires University School of Dentistry (UBACyT20020120100324BA). It complied with legal rules of Buenos Aires City Government's Ministry of Education. Informed consent authorizing participation in the study was obtained from children's parents or legal guardians, and each child was asked for formal assent.

### RESULTS

The study variable categories with highest frequency were: palm grip (71.51%), perpendicular orientation (85.8%), horizontal movement (95.6%), separate addressing of jaws (68%), and including all 6 sextants (50.6%). The sequence in which the children brushed the 6 sextants of the mouth showed a wide variation and no pattern could be defined in this regard. Post-brushing biofilm level beneath the risk threshold was achieved by 42.7% (Table 3).

**Table 2: Number of subjects in clusters.**

Cluster	N
1	54
3	41
10	36
11	44
13	41
<b>Total</b>	<b>216</b>
Not classifiable	100
<b>Total</b>	<b>316</b>

**Table 3: Distribution of brushing variables in the whole sample.**

Variables		Frequency	%
Toothbrush grip	Palm	226	71.5%
	Pen	80	28.5%
Orientation of the active part of the toothbrush with respect to tooth axis	Perpendicular	271	85.8%
	Other	45	14.2%
Predominant movement	Horizontal	302	95.6%
	Other	14	4.4%
Simultaneous addressing of both jaws	Yes	101	32.0%
	No	215	68.0%
Inclusion of all 6 sextants	No	156	49.4%
	Yes	160	50.6%
Biofilm level	0% to 20%	135	42.7%
	20% to 100%	181	57.3%

**TEACHING AND MONITORING HYGIENE TECHNIQUE**

NAME: \_\_\_\_\_ SCHOOL: \_\_\_\_\_ GRADE: \_\_\_\_\_ AGE: \_\_\_\_\_

1) Ask child to brush his/her teeth as usual.

2) Watch and record:

**BRUSH GRIPPING:**     palm                       pen                       other: .....

**POSITION OF BRUSH WITH RESPECT TO TOOTH AXIS:**

oriented to occlusal       perpendicular       oriented to gingival.

**TOOTHBRUSHING MOVEMENT:**

Rotational       Horizontal       Transversal       other: .....

**SEQUENCE:** (number sequential addressing of sextants)


**ADDRESSING JAWS:**     both jaws simultaneously       each jaw separately

**BRUSHING DENTAL SURFACES:**     Only buccal       Buccal and occlusal

Bucal, occlusal and lingual/palatal

**BRUSHES TONGUE:**     YES       NO                      TOTAL BRUSHING TIME: ..... seconds

3) Perform plaque disclosure. Record:                      Postbrushing plaque level:  %

16	15	14	13	12	11	21	22	23	24	25	26
46	45	44	43	42	41	31	32	33	34	35	36

4) Record changes in technique taught to child.

Fig. 1: Ad-hoc chart for recording observations on tooth brushing procedure.

Table 4 shows the distribution of categories in each cluster.

Table 5 shows the significant differences found upon comparing proportions of study variables among clusters.

Cluster 13 had the highest proportion of children achieving acceptable biofilm removal (Fig.2).

Figure 3 shows duration of brushing. No statistically significant difference was found among clusters (Fig. 3).

Cluster 13 differed from the other four in terms of proportion of children that included all sextants during brushing. The following brushing features prevailed in the cluster of children with best biofilm removal: palm grip; perpendicular orientation of

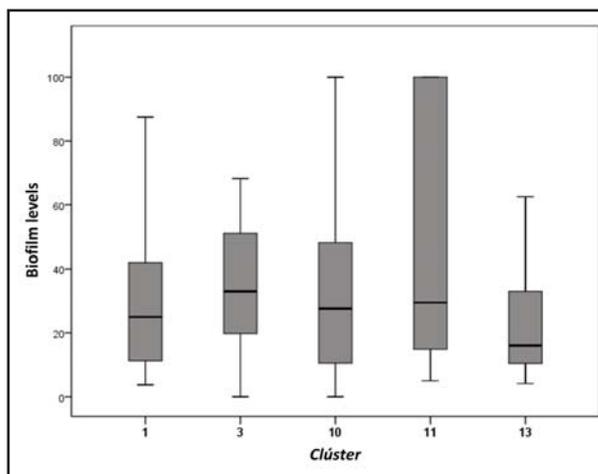


Fig. 2: Post-brushing biofilm level in selected clusters.

**Table 4: Frequency of brushing variable categories broken down according clusters.**

Variables		Cluster 1		Cluster 2		Cluster 3		Cluster 4		Cluster 5	
		Frequency	%								
Orientation of the active part of the toothbrush with respect to tooth axis	perpendicular	52	96.3	34	82.9	31	86.1	39	88.6	40	97.6
	Other	2	3.7	7	17.1	5	13.9	5	11.4	1	2.4
Predominant movement	Horizontal	52	96.3	40	97.6	35	97.2	43	97.7	40	97.6
	Other	2	3.7	1	2.4	1	2.8	1	2.3	1	2.4
Simultaneous addressing of both jaws	Yes	11	20.4	10	24.4	21	58.3	9	20.5	3	7.3
	No	43	79.6	31	75.6	15	41.7	35	79.5	38	92.7
Inclusion of all 6 sextants	No	14	25.9	32	78.0	23	63.9	35	79.5	4	9.8
	Yes	40	74.1	9	22.0	13	36.1	9	20.5	37	90.2
Biofilm level	0 to 20%	22	40.7	11	26.8	16	44.4	15	34.1	24	58.5
	20% to 100%	32	59.3	30	73.2	20	55.6	29	65.9	17	41.5

**Table 5: Comparison of proportions of study variables among clusters.**

Variables		Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5
		A	B	C	D	E
Orientation of the active part of the toothbrush with respect to tooth axis	perpendicular					
	Other					
Predominant movement	Horizontal					
	Other					
Simultaneous addressing of both jaws	Yes			ABDC		
	No	C	C		C	C
Inclusion of all 6 sextants	No		AE	AE	AE	
	Yes	BCD				BCD
Biofilm level	0% to 20%					B
	20% to 100%					

brush head with respect to tooth axis; horizontal movement; brushing each jaw separately. The variable that showed significant differences with regard to other clusters was including all 6 sextants in the procedure (Fig. 4). Risk assessment determined OR = 2.538 (CI 95% 1.603 – 4.017).

## DISCUSSION

There are few studies of toothbrushing practices without prior professional intervention, and even fewer on schoolchildren populations.

Our study identified 15 clusters according to proximity, reflecting the wide range of variable combination. The most frequent variables among children achieving best biofilm removal were palm gripping of brush, perpendicular orientation of toothbrush head with respect to tooth axis, predominantly horizontal movement, separate addressing of jaws and inclusion of all 6 sextants of the mouth during the oral hygiene procedure.

**Movement:** Observation of toothbrushing in schoolchildren without prior formal instruction showed predominance of horizontal movement (95.6%), in agreement with Andrade (1997)<sup>16</sup>.

**Gripping:** Our results agree with those reported by Das (2009) in a study on 3- to 11-year-olds without previous instruction in tooth brushing, with palm gripping being the most frequent<sup>17</sup>.

**Orientation:** Most children oriented the active part of the brush perpendicular to tooth axis.

**Duration:** Duration of brushing in our study ( $X = 48.78 \text{ sec.} \pm 27.36$ ) was similar to duration reported in other studies. Mc Greggor and Rugg-Gunn (1979) found that average duration of brushing in schoolchildren was 60.3 seconds<sup>18</sup>. Das (2009) reported duration of toothbrushing in children aged 6 to 8 years was 48 seconds, increasing significantly with age<sup>17</sup>. Our study found no statistically significant difference between groups with better and worse biofilm control performance.

**Biofilm:** A frequent clinical finding has been that most schoolchildren have unacceptable levels of oral hygiene. Sandstrom (2011) found that only 19% of children removed dental biofilm acceptably<sup>19</sup>. However, in our study, 42.7% of the children achieved a post-brushing biofilm level beneath the cut-off point.

**Sextants:** All 6 sextants were included by 50.6% of the children who took part in this study, but the sequence in which they were brushed was disorderly, often leading to repeatedly brushing some sextants and omitting others. Andrade (1997) reports that 6-year-olds with no prior brushing instruction tend to brush only the molar zone, in contrast to younger children, who only brush the front teeth<sup>16</sup>.

We found that children who did not include all 6 sextants during tooth brushing are at a 2.5 higher risk of having unacceptable biofilm levels than those who include all 6 sextants (OR = 2.538 (CI 95% 1.603 – 4.017), while the other variables analyzed showed lit-

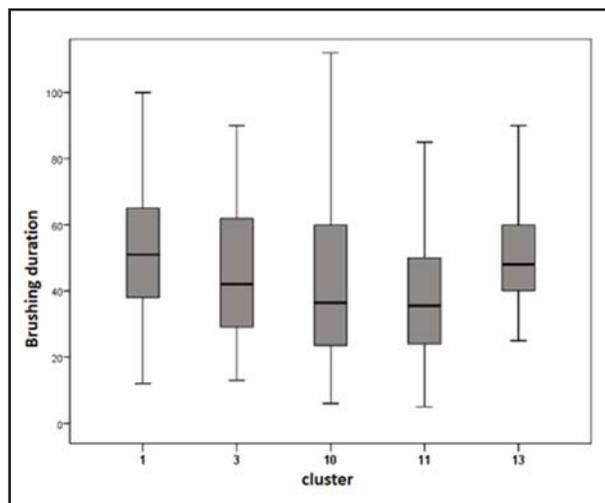


Fig. 3: Toothbrushing duration in selected clusters.

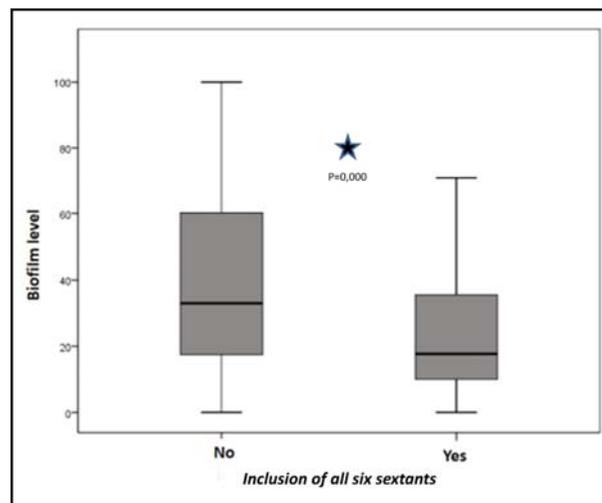


Fig. 4: Biofilm level according to inclusion of sextants for the whole sample.

tle variability. These findings suggest that educational interventions, particularly if they are collective, should emphasize systematization of brushing by establishing a sequence for brushing the whole mouth as one of the aspects of mechanical hygiene.

Clearly, some subjects are able to achieve a high level of biofilm control, while others are not. In this regard, Frandsen (1985) remarks "efficiency in oral hygiene practices is more likely to be improved based on

research and development related to conditions determining individual performance than by attempting to improve treatments and techniques<sup>8</sup>."

## CONCLUSIONS

Including all six sextants may be a determinant variable for effective biofilm removal by toothbrushing in schoolchildren, and its systematization should be emphasized in oral hygiene education.

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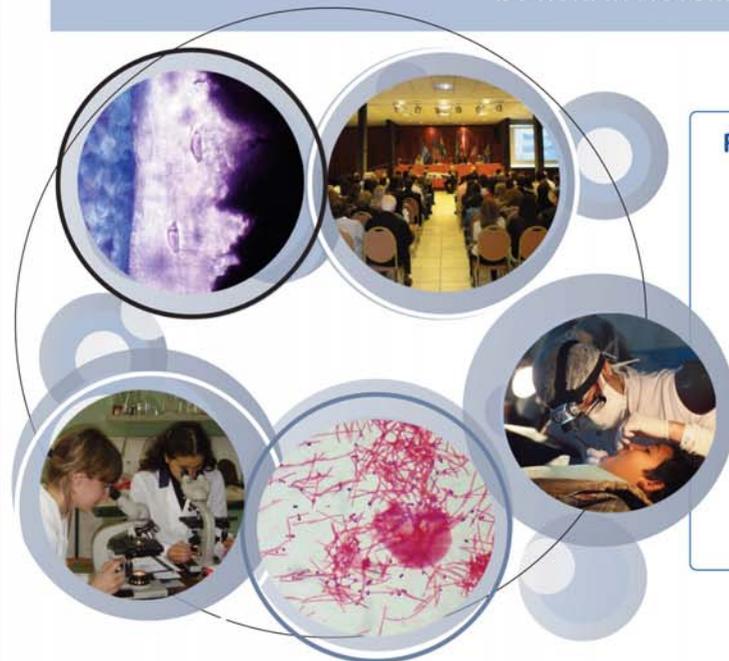
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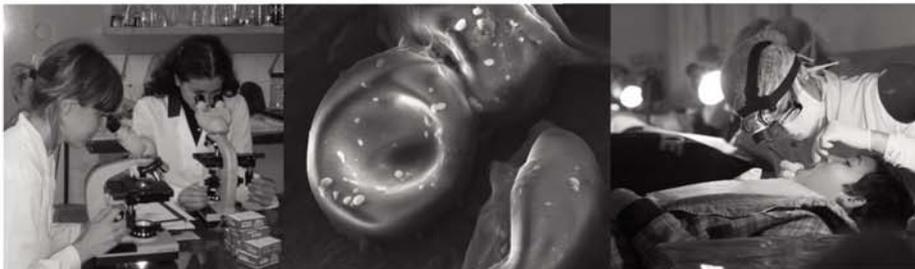
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‡ Resultados de un estudio de 6 meses para evaluar las mejoras en caries del esmalte usando el método QLFTM (Fluorescencia Cuantitativa Inducida por Luz) contra una crema dental regular sólo con flúor, ambas con 1450 ppm de flúor.

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