ISSN 1852-4834 on line version
versión electrónica

ACTA ODONTOLOGICA LATINOAMERICANA

Vol. 28 Nº 3 2015

aol

Colgate®

Recomiende a sus pacientes el alivio instantáneo* y duradero de la sensibilidad.

Colgate Sensitive Pro-Alivio.

*Clínicamente comprobado. Para alivio instantáneo de la sensibilidad, masajee una pequeña cantidad directamente en el diente sensible por un minuto. Para alivio duradero, se recomienda cepillarse 2 veces al día.

www.colgateprofesional.com.ar / www.colgateprofesional.com.uy / www.colgateprofesional.cl

Honorary Editor
Editor honorario
Rómulo Luis Cabrini
(Universidad de Buenos Aires, Argentina)

Scientific Editor
Editor Científico
María E. Itoiz
(Universidad de Buenos Aires, Argentina)

Associate Editors
Editores Asociados
Carlos E. Bozzini
M. Beatriz Guglielmotti
Ricardo Macchi
Angela M. Ubios
(Universidad de Buenos Aires, Argentina)
Amanda E. Schwint
(Comisión Nacional de Energía Atómica, Argentina)

Assistant Editors
Editores Asistentes
Patricia Mandalunis
Sandra J. Renou
(Universidad de Buenos Aires, Argentina)

Technical and Scientific Advisors
Asesores Técnico-Científicos
Lilian Jara Tracchia
Luciana M. Sánchez
Tammy Steimetz
Delia Takara
(Universidad de Buenos Aires, Argentina)

Editorial Board
Mesa Editorial
Enri S. Borda (Universidad de Buenos Aires, Argentina)
Noemí E. Bordoni (Universidad de Buenos Aires, Argentina)
Fermín A. Carranza (University of California, Los Angeles, USA)
José Carlos Elgoyhen (Universidad del Salvador, Argentina)
Fernando Goldberg (Universidad del Salvador, Argentina)
Andrea Kaplan (Universidad de Buenos Aires, Argentina)
Andrés J.P. Klein-Szanto (Fox Chase Cancer Center, Philadelphia, USA)
Héctor E. Lanfranchi Tizeira (Universidad de Buenos Aires,Argentina)
Susana Piovano (Universidad de Buenos Aires, Argentina)
Guillermo Raiden (Universidad Nacional de Tucumán, Argentina)
Sigmar de Mello Rode (Universidade Estadual Paulista, Brazil)
Cassiano K. Rösing (Federal University of Rio Grande do Sul, Brazil)

Publisher
Producción Gráfica y Publicitaria
ImageGraf / e-mail: info@imagegraf.com.ar

Acta Odontológica Latinoamericana is the official publication of the Argentine Division of the International Association for Dental Research.

Revista de edición argentina inscripta en el Registro Nacional de la Propiedad Intelectual bajo el N° 284335.
Todos los derechos reservados.
Copyright by:
ACTA ODONTOLOGICA LATINOAMERICANA
www.actaodontologicalat.com

POLÍTICA EDITORIAL

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.

EDITORIAL POLICY

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

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

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

Bibliographic reviews on subjects of special interest will only be published by special request of the journal.

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

By submitting a manuscript the authors agree that the copyright for their article is transferred to the publisher if and when the article is accepted for publication. To achieve the highest possible standard in scientific content, all articles will be refereed by two specialists appointed by the Editorial Board. To favour international diffusion of the journal, articles will be published in English with an abstract in Spanish or Portuguese.

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

Acta Odontológica Latinoamericana : an international journal of applied and basic dental research. – Vol. 1, no. 1 (1984) - Buenos Aires

Cuatrimestral, 1984-1986 ; irregular, 1987-1993, semestral, 1996-2008, cuatrimestral, 2009-
Artículos en inglés, sumarios en inglés y castellano o portugués.
Variante de título: AOL.
Título clave abreviado: Acta Odontol. Latinoam.
Director : Rómulo Luis Cabrini (1984-2015); María E. Itoiz (2015-
Indizada en **MEDLINE/PubMed** : Vol. 1, nº 1 (1984) – ; **SciELO:** Vol 26 (2013)-
Se encuentra incorporada a **Latindex** (categoría 1, directorio y catálogo), y **Núcleo Básico de Revistas Científicas Argentinas** (2007-)por Resolución nº 1071/07 CONICET
Registrada en: *The Serials Directory, Ulrich's Periodicals Directory* y *SCImago Journal.*
Dirección electrónica: http://www.actaodontologicalat.com/
ISSN 1852-4834 versión electrónica

Este número se terminó de editar el mes de Diciembre de 2015

CONTENTS / *ÍNDICE*

IMPAIRMENT OF BONY CRYPT DEVELOPMENT ASSOCIATED WITH HEXAVALENT CHROMIUM EXPOSURE DURING TOOTH ERUPTION
ALTERACIÓN DEL DESARROLLO DE LA CANASTILLA ÓSEA ASOCIADA A LA EXPOSICIÓN DE CROMO HEXAVALENTE DURANTE LA ERUPCIÓN DENTARIA
Luciana M. Sánchez, Marianela Lewicki, Romina C. De Lucca, Ángela M. Ubios 203

***ENTEROCOCCUS FAECALIS* BIOFILM. FORMATION AND DEVELOPMENT IN VITRO OBSERVED BY SCANNING ELECTRON MICROSCOPY**
BIOFILM DE ENTEROCOCCUS FAECALIS. PRODUCCIÓN IN VITRO Y DESARROLLO OBSERVADO CON MICROSCOPÍA ELECTRÓNICA DE BARRIDO
María de los Á. Bulacio, Lucas R. Galván, Cristina Gaudioso, Rosa Cangemi, Marta I. Erimbaue 210

EFFECT OF BISPHOSPHONATES ON THE LEVELS OF RANKL AND OPG IN GINGIVAL CREVICULAR FLUID OF PATIENTS WITH PERIODONTAL DISEASE AND POST-MENOPAUSAL OSTEOPOROSIS
EFECTO DEL CONSUMO DE BIFOSFONATOS EN LOS NIVELES DEL LIGANDO DEL RECEPTOR ACTIVADOR DEL FACTOR NUCLEAR KAPPA-B Y OSTEOPROTEGERINA EN FLUIDO CREVICULAR DE PACIENTES CON ENFERMEDAD PERIODONTAL Y OSTEOPOROSIS POST-MENOPÁUSICA
María E. Verde, Daniela Bermejo, Adriana Gruppi, Miriam Grenón 215

SEMI-LONGITUDINAL STUDY OF THE MCNAMARA CEPHALOMETRIC TRIANGLE IN CLASS II AND CLASS III SUBJECTS GROUPED BY CERVICAL VERTEBRAE MATURATION STAGE
ESTUDIO SEMI-LONGITUDINAL DEL TRIÁNGULO CEFALOMÉTRICO DE MCNAMARA EN SUJETOS DE CLASE II Y CLASE III AGRUPADOS POR ESTADIO DE MADURACIÓN DE VERTEBRAS CERVICALES
Luis E. Arriola-Guillén, Fernando D. Fitzcarrald, Carlos Flores-Mir 222

USE OF HUMAN FASCIA LATA IN RAT CALVARIAL BONE DEFECTS
FASCIA LATA HUMANA EN DEFECTOS ÓSEOS DE CALOTA DE RATA
Mariano A.R. Amer, Pablo A. Rodríguez, Sandra J. Renou, María B. Guglielmotti 231

ASSESSMENT OF MANDIBULAR MOVEMENTS IN 10 TO 15 YEAR-OLD PATIENTS WITH AND WITHOUT TEMPOROMANDIBULAR DISORDERS
VALORACIÓN DE MOVIMIENTOS MANDIBULARES EN PACIENTES DE 10 A 15 AÑOS CON Y SIN TRASTORNOS TÉMPORO-MANDIBULARES
Silvina G. Cortese, Ana M. Biondi, Diana E. Fridman, Ingrid Guitelman, Catalina L. Farah 237

EFFICACY OF CORE NEEDLE BIOPSY TECHNIQUE FOR JAWBONE DISEASES
EFICACIA DE LA TÉCNICA DE PUNCIÓN ÓSEA PARA BIOPSIA DE LESIONES DE MAXILARES
Federico Stolbizer, Romulo L. Cabrini, Alicia Keszler 245

FAMILY IMPACT SCALE (FIS): CROSS-CULTURAL ADAPTATION AND PSYCHOMETRIC PROPERTIES FOR THE PERUVIAN SPANISH LANGUAGE
ESCALA DE IMPACTO FAMILIAR (FIS): ADAPTACIÓN TRANSCULTURAL Y PROPIEDADES PSICOMÉTRICAS AL IDIOMA ESPAÑOL DE PERÚ
Jenny Abanto, Ursula Albites, Marcelo Bönecker, Saul M. Paiva, Jorge L. Castillo, Denisse Aguilar-Gálvez 251

LINEAR DIMENSIONAL STABILITY OF IRREVERSIBLE HYDROCOLLOID MATERIALS OVER TIME
DETERMINACIÓN DE LA ESTABILIDAD DIMENSIONAL LINEAL DE HIDROCOLOIDES IRREVERSIBLES
Analía B. Garrofé, Beatriz A. Ferrari, Mariana Picca, Andrea E. Kaplan 258

QUANTITATIVE SENSORY TESTING OF THE EFFECT OF DESENSITIZING TREATMENT AFTER DENTAL BLEACHING
TESTE SENSORIAL QUANTITATIVO DO EFEITO DE UM TRATAMENTO DESSENSIBILIZANTE APÓS O CLAREAMENTO DENTAL
Vanessa Rahal, Marjorie O. Gallinari, Jorge Perdigão, Luciano T. A. Cintra, Paulo H. dos Santos, André L. F. Briso 263

Acta Odontológica Latinoamericana
A partir del Volumen 27 (2014) ACTA ODONTOLÓGICA LATINOAMERICANA se edita en formato digital con el Sistema de Gestión de Revistas Electrónicas (Open Journal System, OJS), como revista de acceso abierto (Open Access).

Comité Editorial

Acta Odontológica Latinoamericana
As of Volume 27 (2014) ACTA ODONTOLÓGICA LATINOAMERICANA is published in digital format with the Open Journal System (OJS) as an open access journal.

Editorial Board

Contact us - Contactos: Cátedra de Anatomía Patológica, Facultad de Odontología, Universidad de Buenos Aires
M.T. de Alvear 2142- (1122) Buenos Aires, Argentina - Fax: (54-11) 4 508-3958
mitoiz@odon.uba.ar - http://www.actaodontologicalat.com/contacto.html
La Pampa 2487-(1428) Buenos Aires-Argentina - Fax:(54-11) 4784-7007; fliacabrini@fibertel.com.ar

IMPAIRMENT OF BONY CRYPT DEVELOPMENT ASSOCIATED WITH HEXAVALENT CHROMIUM EXPOSURE DURING TOOTH ERUPTION

Luciana M. Sánchez, Marianela Lewicki, Romina C. De Lucca, Ángela M. Ubios

Department of Histology and Embryology, School of Dentistry, University of Buenos Aires. Buenos Aires, Argentina

ABSTRACT

Improperly treated hexavalent chromium-containing industrial wastes contaminate drinking water, potentially affecting children taking breast milk or baby bottles prepared with infant formula. Thus, the aim of the present work was to determine the effect of this toxic on bone activity in the developing alveolus during tooth eruption of suckling Wistar rats intoxicated with potassium dichromate. Experimental animals received a daily dose of 12.5mg/kg body weight of potassium dichromate by gavage for 10 days; controls received an equivalent volume of saline solution. Histologic and histomorphometric studies of the mandible were performed. The data were statistically analyzed using Student's t test; statistical significance was set at a value of $p < 0.05$. Experimental animals exhibited delayed tooth eruption, decreased periodontal width and bone volume, a lower percentage of bone formation surfaces, and higher percentage of quiescent surfaces ($p < 0.05$) compared to controls. The delay in tooth eruption observed after exposure to hexavalent chromium is the result of a lower rate of bone remodeling in the developing alveolus. The obtained results show the importance of controlling toxic substances in drinking water, since their effects may alter the growth and development of subjects who were exposed during early infancy.

Key words: *tooth eruption; hexavalent chromium; bone remodeling; drinking water.*

ALTERACIÓN DEL DESARROLLO DE LA CANASTILLA ÓSEA ASOCIADA A LA EXPOSICIÓN DE CROMO HEXAVALENTE DURANTE LA ERUPCIÓN DENTARIA

RESUMEN

Desechos industriales que contienen cromo hexavalente inadecuadamente tratados contaminan el agua de consumo pudiendo afectar a los niños por vía de la leche materna o de la preparación de mamaderas. Por lo tanto, el objetivo del presente trabajo fue determinar el efecto de este tóxico en la actividad del hueso en el alveolo en desarrollo durante la erupción dentaria de ratas Wistar lactantes expuestas a dicromato de potasio. Los animales experimentales recibieron una dosis diaria de 12,5 mg / kg de peso corporal de dicromato de potasio por alimentación forzada durante 10 días; mientras que los controles, un volumen equivalente de solución salina. Se llevaron a cabo estudios histológicos e histomorfométricos de la mandíbula. Los datos fueron analizados estadísticamente utilizando la prueba t de Student; estableciéndose un valor de $p<0,05$ como estadísticamente significativo. Los animales expuestos a cromo hexavalente mostraron retraso en la erupción dentaria, menor espacio periodontal y volumen óseo; encontrándose disminuidas las superficies en formación y en reabsorción óseas y aumentadas las superficies en reposo ($p < 0,05$) en comparación con los controles. El retraso en la erupción dentaria observado luego de la exposición a cromo hexavalente es el resultado de una menor remodelación ósea en el alveolo en desarrollo. Los resultados obtenidos muestran la importancia del control de sustancias tóxicas en el agua potable, ya que sus efectos pueden alterar el crecimiento y el desarrollo de los individuos que fueron expuestos durante la infancia temprana.

Palabras clave: *erupción dentaria; cromo hexavalente; remodelación ósea; agua de consumo.*

INTRODUCTION

Hexavalent chromium compounds produced by the chemical industry are used for the manufacture of dyes and pigments, leather tanning, and wood preserving. Wastes from electroplating, petrochemical industry, leather tanning, and textile industry can be released into the air or the soil, or be discharged into waterways, contaminating drinking water [1]. The general population can be exposed to hexavalent chromium directly through skin contact, by inhaling air, or by drinking or eating foods contaminated with chromium [2,3]. After entering the cell, Cr VI is reduced to Cr III, resulting in the formation of reactive intermediates which contribute to the cytotoxicity, genotoxicity, and carcinogenicity [4].

In the year 2010 the US Environmental Protection Agency (EPA) [5] established that the maximum allowable concentration of total chromium which includes all forms of chromium including chromium-6 in drinking water should not exceed 0.1mg/l. or 100 parts per billion (ppb). Nevertheless, these recommendations are not met in some countries in America, Europe and Asia [6-11]. There are studies in the literature associating exposure to hexavalent chromium and risk of bone damage [12,13]. Sankaramanivel et al [14] reported that Cr VI has been found to enter the inorganic bone matrix of vertebrae, femur and calvaria of adults male rats, altering the tissue and interfering with bone formation and resorption, thus, leading to altered bone turnover.

According to the Agency for Toxic Substances and Disease Registry [15], babies could be exposed to high environmental levels of chromium through inhalation and consumption of contaminated foods -including breast milk- and water -used to prepare baby formula.

Very few studies have investigated the effects of chromium exposure on children. However, it is likely that children would have the same health effects as adults. Soudani et al [16] found that exposure of rat dams to potassium dichromate before and after delivery affected growth and decreased bone mineral content of their progeny and De Lucca et al.[17] demonstrated a decrease in body growth of suckling rats receiving potassium dichromate solutions.

The association between other toxic substances and bone alterations is well documented: iron decreases bone formation and inhibits endochondral ossification [18], lead replaces the calcium in the hydroxyapatite crystals and also impairs body growth [19] and uranium affects bone remodeling, decreasing mandibular growth and delaying tooth eruption [20,21].

Tooth eruption is a highly dynamic biological process, in which bone tissue plays a crucial role. Little is known about bone remodeling in the walls of the alveolus as the tooth drifts during tooth eruption. Studies in rat molars, which, like human teeth, are teeth of limited eruption, have shown that bone resorption and formation are essential during the intraosseous and mucosal penetration stages of tooth eruption, when the walls of the dental alveolus develop [22,23]. In their 2012 study in Jaipur, India, Tiwari et al [24] demonstrated the presence of hexavalent chromium in the blood of children working in gem polishing industries. It is of note that the studied children were at the age when the permanent second molars erupt (10-12 years).

Although De Lucca et al [17] demonstrated that the exposure of suckling rats to hexavalent chromium resulted in decreased body and mandibular growth and delayed tooth eruption; it remains to be clarified whether these observations are the result of an alteration in bone remodeling.

Thus, the aim of the present work was to determine the effect of hexavalent chromium exposure on the developing alveolus during tooth eruption in suckling Wistar rats intoxicated with potassium dichromate.

MATERIAL AND METHODS

Sixteen 4-day-old suckling Wistar rats were assigned to one of two groups: an experimental and a control group. Under topical anesthesia [Xylocaine (Xilocaína®, Astra Zeneca Argentina)], experimental animals received 12.5mg/kg body weight of potassium dichromate (Biopack, Argentina) daily by gavage through a flexible PVC tube. Control animals received an equivalent dose of saline solution under the same conditions as experimental pups.

The litters were adjusted to 8 pups per dam and were housed with their mother in individual cages with wood-chip bedding, and kept on a controlled light-dark cycle (lights on at 7 am and off at 7 pm) and under constant humidity (40-70%). The mothers were fed a solid diet and water at libitum. After each procedure, the pups were returned to the cage with their mother.

All the pups were euthanized on day 15 of the experiment and the mandibles were resected.

The hemi-mandibles were fixed in 4% buffered formalin for 48 hours, decalcified in 10% EDTA pH 7, and embedded in paraffin. Buccal-lingual sections of the hemi-mandibles at the level of the mesial root of the first lower molar were obtained and stained with hematoxylin-eosin in order to perform histologic and histomorphometric studies under a stereoscopic microscope.

Digital microphotographs of the histologic sections of the hemi-mandibles were analyzed using the Image Pro® Plus software, version 5.1 (Media Cybernetics) to measure the histomorphometric parameters listed below, based on stereologic principles [25] and using current nomenclature as stated by Parfitt [26] and revised by Dempster et al [27].

Parameters measured in the alveolar bone of the developing alveolus (Fig. 1):

* The degree of tooth eruption, expressed in millimeters, was determined as the distance between the highest point of the bone crest on the buccal aspect of the developing alveolus and the cementum-enamel junction. Therefore, the result is 0 when tooth eruption is complete and is a negative number when the tooth is not fully erupted.

The following parameters were measured on both the buccal and lingual aspects. To simplify reading, only measures on the buccal aspect are explained in Fig. 1.

* Periodontal width was measured at three sites: B-B´, C-C´and D-D´; values were averaged and results are expressed in microns.
* Bone volume, defined as the fraction of total volume corresponding to trabecular bone, was measured in the area demarcated by the black lines. Total volume is defined as the volume of trabecular bone tissue plus the volume of developing bone marrow; results are expressed as a percentage.
* Bone activity was evaluated in three different regions: on the wall of the developing alveolus from the highest point of the bone crest to the diaphragm of the Hertwig's root sheath; on the wall of the fundus of the developing alveolus, from the diaphragm of Hertwig's epithelial root sheath on the buccal aspect (Point C) to the diaphragm of Hertwig's epithelial root sheath on the lingual aspect (Point C´´´); and on the endosteal walls, inside the area demarcated by the black lines, by determining the following parameters:

- *ObS/BS* (%): Bone formation surfaces (surfaces covered with active osteoblasts).
- *ES/BS* (%): Bone resorption surfaces (surfaces covered with active osteoclasts).
- *LCS/BS* (%): Resting bone surfaces, covered with bone lining cells.

Statistical analysis: The data were statistically analyzed using Student's *t* test; statistical significance was set at a value of $p < 0.05$.

Ethical principles: All procedures were performed in keeping with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals (NIH publication 85-123 Rev.2010) and the study was approved by the Ethics Committee of the School of Dentistry of the University of Buenos Aires (FOUBA-UBACYT 2011-2014-3).

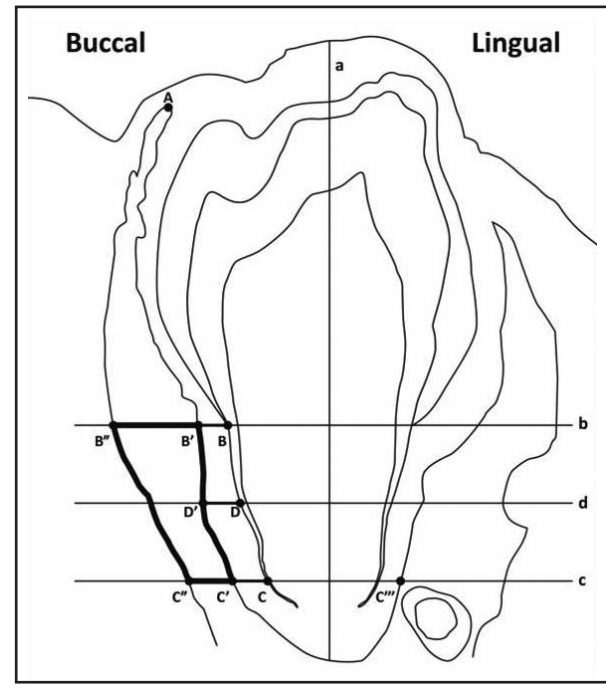


Fig. 1: Method used for the histomorphometric study of the developing tooth alveolus.
Buccal-lingual section.
Line a drawn through the longitudinal axis of the developing tooth.
Line b drawn perpendicular to line "a" through the cementum-enamel junction.
Line c drawn perpendicular to line "a" through the diaphragm of Hertwig's epithelial root sheath.
Line d drawn parallel to and equidistant from lines "b" and "c".
Point A is the highest point of the bone crest.
Point B at the cementum-enamel junction.
Point C at the diaphragm of Hertwig's epithelial root sheath.
Point D on line "d", equidistant from points B and C on the surface of the developing root.
Point B´ drawn where line "b" goes through the periodontal plate of the developing alveolus.
Point C´ drawn where line "c" goes through the periodontal cortical plate of the developing alveolus.
Point D´, where line "d" goes through the periodontal cortical plate of the developing alveolus.
Point B´´, on line "b", 100 microns from point B´.
Point C´´, on line "c", 100 microns from point C´.
- The degree of tooth eruption was measured from point A to point B´.
- Periodontal width was determined, measuring segments B-B´ C- C´ D-D´.
- Bone volume was measured in the area delimited by points B´ C´ B´´ and C´´.
- Bone activity was assessed in three different regions of the alveolar wall:
- From point A to point C´
- From point C´ on the buccal aspect to point C´ on the lingual aspect
- On the endosteal walls in the area delimited by points B´ C´ E and F

RESULTS

Our study showed that exposure to potassium dichromate in the form of chromium VI caused a significant delay in the eruption of the first lower molar, which was associated with a clear alteration in the development of the alveolus.

The bone of the developing alveolus of experimental animals exhibited thinner and more spaced trabeculae than that of controls. Chromium-exposed animals showed fewer osteoclasts and active osteoblasts and more bone lining cells on the endosteal surfaces, the wall of the developing bone crest, and on the fundus of the alveolus (Fig. 2). The histomorphometric study showed significantly decreased bone volume in the alveolus of experimental animals compared to controls, on both the buccal and lingual aspects (Fig. 3).

The surfaces of the endosteal bone trabeculae on the buccal and lingual aspects and fundus of the alveolus of the experimental group exhibited a lower percentage of bone formation and resorption surfaces and a higher percentage of resting surfaces than in the corresponding controls (Fig. 4).

The distance between the highest point of the bone crest on the buccal aspect of the developing alveolus and the cementum-enamel junction was greater in experimental animals than in controls, showing a significant delay in the eruption of the first lower molar. Hence, the result of the histomorphometric analysis was a negative number (Fig. 5).

In addition, the periodontal space was narrower in the potassium dichromate-exposed animals

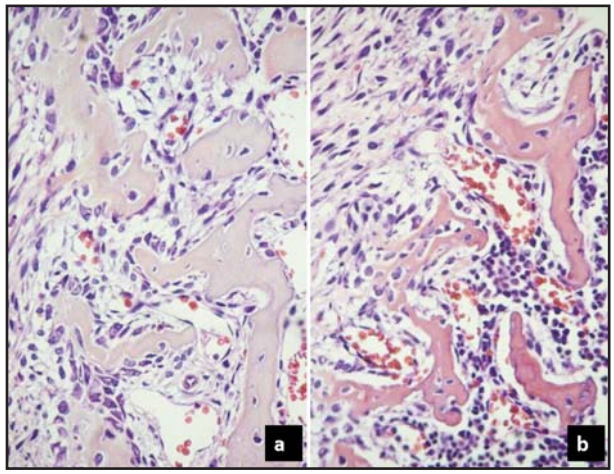


Fig. 2: Bone activity in the endosteal walls of the developing alveolus. Orig. Mag. X400. a: Control; b: Experimental. Note the large areas of bone formation with a large number of osteoblasts in the control section. Quiescent bone surfaces with inactive osteoblasts predominate in the experimental section; the latter also shows fewer and thinner trabeculae than the control section.

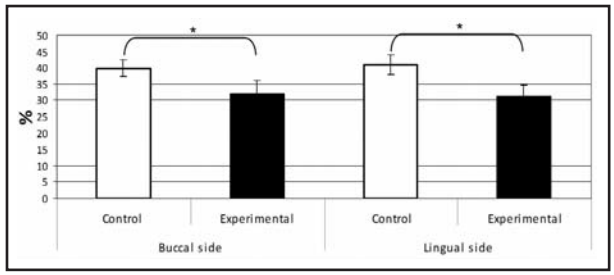


*Fig. 3: Histomorphometric values of alveolar bone volume. Bone volume on both the buccal and lingual aspects was significantly lower in experimental animals than in controls (*p<0.05).*

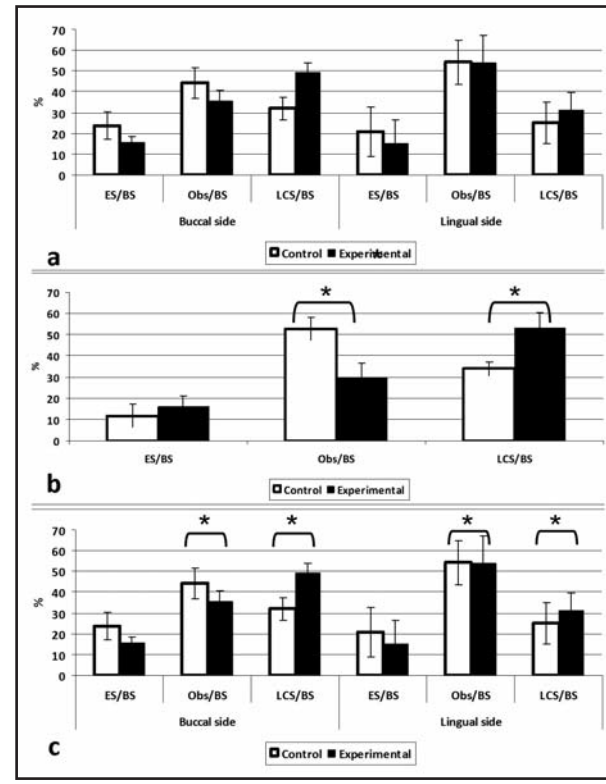


Fig. 4: Histomorphometric study of bone activity.
Bone activity in the wall of the developing alveolus.
Bone activity in the fundus of the alveolus.
Bone activity inside the developing alveolus.
A lower percentage of bone formation and resorption surfaces and a higher percentage of resting surfaces were observed in the buccal and lingual plates of the developing alveolar bone (c) and in the fundus of the alveolus (b) of experimental animals, as compared to controls. The differences between groups were statistically significant (p<0.05). No significant differences in bone activity in the developing alveolus (a) were observed between groups.*

than in controls, and the histomorphometric study showed it was significantly lower in experimental animals compared to controls, on both the buccal and lingual aspects (Fig. 6).

DISCUSION

The results of the present study showed that hexavalent chromium has a toxic effect on the bone cells involved in the bone remodeling process that takes place in the bone tissue of the developing tooth alveolus. According to De Lucca et al [17] this effect can be observed morphometrically as a delay in tooth eruption in animals exposed to potassium dichromate. It is well documented that bone remodeling involves the coupled action of osteoblasts (bone matrix-forming cells) and osteoclasts (cells that resorb the bone matrix).

In vitro studies have reported that exposure to hexavalent chromium can affect human osteoblast and osteoclast survival and function [28]. Thompson and Puleo [29] reported that chromium interferes in the differentiation and function of osteoblasts derived from mesenchymal cells. Ning and Grant [30] showed that hexavalent chromium reduced to trivalent chromium is a potent inducer of cytotoxicity in osteoblasts. Lohman et al [31] found changes in cell morphology and in the differentiation capacity of osteoblasts. In addition, a study by Anisian et al [32] showed that high concentrations of chromium decreased osteoblast activity. Nichols and Puleo [33] found that sub-lethal and physiological concentrations of hexavalent chromium affected the formation and function of osteoclasts. Thus, chromium would interfere in the differentiation of osteoclastic cells derived from precursor cells in the bone marrow, and would inhibit the Ca^{2+} receptors in osteoclasts. The receptor binding site is highly sensitive to di- and tri-valent cations. Hence, given that hexavalent chromium reduces to trivalent chromium, the latter would bind to the receptor, increasing the cytosolic concentration of calcium and decreasing bone resorption. Neale et al [34] demonstrated the inhibitory effect of chromium on osteoclastogenesis in human monocytes cultured with chromium particles.

Previous studies conducted at our laboratory showed that bone resorption and formation are indispensable during the intraosseous and mucosal penetration stages of tooth eruption, which is when the walls of the alveolus develop [21,22]; the present results confirm those findings.

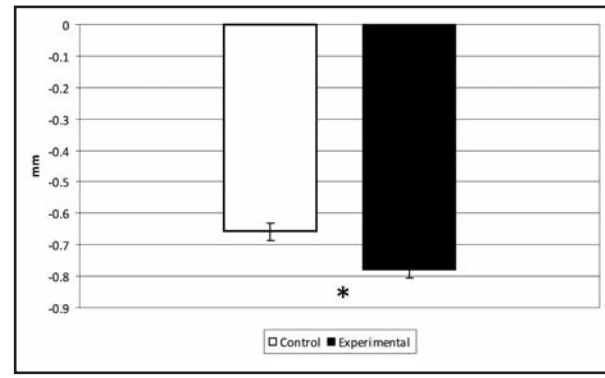


Fig. 5: Morphometric values of tooth eruption. The degree of tooth eruption was significantly lower in experimental animals compared to controls (p<0.05).*

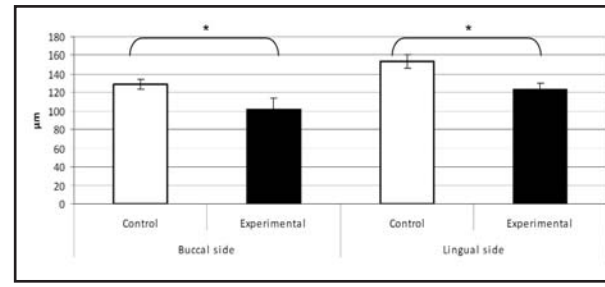


Fig. 6: Histomorphometric values of periodontal width. Periodontal width on both the buccal and lingual aspects was significantly lower in experimental animals as compared to controls (p<0.05).*

The results of the our study show that exposure to hexavalent chromium leads to significantly decreased bone resorption and formation in the endosteal surfaces of the developing tooth alveolus and in the fundus of the alveolus, as shown by the presence of fewer osteoclasts and active osteoblasts. The larger proportion of areas of resting bone on the endosteal surfaces and fundus of the developing alveolus in experimental animals is similar to what occurs in adynamic bone disease. The latter disease has been observed for example, in cases of aluminum toxicity in humans [35] and in an experimental model of iron overload [18].

The present study showed decreased bone formation and resorption, and a predominance of bone quiescence in the developing alveolus of animals exposed to potassium dichromate as compared to controls.

These findings show that hexavalent chromium affects bone turnover, as shown by the lower proportion of both areas of bone resorption and

bone formation and the predominance of resting bone, which in turn results in the decrease of bone remodeling. These observations explain the delay in tooth eruption.

Hexavalent chromium has been found to induce damage to the cytoskeleton[36] and DNA alterations[37] in exposed fibroblast cultures. These findings could explain the decreased periodontal width observed in the present study, which would be related to the inhibition of formation of fibroblasts that impairs periodontal ligament remodeling.

The results obtained in the present study allow concluding that because hexavalent chromium inhibits osteoclasts and osteoblast function, the delay in tooth eruption observed in animals exposed to hexavalent chromium would be due to a lower rate of bone remodeling in the developing tooth alveolus. Taking into account that in addition to the well known health consequences of hexavalent chromium exposure the latter can also affect tooth eruption in children who intake water contaminated with this toxic substance, it is crucial to create further awareness worldwide about the importance of avoiding environmental contamination, and ensuring compliance with waste-water treatment regulations with the aims to protect future generations.

ACKNOWLEDGMENTS

The authors thank Ht. Ivana Sánchez Rojas for her technical assistance and Ignacio Emanuel Sánchez for his collaboration in preparing the graphs of the manuscript. This study was supported by GRANT 2011-2104 UBACYT 20020100100196 (University of Buenos Aires).

CORRESPONDENCE

Luciana M. Sánchez
Marcelo T. De Alvear 2142, 1° "A".
CP 1122AAH. Buenos Aires, Argentina.
e-mail: lucianamsanchez@hotmail.com

REFERENCES

1. Zhitkovich A. Chromium in drinking water: Sources, metabolism and cancer risks. Chem Res Toxicol 2011; 24:1617-1629.
2. Nath K, Kumar N. Hexavalent chromium: toxicity and its impact on certain aspects of carbohydrate metabolism of the freshwater teleost, Colisa fasciatus. Sci Total Environ 1988; 72:175-181.
3. Bocio A, Nadal M, Domingo JL. Human exposure to metals through the diet in Tarragona, Spain: temporal trend. Biol Trace Elem Res 2005; 104:193-201.
4. O´Brien TJ Ceryak S. Patierno SR. Complexities of chromium carcinogenesis: role of cellular response, repair and recovery mechanisms. Mutat 2003; 533: 3-36.
5. U.S. EPA. Toxicological review of hexavalent chromium. US Environmental Protection Agency 18549- 29-9, 1998. Washington DC. 70 pp.
6. Arauzo M, Rivera M, Valladolid M, Norea C, Cedenilla O. Contaminación por cromo en el agua intersticial, en el agua del cauce y en los sedimentos del rio Jarama. Limnetica 2003; 22: 87-100.
7. Colombo JC, Barreda A, Bilos C, Cappelletti N, Demichelis S, Lombardi P, Migoya MC, Skorupka C, Suarez G. Oil spill in the Rio de la Plata estuary, Argentina: 1. Biogeochemical assessment of waters, sediments, soils and biota. Environ Pollut 2005; 134:277-289.
8. Banu SK, Samuel JB, Arosh JA, Burghardt RC, Aruldhas MM. Lactational exposure to hexavalent chromium delays puberty by impairing ovarian development, steroidogenesis and pituitary hormone synthesis in developing Wistar rats. Toxicol Appl Pharmacol 2008; 232:180-189.
9. Cuberos E., Rodriguez AI, Prieto E. Chromium levels and their relationship with alterations in the health of tannery workers living and working in Bogotá, Colombia. Rev Salud Pública (Bogotá) 2009; 11:278-289.
10. Tziritis E, Kelepertzis E, Korres, G, Perivolaris D, Repani S. Hexavalent chromium contamination in croundwaters of Thiva Basin, central Greece. Bull Environ Contam Toxicol 2012; 89:1073-1077.
11. Greenpeace. Informe: Cueros tóxicos. Nuevas evidencias de contaminación de curtiembres en la cuenca Matanza-Riachuelo 2012; 27 pp.
12. Costa M. Toxicity and carcinogenicity of Cr (VI) in animal models and humans. Crit Rev Toxicol 1997; 27: 431-442.
13. Sutherland JE, Zhitkovich A, Kluz T, Costa M. Rats retain chromium in tissues following chronic ingestion of drinking water containing hexavalent chromium. Biol Trace Elem Res 2000; 74:41-53.
14. Sankaramanivel S, Jeyapriya R, Hemalatha D, Djody S, Arunakaran J, Srinivasan N. Effect of chromium on vertebrae, femur and calvaria of adult male rats. Hum Exp Toxicol 2006; 25:311-318.
15. Agency for Toxic Substances and Disease Registry. Toxicological Profile for Chromium. U.S. Department of Health and Human Services, Washington 2012. D.C. 497 pp.
16. Soudani N, Ben Amara I, Troudi A, Bouaziz H, Boudawara T, Zeghal N. Oxidative stress induced by chromium (VI) in bone of suckling rats. Toxicol Ind Health 2011; 27:724-734.
17. De Lucca RC, Dutrey PL, Villarino ME, Ubios AM. Effect of different doses of hexavalent chromium on mandibular growth and tooth eruption in juvenile Wistar rats. Exp Toxicol Pathol 2009; 61:347-352.
18. Mandalunis P, Ubios A. Experimental renal failure and iron overload: a histomorphometric study in rat tibia. Toxicol Pathol 2005; 33:398-403.

19. Conti MI, Terrizzi AR, Lee CM, Mandalunis PM, Bozzini C, Piñeiro AE, Martinez MP. Effects of lead exposure on growth and bone biology in growing rats exposed to simulated high altitude. Bull Environ Contam Toxicol 2012; 88: 1033-1037.
20. Ubios AM, Braun EM, Cabrini RL. Effect of diphosphonates on abnormal mandibular growth of rats intoxicated with uranium. Health Phys 1998; 75:610-613.
21. Pujadas Bigi MM, Lemlich L, Mandalunis PM, Ubios AM. Exposure to oral uranyl nitrate delays tooth eruption and development. Health Phys 2003; 84:163-169.
22. Villarino ME Goya JA, De Lucca RC, Ubios AM. Alterations of tooth eruption and growth in pups suckling from diabetic dams. Pediatr Res 2005; 58:695-699.
23. Wise GE, He H, Gutiérrez DL, Ring S, Yao, S. Requirement of alveolar bone formation for eruption of rat molars. Eur J Oral Sci 2011; 119:333-338.
24. Tiwari R, Saha A, Sathwara N, Parikh J. Blood chromium levels of children working in gem-polishing industries in India. Toxicol Ind Health 2012; 28:170-173.
25. Weibel E, Elias H. Quantitative methods in morphology. In: Weibel ER, Elias H (eds). Introduction to stereology and morphology. Springer Verlag, Berlin 1967; 3-19.
26. Parfitt AM. Bone histomorphometry: standardization of nomenclature, symbols and units (summary of proposed system). Bone 1988; 9:67-69.
27. Dempster DW, Compston JE, Drezner MK, Glorieux FH, Kanis JA, Malluche H, Meunier PJ, Ott SM, Recker RR, Parfitt AM. Standardized nomenclature, symbols, and units for bone histomorphometry: A 2012 update of the report of the ASBMR Histomorphometry Nomenclature Committee. J Bone Miner Res 2013; 28:2-17.
28. Andrews R, Shah K, Wilkinson J, Gartland A. Effects of cobalt and chromium ions at clinically equivalent concentrations after metal-on-metal hip replacement on human osteoblasts and osteoclasts: Implications for skeletal health. Bone 2011; 49:717-723.
29. Thompson GJ, Puleo DA. Effects of sub-lethal metal ion concentrations on osteogenic cells derived from bone marrow stromal cells. J Appl Biomater 1995; 6:249-258.
30. Ning J, Grant MH. Chromium (VI)-induced cytotoxicity to osteoblast-derived cells. Toxicol in Vitro 1999; 13: 879-887.
31. Lohmann CH, Schwartz Z, Köster G, Jahn U, Buchhorn GH, MacDougall MJ, Casasola D, Liu Y, Sylvia VL, Dean DD, Boyan BD. Phagocytosis of wear debris by osteoblasts affects differentiation and local factor production in a manner dependent on particle composition. Biomaterials 2000; 21:551-561
32. Anissian L, Stark A, Dahistrand H, Granberg B, Good V, Bucht E. Cobalt ions influence proliferation and function of human osteoblast-like cells. Acta Orthop Scand 2002; 73:369-374.
33. Nichols KG,Puleo DA. Effect of metal ions on the formation and function of osteoclastic cells in vitro. J Biomed Mater Res 1997; 35:265-271.
34. Neale SD, Haynes DR, Howie DW, Murray DW, Athanasou NA. The effect of particle phagocytosis and metallic wear particles on osteoclast formation and bone resorption in vitro. J Arthroplasty 2000; 15:654-662.
35. Malluche HH. Aluminium and bone disease in chronic renal failure. Nephrol Dial Transplant 2002; 17:21-24.
36. Rudolf E, Cervinka M. Nickel modifies the cytotoxicity of hexavalent chromium in human dermal fibroblasts. Toxicol Lett 2010; 197:143-150.
37. Liu W, Chaspoul F, Botta C, De Méo M, Gallice, P. Bioenergetics and DNA alteration of normal human fibroblasts by hexavalent chromium. Environ Toxicol Pharmacol 2010; 29:58-63.

ENTEROCOCCUS FAECALIS BIOFILM. FORMATION AND DEVELOPMENT *IN VITRO* OBSERVED BY SCANNING ELECTRON MICROSCOPY

María de los Á. Bulacio[1], Lucas R. Galván[1], Cristina Gaudioso[2], Rosa Cangemi[2], Marta I. Erimbaue[1]

[1] Department of Endodontics, School of Dentistry, National University of Tucumán, Tucumán, Argentina..
[2] Department of Microbiology, School of Biochemistry, National University of Tucumán, Tucumán, Argentina.

ABSTRACT
Biofilm produced by Enterococcus faecalis isolated from root canals was detected by growing it on microplates and using 10% crystal violet stain, elution with alcohol and three procedures: no fixation, heat fixation and 10% formaldehyde fixation. The biofilm was evaluated using a Versamax Microplate Reader (USA).
Twenty sterile root portions were incubated in TS broth with E. faecalis (10^8) for 48 hours, 4, 7, 14 and 30 days, after which they were processed and observed by scanning electron microscopy (SEM).
Significantly more biofilm was found on the microplates for formaldehyde fixation than for heat fixation or no fixation (ANOVA $p<0.0001$). SEM showed E. faecalis growth at all times and biofilm development as from 14 days' incubation.
Fixation with 10% formaldehyde was the most appropriate technique for detecting E. faecalis biofilm development on microplates. SEM confirmed biofilm formation after 14 days incubation.

Key words: *biofilm; Enterococcus faecalis; scanning electron microscopy.*

BIOFILM DE *ENTEROCOCCUS FAECALIS*. PRODUCCIÓN *IN VITRO* Y DESARROLLO OBSERVADO CON MICROSCOPÍA ELECTRÓNICA DE BARRIDO

RESUMEN
Se realizó la detección de biofilm de Enterococcus faecalis aislados de conductos radiculares, desarrollándolos en microcubetas realizando tinción con Cristal Violeta 10 %, elución con alcohol y tres procedimientos: sin fijar, fijación con calor y fijación con formaldehido 10%. Se evaluó el biofilm formado realizando la lectura con lector de microplaca Versamax-Microplate-Reader (USA).
Se incubaron 20 porciones radiculares estériles en el medio TS caldo con E. faecalis (10^8) durante 48 hs, 4, 7, 14 y 30 días. Transcurrido cada tiempo experimental se procesaron y observaron al microscopio electrónico de barrido (MEB).
Se evidenció significativamente mayor formación de biofilm en microplacas cuando se realizó fijación con formaldehido, que al fijar con calor y sin fijar (ANOVA $p<0,0001$). Al MEB se observó crecimiento del E. faecalis en todos los tiempos y desarrollo de biofilm a partir de 14 días de incubación.
La fijación con formaldehido 10% fue la técnica más apropiada para detectar biofilm de E. faecalis desarrollado en microplacas. Mediante el MEB se confirmó la formación de biofilm después de 14 días de incubación.

Palabras clave: *biofilm; Enterococos faecalis; microscopía electrónica de barrido.*

INTRODUCTION

Enterococci are natural inhabitants of the oral cavity and normal intestinal flora, and are recognized as opportunistic pathogens. *Enterococcus faecalis* is the most common species, and is responsible for 80 - 90% of human infections[1].

Research has shown that areas in the canal which are inaccessible to instruments and irrigants may remain contaminated after biomechanical preparation and be responsible for persistent infection[2].

Cases of apical periodontitis resistant to conventional treatment[3] are attributed to the presence of biofilm inside the canal and at the apical end of teeth with pulp necrosis and chronic periapicial lesion[4].

SEM observations of *E. faecalis* have shown that it adheres to collagen structures, colonizes dentin surfaces and may progress towards dentin tubules and form mature biofilm[5].

The aim of this study was to compare detection on microplates of biofilm formed by *Enterococcus faecalis* isolated from root canals, in Tryptone Soya (TS) broth supplemented with 1% glucose and 10% human serum, using three different techniques: no

fixation, heat fixation and 10% formaldehyde fixation, and observing the development of biofilm on root portions under scanning electron microscopy (SEM) at 48 hours, 4, 7, 14 and 30 days.

MATERIALS AND METHODS

The study included 10 patients who attend the Endodontics Department at the School of Dentistry at the National University of Tucumán and provided informed consent, with indication for retreatment of teeth due to the persistence of chronic-type periapical lesion.

After clinical-radiographic diagnosis, the patients rinsed their mouths with 0.12% chlorhexidine solution (Plac Out. Microsules Bernabó, Argentina). The area was completely isolated and the operative field disinfected with 1% chlorhexidine solution. Caries was eliminated using hand instruments and irrigation with 1% chlorhexidine. The access cavity was prepared with water-cooled high-speed diamond burs. The operative field was disinfected and the canal filling material was removed with H-files, followed by irrigation with 1 mL distilled water. Samples were taken with two standard N° 20 paper points placed inside the root canal for one minute, and then placed individually in test tubes containing TS broth and transferred immediately to the laboratory.

In the laboratory we performed a semi-quantitative study by dilution in the following media: Azide agar for Enterococcus and Staphylococcus, MacConkey agar for Gram-negative bacilli, Blood agar for Streptococcus. The different colonies were counted and *E. faecalis* was identified according to morphological and physiological features[6].

The three strains with greatest development on plates were selected and their ability to produce biofilm was studied.

Biofilm formation on microplates

The culture medium used was Trypticase Soya (TS) broth supplemented with 1% glucose and 10% human serum. Three *E. faecalis* strains isolated from the canals were selected and called strains 1, 2 and 3.

Microplates were loaded with 200 µl. of the medium with inoculum at a concentration of 10^8, incubated at 37°C for 24 hours, without stirring, in triplicate. To detect biofilm production, the microplates were stained with 10% crystal violet (C.V.), washed with 95% ethanol to remove excess dye and subject to one of three different procedures:

- Without fixation
- Heat fixation
- Formaldehyde fixation

The biofilm formed was evaluated by measuring absorbance using a Versamax Microplate Reader (USA).

Biofilm development observed with scanning electron microscope (SEM)

Twenty disks were cut from the middle third of the roots of recently extracted upper central incisors using a water-cooled, high-speed N° 1090 cylindrical bur (Sorensen, Brazil). The disks were sterilized and placed individually in TS broth enriched with 1% glucose and 10% human serum, and inoculated with *E. faecalis* at a concentration of 10^8. The media were refreshed every 72 hours with the same inoculum concentration. Five experimental incubation times were used: 48 hs, 4, 7, 14 and 30 days, after which the samples were removed from the medium and placed individually in a solution of 8% paraformaldehyde, phosphate buffer and 16% glutaraldehyde and fixed for 48 hours at 4°C. They were washed 3 times for 10 minutes with phosphate buffer. A 1/1 osmium/phosphate buffer solution (2% OsO_4) was added and left overnight. The osmium was discarded and the samples were washed twice for 10 minutes with 30% ethanol. The samples were dehydrated by passing through a series of alcohol concentrations: ethanol 50%, 70%, 80%, 90%, and 3 times 100%, with 10 minutes for each step. The 100% ethanol was discarded and 100% acetone added for 1 hour[7]. The samples were mounted on an aluminum specimen holder, sputter-coated with gold and viewed under high vacuum with a JEOL JSM-35CF microscope (Tokyo, Japan). Microphotographs were taken at 10000 X.

RESULTS

Biofilm forming assay on microplates

Strain 2 had the greatest development in TS broth supplemented with 1% glucose and 10% human serum for all three techniques used (Table 1), so we selected it for the SEM study. The control value (0.0360) was taken from the reading for the microplate without biofilm.

Table 1: Analysis of Variance. Absorbance reading on biofilms in nm for three Enterococcus faecalis strains using three techniques. The mean is the average of the readings for the four wells seeded with the same strain.

Treatment	Strein	Mean	95% Confidence Interval		ANOVA
			Lower Limit	Upper Limit	
No fixation	1	0.1463	0.14	0.1525	$p < 0.0001$
	2	0.1613	0.1446	0.178	
	3	0.1263	0.1211	0.1315	
Heat	1	0.1288	0.1225	0.135	$p = 0.0017$
	2	0.1393	0.1372	0.1414	
	3	0.1289	0.1237	0.134	
Formaldehyde	1	0.2587	0.216	0.3014	$p < 0.0001$
	2	0.7303	0.5714	0.8892	
	3	0.1681	0.1573	0.1788	

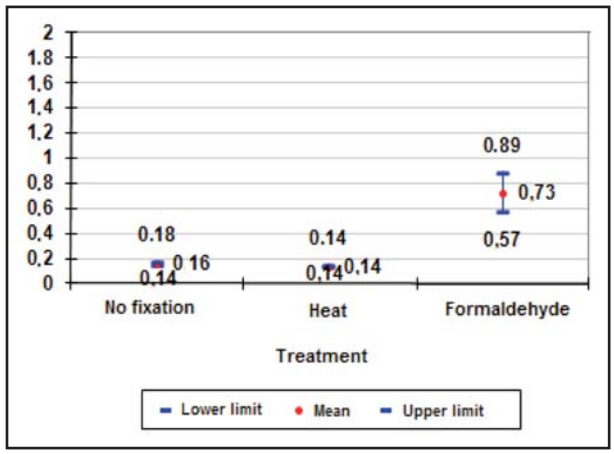


Fig. 1: Biofilm obtained. Absorbance (nm) measurements of E. faecalis strain 2, with three different fixation procedures.

The values obtained with strain No. 2 for the formaldehyde fixation treatment were significantly higher than for the other treatments (ANOVA, $p < 0.0001$), Fig. 1.

Biofilm development observed with scanning electron microscope

Figures 2 to 6 show the development of *Enterococcus faecalis* under SEM at the different times. Biofilm is apparent as from 14 days.

DISCUSSION

This study used the ability of *E. faecalis* to form biofilm on polystyrene[6], which occurs due to the presence of a surface protein, the Esp-encoding gene

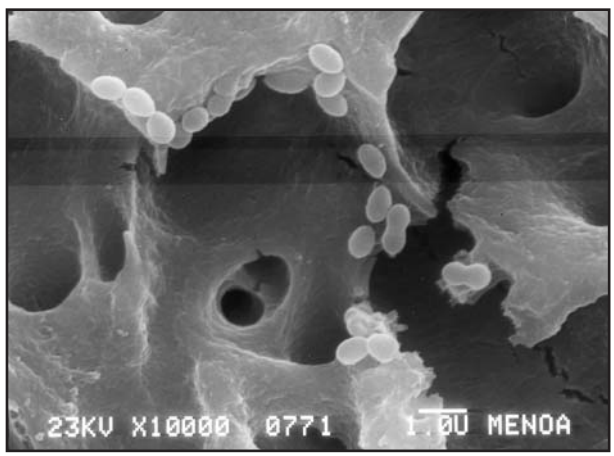


Fig. 2: SEM. E. faecalis development at 48 hs. The reference line corresponds to 1 µm.

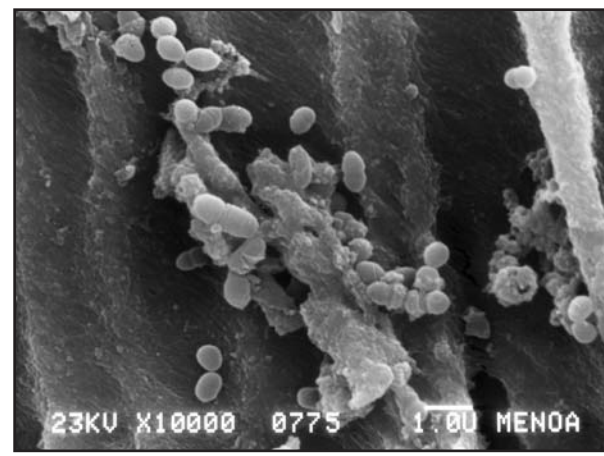


Fig. 3: SEM. E. faecalis development at 4 days. The reference line corresponds to 1 µm

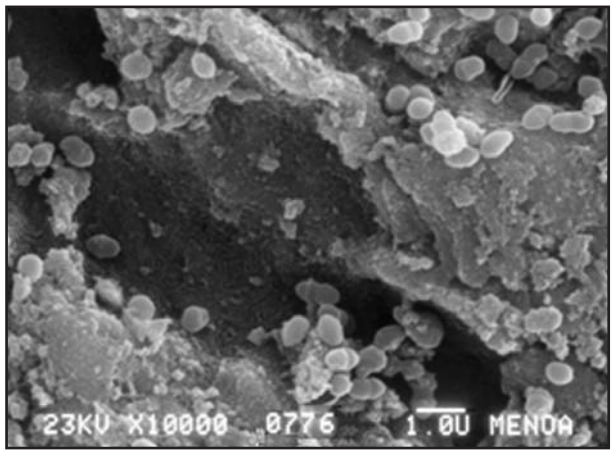


Fig. 4: SEM. E. faecalis development at 7 days. The reference line corresponds to 1 µm.

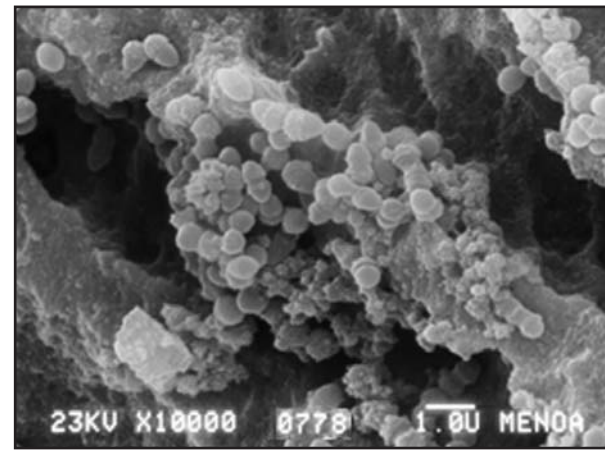


Fig. 5: SEM. E. faecalis biofilm at 14 days. The reference line corresponds to 1 µm.

(*esp)*[8], although Kristich et al.[9] claim that *E. faecalis* is able to form biofilms in absence of this gene.

Biofilm production often depends on factors such as cell density and glucose concentration[10], with several authors having proved that glucose supplement increases biofilm formation by *E. faecalis*[9-11] and other papers reporting that biofilms are denser in presence of glucose[12]. Our study took this factor into account.

Not all strains have the same biofilm-producing capacity. Our study used 3 *E. faecalis* strains isolated from root canals with endodontic failure. We selected the strain with the greatest capacity to produce biofilm on microplates, and used it to contaminate root dentin and observe biofilm development under SEM.

Duggan and Sedgley[13] found no statistically significant difference in the formation of biofilm by *E. faecalis* obtained from root canals, oral cavity and other parts of the body, using a similar culture medium and evaluating the optical density of the microplates, suggesting that the origin of the strain did not influence the result of their study.

Zheng et al.[14] found that 75.47% of the *E. faecalis* strains isolated from root canals with endodontic failure were able to develop biofilm.

Wang et al.[15] also found high biofilm forming rates by *E. faecalis* isolated from root canals with endodontic failure, employing growth on microplates and SEM observations.

Wilcox[16] report that early formaldehyde fixation may help show biofilm formation, and this technique was employed successfully in our study.

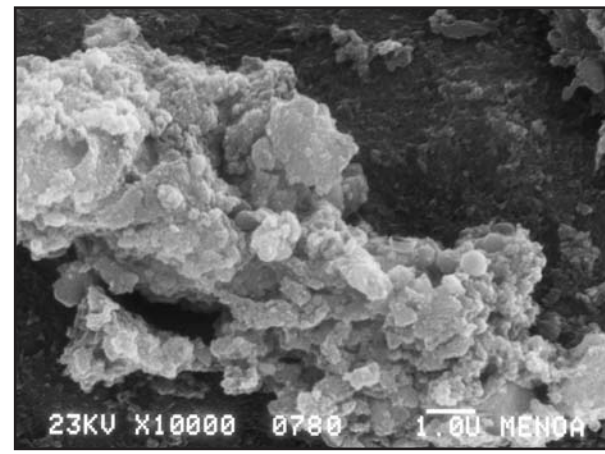


Fig. 6: SEM. Mature E. faecalis biofilm at 30 days. The reference line corresponds to 1 µm.

Our study found that *E. faecalis* biofilm was observable at 14 days and mature biofilm at 30 days. Guerreiro-Tanomaru et al.[17] report similar results. They used different substrates such as bovine dentin and bone, hydroxyapatite and gutta-percha, and reported biofilm development at 14 and 21 days, which they observed by using staining methods and confocal microscopy, finding the greatest development on hydroxyapatite. Wu et al.[18] inoculated *E. faecalis* on human teeth for 4 weeks to obtain biolfilm. Somayaji et al.[19] used SEM and found a high percentage of biofilm development 45 days.

Du et al.[20] made observations under confocal microscope on pieces of dentin incubated with *E. faecalis* for 1 and 21 days, finding young and a mature biofilm, respectively, at those times. Our study shows

that under the experimental conditions applied, it is possible to generate mature *E. faecalis* biofilm at 30 days' incubation on the surface of human root dentin.

CONCLUSIONS

Enterococcus faecalis isolated from root canals was able to produce biofilm on microplates, which was best seen using 10% crystal violet stain, elution with alcohol and fixation with 10% formaldehyde.

Mature *E. faecalis* biofilm formation was confirmed by SEM after 30 days' incubation, using pieces of root dentin in TS broth supplemented with 10% human serum and 1% glucose.

ACKNOWLEDGMENTS

Partially supported by CIUNT,Tucuman,Argentina.
We thank Dr. Marcela D'Urso for the statistical analysis.

CORRESPONDENCE

Dr.María de los Ángeles Bulacio.
Crisóstomo Álvarez 1487. CP 4000
San Miguel de Tucumán, Tucumán. Argentina
maritabulacio@hotmail.com

REFERENCES

1. Ruoff Kl, de la Maza L, Murtagh MJ, Spargo JD, Ferraro MJ. Species identities of enterococci isolated from clinical specimen. J Clin Microbiol 1990;28:435-437.
2. Nair PNR. Biology and pathology of apical periodontitis. In: Estrela C. Endodontic Science. 2nd ed. São Paulo: Artes Médicas 2009 p. 285-347
3. Ferreira FB, Ferreira AL, Gomes BP, Souza-Filho FJ. Resolution of persistent periapical infection by endodontic surgery. Int Endod J 2004; 37:61-69.
4. Leonardo MR. Endodoncia. Tratamiento de Conductos Radiculares. Principios Técnicos y Mecánicos. Editora Artes Médicas, Argentina 2005: p437.
5. Shehab ED, Soha AE. Development of an intracanal mature *Enterococcus faecalis* biofilm and its susceptibility to some antimicrobial intracanal medications; an in vitro study. Eur J Dent 2012;6:43-50.
6. Holt JG, Krieg NR, Sneath PHA, Staley JT, Williams ST, editors. Bergey's Manual of Determinative Bacteriology. Baltimore: Williams and Wilkins; 1994 p. 538.
7. Karnovsky MJ. A formaldehyde-glutaraldehyde fixative of high osmolality for use in electron microscopy. J Cell Biol 1965;27:137-8A.
8. Toledo-Arana A, Valle J, Solano C, Arrizubieta MJ, Cucarella C, Lamata M, Amorena B, Leiva Penade JR, Lasa I. The Enterococcal Surface Protein, Esp, Is Involved in *Enterococcus faecalis* Biofilm Formation. Appl Environ Microbiol. 2001; 67:4538-4545.
9. Kristich CJ, Li YH, Cvitkovitch DG, Dunny GM. Esp-independent biofilm formation by *Enterococcus faecalis*. J Bacteriol 2004; 186:154-163.
10. Pillai SK, Sakoulas G, Eliopoulos GM, Moellering RC Jr, Murray BE, Inouye RT. Effects of glucose on fsr-mediated biofilm formation in *Enterococcus faecalis*. J Infect Dis 2004; 190:967-97011.
11. Seneviratne CJ, Yip JW, Chang JW, Zhang CF, Samaranayake LP. Effect of culture media and nutrients on biofilm growth kinetics of laboratory and clinical strains of *Enterococcus faecalis*. Arch Oral Biol. 2013; 58:1327-1334.
12. Cerca N, Pier GB, Vilanova M, Oliveira R, Azeredo J. Quantitative analysis of adhesion and biofilm formation on hydrophilic and hydrophobic surfaces of clinical isolates of *Staphylococcus epidermidis*. Res Microbiol 2005;156:506-514.
13. Duggan JM, Sedgley CM. Biofilm formation of oral and endodontic *Enterococcus faecalis*. J Endod. 2007; 33:815-818.
14. Zheng J, Wang L, Song Q, Li J, W Niu . The correlation analysis on *Enterococcus faecalis* biofilm and clinical manifestation in retreatment of root canal. Hua Xi Kou Qiang Yi Xue Za Zhi 2012; 65-67, 72.
15. Wang L, Dong M, Zheng J, Song Q, Yin W, Li J, Niu W. Relationship of biofilm formation and gelE gene expression in *Enterococcus faecalis* recovered from root canals in patients requiring endodontic retreatment. J Endod 2011; 37:631-636.
16. Wilcox MH. Comparison of formalin and Bouin's reagent for fixation of coagulase negative staphylococcal biofilm. J Clin Pathol 1994; 47:1044-1046.
17. Guerreiro-Tanomaru JM, de Faria-Júnior NB, Duarte MA, Ordinola-Zapata R, Graeff MS, Tanomaru-Filho M. Comparative analysis of *Enterococcus faecalis* biofilm formation on different substrates. J Endod 2013;39: 346-350.
18. Wu D , Fan W , Kishen A , Gutmann JL, Fan B. Evaluation of the antibacterial efficacy of silver nanoparticles against *Enterococcus faecalis* biofilm. J Endod 2014;40: 285-290.
19. Somayaji K, Acharya SR, Bairy I, Prakash PY, Rao MS, Ballal NV. In vitro scanning electron microscopic study on the effect of doxycycline and vancomycin on enterocociccal induced biofilm. Iran Endod J 2010;5: 53-5820.
20. Du T, Wang Z , Shen Y, Ma J, Cao Y, Haapasalo M. Effect of Long-term Exposure to endodontic disinfecting solutions on young and old Enterococcus faecalis biofilms in dentin canals. J Endod 2014; 40:509-514.

EFFECT OF BISPHOSPHONATES ON THE LEVELS OF RANKL AND OPG IN GINGIVAL CREVICULAR FLUID OF PATIENTS WITH PERIODONTAL DISEASE AND POST-MENOPAUSAL OSTEOPOROSIS

María E. Verde[1], Daniela Bermejo[2], Adriana Gruppi[2], Miriam Grenón[1]

[1] Periodontology Department, School of Dentistry, National University of Córdoba, Córdoba, Argentina.

[2] Center of Biochemistry and Immunology Research. School of Chemistry Sciences. National University of Cordoba, Córdoba, Argentina.

ABSTRACT

The Receptor activator of nuclear factor-kappa B ligand (RANKL)/RANK/Osteoprotegerine (OPG) system has been proposed as essential for osteoclast biology and identified as key part in regulating the physiology and pathology of the skeletal system. The study of the RANKL/RANK/OPG system has increased the understanding of the mechanisms involved in the bone remodeling process, especially in postmenopausal osteoporosis and periodontal disease.

Bisphosphonates have become the mainstay of the treatment and prevention of post-menopausal osteoporosis. They inhibit the formation and dissolution of calcium phosphate crystals in bone and also osteoclasts, thus reducing bone turnover.Current investigations relate osteoporosis with the appearance and progression of periodontal disease. Although the etiology of both is different, the bone loss present in both shares several characteristics. Thus, therapy used for osteoporosis can be considered of value in the treatment of periodontal disease.

The aim of this study was to evaluate the levels of RANKL, OPG and their relationship in gingival crevicular fluid (GCF) in patients with periodontal disease and postmenopausal osteoporosis/ osteopenia in relation to consumption of bisphosphonates.

We studied 66 periodontal active sites obtained from 17 post-menopausal women patients aged between 45-70 years old with osteoporosis/osteopenia and periodontal disease. GCF samples were collected using sterile filter paper strips. To determine the concentration of RANKL and OPG, a commercial ELISA assay was used. The values of RANKL, OPG and their ratio (RANKL/ OPG) were compared with Mann-Whitney U Test.

The values of RANKL, OPG and their ratio obtained in patients with osteoporosis/osteopenia and periodontal disease with or without bisphosphonates treatment showed no differences. Bisphosphonates do not alter the concentration of RANKL and OPG and their ratio in the GCF of patients with osteoporosis/ osteopenia and periodontal disease, probably because these cytokines may not be the main target of bisphosphonates to inhibit bone resorption in periodontal disease.

Key words: *periodontal disease; RANKL; OPG; bisphosphonates; osteoporosis.*

EFECTO DEL CONSUMO DE BIFOSFONATOS EN LOS NIVELES DEL LIGANDO DEL RECEPTOR ACTIVADOR DEL FACTOR NUCLEAR KAPPA-B Y OSTEOPROTEGERINA EN FLUIDO CREVICULAR DE PACIENTES CON ENFERMEDAD PERIODONTAL Y OSTEOPOROSIS POST-MENOPÁUSICA

RESUMEN

El sistema: Receptor activador del factor nuclear kappa-B ligando (RANKL)/RANK/Osteoprotegerina (OPG) ha sido propuestos como esencial para la biología osteoclástica, ya que ha sido identificado como participante clave en la regulación fisiológica y patológica del sistema óseo. El estudio del sistema RANKL-RANK-OPG ha facilitado la comprensión de los mecanismos intervinientes en el proceso de remodelación ósea, especialmente en la osteoporosis post-menopáusica y la enfermedad periodontal.

Los bisfosfonatos se han convertido en el pilar principal del tratamiento y prevención de la osteoporosis post-menopáusica. Ellos inhiben la formación y disolución de los cristales de fosfato de calcio en el hueso y también inhiben a los osteoclastos reduciendo el recambio óseo.

Actualmente, varios trabajos de investigación asocian la osteoporosis con el inicio y la progresión de la enfermedad periodontal. Aunque la etiología de ambas es diferente, la pérdida de masa ósea comparte varias características y la terapéutica utilizada para la osteoporosis puede ser considera de valor para el tratamiento de la enfermedad periodontal.

El objetivo de este estudio fue evaluar el efecto del consumo de bifosfonatos en fluido crevicular (FC) sobre los niveles de RANKL, OPG y la relación RANKL/OPG en pacientes post-menopáusicas con enfermedad periodontal y osteoporosis/ osteopenia.

Se estudiaron 66 sitios periodontalmente activos obtenidos de pacientes mujeres post-menopáusicas con edades entre 45-70 años de edad con enfermedad periodontal y osteoporosis/ osteopenia. La toma del FC se realizó mediante tiras de papel de filtro estériles. Para determinar la concentración de RANKL y OPG se utilizó el ensayo de ELISA comercial siguiendo las instrucciones del fabricante. Los valores obtenidos de las citoquinas y su relación fueron comparados con el Test U de Mann-Whitney.

No se observaron diferencias en las concentraciones de RANKL y OPG encontradas, ni en su relación, en pacientes con enfermedad periodontal y osteoporosis/osteopenia con y sin tratamiento de bifosfonatos.

Esto sugiere que probablemente estas citoquinas no serian el blanco principal de los bifosfonatos para inhibir la resorción ósea en la enfermedad periodontal.

Palabras clave: *enfermedad periodontal; RANKL; OPG; bifosfonatos; osteoporosis*

INTRODUCTION

Periodontal disease is an inflammatory condition caused by multiple factors where the most significant environmental risk factor is the bacteria that reside in the biofilm. According to the report by Page and Kornman[1], these bacteria are essential but not enough to cause periodontal disease, while factors related to the host, such as heredity and environmental factors like smoking or consumption of certain drugs, are important determinants of the occurrence and severity of this condition.

In the appearance and progression of periodontal disease there is an influence of a wide range of determinants and risk factors, among them osteoporosis/osteopenia and estrogen deficiency are included. Immune and skeletal systems share a variety of regulatory molecules such as cytokines. In the bone marrow, immune cells interact with bone cells. Consequently, the physiology and pathology of one system may affect the other; indeed, abnormal activation of the immune system leads to bone destruction [2].

Members of the tumor necrosis factor alpha superfamily have been proposed as essential for osteoclast biology because they were identified as key parts in the physiological and pathological regulation of the bone system [3]. The members RANKL/RANK/OPG form a system involved in osteoclast formation and activation. The binding of RANKL to its receptor RANK, in the presence of macrophage-colony stimulating factor (M-CSF), provides the fundamental signal to drive the development of osteoclasts from hematopoietic progenitor cells, as well as to activate osteoclasts. OPG binds to RANKL and inhibits bone resorption, preventing both cellular differentiation and function of osteoclasts [4]. Osteoclast recruitment depends on the balance between the RANKL and OPG [5].

The RANK-RANKL-OPG axis is involved in the regulation of bone metabolism in periodontitis and osteoporosis/osteopenia, in which an increase in the relative expression of RANKL and a decrease in OPG can favor the balance to osteoclastogenesis and bone reabsorption [6,7]. Increased RANKL and decreased OPG have been observed in various inflammatory and bone diseases such as osteoporosis, rheumatoid arthritis, periodontal disease, and multiple myeloma [8-10]. Local deregulation of RANKL-OPG levels may lead to alveolar bone reabsorption as has been demonstrated in experimental models of periodontitis [11,12]. Many studies show that, compared to healthy subjects, patients with periodontitis exhibit higher RANKL expression and a reduction of OPG levels in GCF [10,13,14], or gingival tissue [14-17]. Periodontitis and osteoporosis represent main health problems, especially in elderly women. Although the etiology of postmenopausal osteoporosis and periodontal disease is different, the bone loss that occurs in both diseases shares several features [6]. Thus, therapy used for osteoporosis can be considered of value in the treatment of periodontal disease. Among the many drugs for osteoporosis treatment, bisphosphonates are the most commonly prescribed and first-line drugs in most cases [18]. Bisphosphonates have been characterized as modulators of osteoclast function and bone metabolism [19]. Particularly they act on bone tissue, decreasing bone turnover, reducing bone resorption and the number of new bone multicellular units. At cellular level, they reduce the recruitment of osteoblasts and osteoclasts, the adhesion of osteoclasts to the bone as well as the release of cytokines by macrophages. Considering RANKL expression is essential for osteoclast differentiation; the effect of bisphosphonates on osteoclast differentiation could be related to a decrease in the expression of RANKL or by an increase of OPG. On the basis of these properties, several generations of bisphosphonates have been successfully developed in the treatment of post-menopausal osteoporosis, osteopenia and Paget's disease [20].

Considering that RANKL/RANK/OPG system mediates periodontal disease and osteoporosis/osteopenia and that bisphosphonates are established as one of the effective drugs for osteoporosis treatment and that they can act on the RANKL/RANK/OPG mentioned, the aim of this study was to investigate the effect of bisphosphonate treatment on the levels of RANKL and OPG, and their ratio in GFC of post-menopausal women patients with periodontal disease and osteoporosis/osteopenia.

MATERIALS AND METHODS

Study population and clinical examination

A total of 66 samples of GFC were obtained from active periodontal sites of 17 post-menopausal women aged between 45-70 years old with and without bisphosphonate-treatment (risedronate or ibandronate: 150 mg/ 1 tablet a month for at least 3 months, prior to the study), all with chronic

periodontitis and osteoporosis/osteopenia. All women were recruited from the Cimateric Department, University Hospital of Maternity and Neonatology, National University of Cordoba, Argentina. The study protocol was approved by a Bioethical Institutional Committee and explained to all participants who signed the informed consent forms.

Complete medical and dental histories from all patients were considered. None of them had had any systemic illness nor taken therapeutic medication such as antibiotics or anti-inflammatory drugs that could affect the periodontal status for at least 6 months before the study, and also they did not have a history of aggressive periodontitis and they had not received periodontal treatment before they entered the study. The selection of the patients was made according to criteria proposed by the World Health Organization [21]. To determine the clinical periodontal status, all subjects were subject to a clinical periodontal examination including the measurement of probing pocket depth (PPD) and clinical attachment level (CAL) at six sites around each tooth with a manual probe, using a Marquis probe. Dichotomous measurement of supragingival plaque index (PI) and bleeding index (BI) were also recorded.

Diagnosis of osseous and periodontal disease

The presence of 1 or more sites with PPD ≥ 4 mm and ≥ 4 mm CAL [22] in a patient was defined as/considered to be periodontitis. Sites with bleeding on probing were initially defined as active sites [8].

Dual X-ray absorptiometry (DXA) was used to evaluate the bone mineral density, since this method is considered "gold standard" for the diagnosis-prognosis of osteoporosis, monitoring the natural history of the disorder and response to treatment [21].

The diagnosis of osteoporosis/osteopenia was verified from the densitometry reports from proximal femur and lumbar column, following the criteria established by the WHO at the Consensus Development Conference [23].

Osteopenia was defined as a bone mineral density T score (difference between the measured bone mineral density and the mean value for young white women in standard deviations [SDs]) of less than –1 SD or at least –2.5 SD. Osteoporosis was defined by a bone mineral density T score of less than –2.5 SD [23]. The individuals with osteopenia were grouped with those with osteoporosis to form an osteopenia/osteoporosis group.

Sampled sites were then categorized into two groups: a group of 12 post-menopausal women with osteoporosis/osteopenia treated with bisphosphonates (n=55 samples) and a group of 5 post-menopausal women with osteoporosis/osteopenia without bisphosphonates therapy (n = 11 samples).

Collection of Gingival Crevicular Fluid

GCF was obtained using sterile filter paper strips (Periopaper; Oraflow, New York, NY, USA). The selected sites were cleared of supragingival plaque, isolated with cotton rolls and dried with a gentle stream of air to prevent saliva contamination. A sterile Periopaper™ strip (ProFlow Inc., Amityville, NY, USA) was gently inserted into the periodontal pocket until mild resistance was felt and it was left in place for 30 seconds. Mechanical irritation was avoided and strips contaminated with blood were discarded.

After GCF collection, strips were placed in an Eppendorf tube and samples were stored at -80°C before laboratory analysis.

4-5 GCF samples were obtained from post-menopausal women from the group with osteoporosis/osteopenia under bisphosphonate treatment and 2-3 GCF samples were obtained from post-menopausal women from the group with osteoporosis/osteopenia without bisphosphonate therapy.

Paper strips for each patient were pooled, and the GCF was extracted and assayed for the content of RANKL and OPG. GCF was extracted from the paper strips with buffer (50 mM phosphate buffer, pH 7.2, containing protease inhibitors), and collected after centrifugation at 15,000 g and 4ºC for 10 min.

Quantification of RANKL and OPG

Concentrations of RANKL and OPG from GCF samples were determined by ELISA, following manufacturer's instructions (Antigenix America Inc., New York, NY, USA) and the ratio of both cytokines was established.

Briefly, diluted GCF samples were loaded into single wells of a 96-well plate coated with 20 ng/ml of anti-human RANKL antibody or with 4 ng/ml of anti-human-OPG antibody, and incubated overnight at 4°C. Plates were washed four times. After the last wash, 0.2 ug/ml of biotin labelled anti-human

RANKL or OPG were added and incubated for 60 minutes at room temperature.
The reaction was developed with the addition of streptavidin coupled to peroxidase and visualized with the addition of 3.3'-5.5'-tetramethylbenzidine which was transformed into a colored product in the presence of the enzyme. The reaction was stopped by the addition of sulfuric acid and color was measured in a microplate spectrophotometer at 450 nm.
The concentration was measured with reference to standard curves using known amounts of recombinant RANKL and OPG.

Data Analysis
Data were expressed as means ± standard deviation. Taking into account the size of samples, a non-parametric analysis (Mann-Whitney Test U) was used. GraphPad Software (version 5.00 for Windows, San Diego California USA) was used to process the data. P-values ≤0.05 were considered statistically significant.

RESULTS
Clinical parameters of the patients
Fifty five periodontally active sites from twelve patients were analyzed in the study group of post-menopausal women with osteoporosis/osteopenia treated with bisphosphonates (age: 56.21 ± 9.24 years old) and eleven periodontal active sites from five patients were studied in the control group of postmenopausal women without treatment with bisphosphonates (age: 56.55 ± 4.68 years old). Table 1 summarizes the clinical characteristics of subjects included in this study. No statistically significant differences in clinical characteristics (age, PPD, CAL, PI and BI) were observed between groups. In the case of clinical records, PPD and CAL were obtained from an average of measured active sites, while the BI and PI were an average of the entire oral cavity.

Levels of RANKL, OPG and RANKL/OPG ratio in GCF from patients treated or not with bisphosphonates
GCF samples obtained from subjects under bisphosphonate treatment (n= 55) showed no significant differences in the levels of RANKL in comparison with the group without bisphosphonates treatment (n = 11), p = 0.88. The values were 26.81 ± 18.66 and 27.78 ± 19.50 pg/ml, respectively (Fig. 1).
OPG showed similar behavior to RANKL. OPG concentrations showed no statistically significant difference (p = 0.62) between both group of patients, whose values were 0.05 ± 0.02 pg/ml in GCF samples from subjects under treatment and 0.04 ± 0.01 pg/ml in GCF samples from patients without treatment (Fig. 2).
As the RANKL/OPG ratio is considered indicative of bone resorption, we investigated if changes in RANKL and OPG levels reflected changes in this ratio. The group under bisphosphonate treatment exhibited a RANKL/OPG ratio of 693.5 ± 561.3 pg/ml, while the group without bisphosphonate treatment showed similar values, 677.7 ± 437.6 pg/ml. There were no significant differences among these groups (Fig. 3).

DISCUSSION
Bisphosphonates have been shown to inhibit the progression of osteoporosis as a result of the reduction of bone loss and they have been characterized as modulators of osteoclast function and bone metabolism [19].

Previous reports suggest that bisphosphonates can reduce not only the maturation and osteoclast function directly, but they can also act indirectly through the synthesis of mediators that interfere with osteoclastogenesis [24-26], further regulating essential signaling molecules involved in osteoclastogenesis such as RANKL [27]. The system RANKL/RANK/OPG acts as a final effector molecular system. RANKL,

Table 1: Baseline characteristics of the study groups (mean ± SD).

	Osteoporosis/ Osteopenia NT	Osteoporosis/ Osteopenia WT	
Clinical parameters	**(n=11)**	**(n=55)**	***P***
Age (years)	**56.55 ± 4.68**	**56.21 ± 9.24**	**0.1898**
PPD (mm site)	3.5 ± 0.7	3.3 ± 1.2	0.493
CAL (mm site)	**3.7 ± 1.19**	**3.2 ± 1.56**	**0.1727**
PI (%)	68.74 ± 25.63	59.14 ± 37.34	0.1815
BI (%)	**54.33 ± 21.88**	**68.12 ± 30.76**	**0.2115**

PPD: Probing pocket depths. CAL: Clinical attachment loss. PI: Plaque index. BI: Bleeding index. NT: Not treated with bisphosphonates. WT: With bisphosphonate treatment.

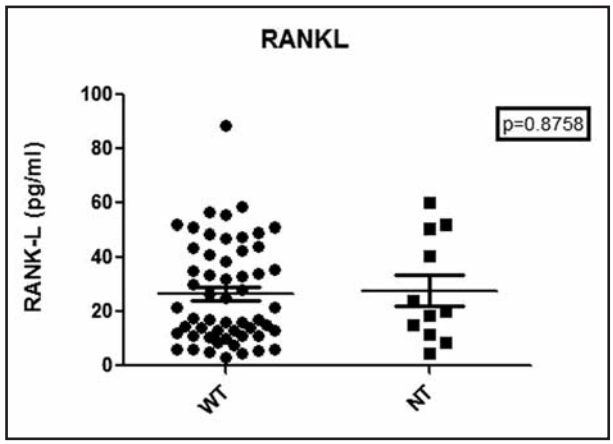


Fig. 1: Distribution levels of RANKL in GCF from active periodontal sites of women with bone disease under bisphosphonate treatment –WT- (n = 55) or without treatment–NT- (n = 11). The individual values represent the concentration of RANKL in GCF [Total RANKL (pg) / volume (ml)] in each patient.

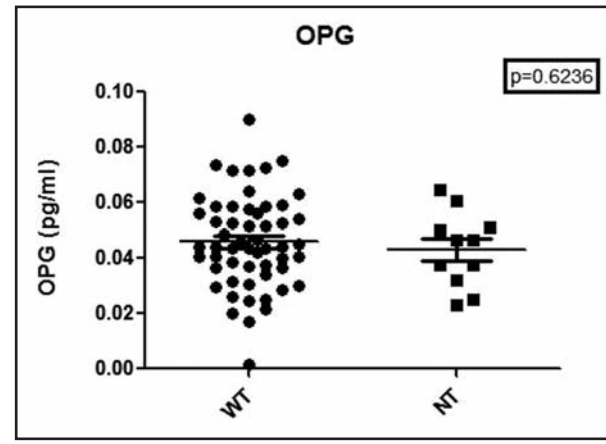


Fig. 2: Distribution levels of OPG in GCF from active periodontal sites of women with bone disease under treatment–WT-(n = 55) or without treatment –NT-(n= 11). The individual values represent the concentration of GCF in OPG [Total OPG (pg) / volume (ml)] in each subject..

RANK, and the decoy receptor OPG, are three key molecules that regulate the recruitment and function of osteoclasts [28]. The binding of RANKL to RANK provides the fundamental signal to drive the development of osteoclasts from hematopoietic progenitor cells, as well as to activate mature osteoclasts. OPG negatively regulates by binding to RANKL, thus inhibits bone reabsorption by preventing both cellular differentiation and function of osteoclasts [4,28]. The recruitment of osteoclasts depends on the balance between RANKL and OPG [5]. To our knowledge, this is the first clinical trial to study the effect of risedronate/ibandronate treatment on RANKL and OPG levels in GCF and their relative ratio in post-menopausal women with osteoporosis/ osteopenia and periodontal disease.

Several studies evaluated the effect of bisphosphonates on the levels of RANKL and OPG; however, the results have been controversial. Tipton et al. [29] demonstrated that the action of alendronate and pamidronate on human gingival fibroblasts, through altering the production of RANKL and OPG, appears to contribute to a microenvironment that favors the inhibition of bone reabsorption due to an increase or no change in the levels of OPG, and a decrease in the production of RANKL. Viereck et al. [30] have demonstrated that bisphosphonates such as pamidronate and zoledronate increase the expression of OPG mRNA in primary human osteoblasts. Pan *et al.* [31] demonstrated that zoledronic acid may inhibit

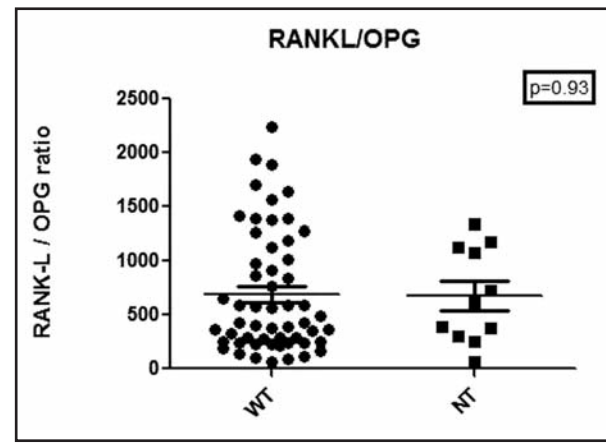


Fig. 3: Distribution levels of RANKL / OPG in GCF from active periodontal sites of women with bone disease under treatment -WT-(n = 55) or without treatment –NT-(n= 11). The individual values represent the concentration in GCF of the RANKL / OPG [Total RANKL (pg) / volume (ml) / total OPG (pg) / volume (ml)] in each subject.

bone reabsorption by reducing the expression of RANKL and increasing transmembrane OPG secretion in cells such as osteoblasts. However, it does not affect gene expression of RANKL and OPG. Dobnig et al. [32] showed a significant increase in serum levels of OPG in patients treated with alendronate and risedronate, whereas serum levels of RANKL were unchanged throughout the treatment period; on the other hand, a positive correlation between changes in serum levels of OPG and BMD was found.

On the contrary, Kim et al. [33] demonstrated that nitrogen-containing bisphosphonates, such as alendronate and pamidronate, do not alter the RANKL and OPG mRNA expression in favor of the inhibition of the osteoclast formation [30]. Alvarez et al. [34] demonstrated that serum OPG decreases after treatment with tiludronate. In contrast, RANKL serum levels and RANKL/OPG ratio are unmodified in patients with Paget's disease. D'Amelio et al. [35] demonstrated that risedronate inhibits the *in vitro* formation of osteoclast by reducing the number and degree of differentiation of osteoclast precursors and by reducing the half-life and the inhibition of the production of pro-osteoclastogenics cytokines. Moreover, risedronate is able to reduce the recruitment of osteoclasts from peripheral blood and this could be the effect of the reduction in cytokine production. Besides, it was observed a reduction in the levels of OPG and RANKL (soluble) in serum during treatment with bisphosphonates. A study by Kwak et al. [36] concluded that risedronate inhibitory effects on osteoclast differentiation are related to decreased expression of RANKL.

According to previous studies, osteotropic agents modulate osteoclast formation through the regulation of RANKL/OPG ratio instead of increasing or decreasing levels of RANKL or OPG alone [37,38]. The increase in the RANKL/OPG ratio results in a favorable condition for osteoclast formation and vice versa.

In this study it was observed that risedronate and ibandronate consumption did not change the GCF RANKL and OPG levels, and RANKL/OPG ratio in patients suffering osteoporosis/osteopenia and periodontal disease. So it could be suggested that RANKL and OPG might not be the main target of bisphosphonates to inhibit bone resorption in periodontal disease, reaffirming the results of Kim et al. [33] and Pan et al. [31].

The discrepancy in the results about the effects of bisphosphonates on the above cytokines can be attributed to several reasons. Firstly, the research design used was different to previous reports. Experimental studies were carried out with mice [33,36] and with human cells *in vitro* [29-31]; however, in this study, the effect of bisphosphonates was evaluated on patients, as in other researches [32,34,35]. Secondly, all drugs analyzed were aminobisphosphonates and they had a different chemical structure. Thirdly, the methods of analysis of the cytokines varied if the gene expression or the presence of proteins was studied.

In conclusion, the present study showed that nitrogen-containing bisphosphonates, ibandronate and risedronate, did not alter the GCF RANKL and OPG levels and their relative ratio in periodontal disease. It could be suggested that RANKL and OPG might not be the main target of bisphosphonates to inhibit bone resorption in periodontal disease.

ACKNOWLEDGMENTS

E.Verde acknowledges the Secretary of Science and Technology – Universidad Nacional de Córdoba (SECyT-UNC) for the PhD scholarship awarded and the Secretary of Science and Technology - School of Dentistry (SECyT-FO) for the financial support for this publication.

The authors acknowledge the assistance of Dr. Monica Ñañez de Lucino from the University Hospital of Maternity and Neonatology.

CORRESPONDENCE

Dr. María Eugenia Verde
Fray Mocho 2242 - B° Parque Corema.
Córdoba Capital. C.P 5009
Argentina
eugenia.verde@unc.edu.ar

REFERENCES

1. Page RC, Kornman KS. The pathogenesis of human periodontitis: an introduction. Periodontol 2000; 1997: 9-11.
2. Takayanagi H. Inflammatory bone destruction and osteoimmunology. J Periodontal Res 2005; 40:287-293.
3. Yasuda H, Shima N, Nakagawa N, Yamaguchi K, Kinosaki M, Mochizuki S, Tomoyasu A, Yano K, Goto M, Murakami A, Tsuda E, Morinaga T, Higashio K,Udagawa N, Takahashi N, Suda T. Osteoclast differentiation factor is a ligand for osteoprotegerin/osteoclastogenesis-inhibitory factor and is identical to TRANCE/RANKL. Proc Natl Acad Sci U S A 1998; 95:3597-3602.
4. Wada T, Nakashima T, Hiroshi N, Penninger JM. RANKL–RANK signaling in osteoclastogenesis and bone disease. Trends Mol Med 2006; 12:17-25.
5. Nagasawa T, Kiji M, Yashiro R, Hormdee D, He L, Kunze M, Suda T, Koshy G, et al. Papel del ligando del receptor activador del factor nuclear K B (RANKL) y de la osteoprotegerina en la salud y la enfermedad periodontal. Periodontol 2000 (Ed Esp) 2008; 18:43-55.
6. Lerner UH. Bone remodeling in post-menopausal osteoporosis. J Dent Res. 2006; 85:584-595.
7. Lerner UH. Inflammation-induced bone remodeling in periodontal disease and the influence of postmenopausal osteoporosis. J Dent Res 2006; 85:596- 607.

8. Armitage, GC. Development of a Classification System for Periodontal Diseases and Conditions. Ann Periodontol 1999; 4:1-6.
9. Joss A, Adler R, Lang NP. Bleeding on probing. A parameter for monitoring periodontal conditions in clinical practice. J Clin Periodontol 1994; 21:402-408.
10. Cochran DL. Inflammation and bone loss in periodontal disease. J Periodontol 2008; 79:1569-1576.
11. Khosla S, Arrighi HM, Melton LJ III, Atkinson EJ, O'Fallon WM, Dunstan CR, Riggs BL. Correlates of osteoprotegerin levels in women and men. Osteoporos Int 2002; 13:394-399.
12. Liu D, Xu JK, Figliomeni L , Huang L, Pavlos NJ, Rogers M, Tan A, Price P et al. Expression of RANKL and OPG mRNA in periodontal disease: possible involvement in bone destruction. Int J Mol Med 2003; 11:17-21.
13. Mogi M. Otogoto J, Ota N, Togari A. Differential expression of RANKL and Osteoprotegerin in gingival crevicular fluid of patients with periodontitis. J Dent Res 2004; 83: 166-169.
14. Teng YT, Nguyen H, Gao X, Kong Y, Gorczynski R, Singh B, Ellen R, Penninger J. Functional human T-cell immunity and osteoprotegerin ligand control alveolar bone destruction in periodontal infection. J Clin Invest 2000; 106:59-67.
15. Taubman TA, Valverde P, Han X, Kawai T. Immune response: the key to bone resorption in periodontal disease. J Periodontol 2005; 76:2033-2041.
16. Vernal R, Chaparro A, Graumann R, Puente J, Valenzuela MA, Gamonal J. Levels of cytokine receptor activator of nuclear factor KB ligand in gingival crevicular fluid in untreated chronic periodontitis patients. J Periodontol 2004; 75:1586-1591.
17. Lu HK, Chen YL, Chang HC, Li CL, Kuo MY. Identification of the OPG/RANKL system in gingival crevicular fluid and tissue of patients with chronic periodontitis.J Periodontal Res 2006; 41:354-360.
18. Russell RG, Rogers MJ. Bisphosphonates: from the laboratory to the clinic and back again. Bone. 1999; 25:97 - 106.
19. Tenenbaum HC, Shelemay A, Girard B, Zohar R, Fritz PC. Bisphosphonates and periodontics: Potential applications for regulation of bone mass in the periodontium and other therapeutic/ diagnostic uses. J Periodontol 2002; 73: 813-822.
20. Russell RG. Bisphosphonates: mode of action and pharmacology. Pediatrics 2007; 119:150-162.
21. WHO Scientific Group on the Prevention and Management of Osteoporosis (2000: Geneva, Switzerland) Prevention and management of osteoporosis: report of a WHO scientific group. (WHO technical report series; 921).
22. AAP - American Academy of Periodontology. Parameter on chronic periodontitis with slight to moderate loss of periodontal support. J Periodontol. 2000; 71:853-855.
23. WHO Study Group. Assessment of fracture risk and its application to screening for postmenopausal osteoporosis. Report of a WHO scientific group. World Health Organization, 1994 (WHO Technical Report Series, No. 843).
24. Sahni M, Guenther HL, Fleisch H, Collin P, Martin TJ. Bisphosphonates act on rat bone resorption through the mediation of osteoblasts. J Clin Invest, 1993; 91: 2004-2011.
25. Vitté C, Fleisch H, Guenther HL. Bisphosphonates induce osteoblasts to secrete an inhibitor of osteoclast-mediated resorption. Endocrinology, 1996; 137:2324-2333.
26. Owens JM, Fuller K, Chambers TJ. Osteoclast activation: potent inhibition by the bisphosphonate alendronate through a nonresorptive mechanism. J Cell Physiol 1997; 172:79-86.
27. Thomas GP, Baker SU, Eisman JA Gardiner EM. Changing RANKL/OPG mRNA expression in differentiating murine primary osteoblasts. J Endocrinol, 2001. 170:451-460.
28. Boyle WJ, Simonet WS, Lacey DL. Osteoclast differentiation and activation. Nature 2003; 423:337-342.
29. Tipton DA, Seshul BA, Dabbous MKh. Effect of bisphosphonates on human gingival fibroblast production of mediators of osteoclastogenesis: RANKL, osteoprotegerin and interleukin-6. J Periodont Res 2011; 46:39-47.
30. Viereck V, Emons G, Lauck V, Frosch KH, Blaschke S, Gründker C, Hofbauer LC. Bisphosphonates pamidronate and zoledronic acid stimulated osteoprotegerin production by primary human osteoblasts. Biochem Biophys Res Commun 2002; 291:680-686.
31. Pan B, Farrugia A, To L, Findlay DM, Green J, Lynch K, Zannettino AC. The nitrogen-containing bisphosphonate, zoledronic acid, influences RANKL expression in human osteoblast-like cells by activating TNF-alpha converting enzyme (TACE). J Bone Miner Res 2004; 19:147-154.
32. Dobnig H, Hofbauer LC, Viereck V, Obermayer-Pietsch B, Fahrleitner-Pammer A. Changes in the RANK ligand/ osteoprotegerin system are correlated to changes in bone mineral density in bisphosphonate-treated osteoporotic patients. Osteoporos Int 2006; 17:693-703.
33. Kim YH, Kim GS, Jeong-Hwa B. Inhibitory action of bisphosphonates on bone resorption does not involve the regulation of RANKL and OPG expression. Exp Mol Med 2002; 34:145-151.
34. Alvarez L, Peris P, Guanabens N, Vidal S, Ros I, Pons F, Filella X, Monegal A, et al. Serum osteoprotegerin and its ligand in Paget's disease of bone: relationship to disease activity and effect of treatment with bisphosphonates. Arthritis Rheum 2003; 48:824-828.
35. D'Amelio P, Grimaldi A, Di Bella S, Tamone C, Brianza SZM, Ravazzoli MGA, Bernabei P, Cristofaro MA, et al. Risedronate reduces osteoclast precursors and cytokine production in postmenopausal osteoporotic women. J Bone Miner Res 2008; 23:373-379.
36. Kwak HB, Kim JY, Kim JY, Choi MK, Kim JJ, Kim KM, Shin YI, Lee MS et al. Risedronate directly inhibits osteoclast differentiation and inflammatory bone loss, Biol Pharm Bull 2009; 7:1193-1198.
37. Hofbauer LC, Khosla S, Dunstan CR, Lacey DL, Spelsberg TC, Riggs BL. Estrogen stimulates gene expression and protein production of osteoprotegerin in human osteoblastic cells. Endocrinology 1999; 140:4367-4370.
38. Hofbauer LC, Heufelder AE. Role of receptor activator of nuclear factor-KB ligand and osteoprotegerin in bone cell biology. J Mol Med 2001; 79:243-253.

SEMI-LONGITUDINAL STUDY OF THE MCNAMARA CEPHALOMETRIC TRIANGLE IN CLASS II AND CLASS III SUBJECTS GROUPED BY CERVICAL VERTEBRAE MATURATION STAGE

Luis E. Arriola-Guillén[1], Fernando D. Fitzcarrald[2], Carlos Flores-Mir[3]

[1] School of Dentistry. Universidad Científica del Sur , Lima, Perú
[2] School of Dentistry. Universidad Científica del Sur , Lima, Perú.
[3] Faculty of Medicine and Dentistry, University of Alberta, Edmonton, Alberta, Canada.

ABSTRACT
The aim was to compare the McNamara cephalometric triangle values in untreated normodivergent Class II and Class III malocclusion subjects of Latin American origin grouped by cervical vertebrae maturation stage to an untreated Class I malocclusion normodivergent control group.
The study was conducted on a sample of 610 pretreatment lateral cephalograms (250 male, 360 female), examined and grouped according to their anteroposterior skeletal relationship (Class I, II or III), cervical vertebrae maturation stage (Pre Pubertal Peak P1 = CS1 and CS2, Pubertal Peak P2= CS3 and CS4, and Post Pubertal Peak P3 = CS5 and CS6) and sex. Co-A, Co-Gn and ENA-Me were measured in each lateral cephalogram. ANOVA and Tukey HSD post-hoc tests were performed to determine differences between the groups. The results showed that in males, the greatest maxillary and mandibular dimensional increases occurred during the P3 stage (CS5 to CS6), while in females, they occurred in the P2 stage (CS3 to CS4). The Co-A and Co-Gn showed significant differences between the malocclusion classes ($p<0.05$). The maxillary lengths in Class II subjects and the mandibular lengths in Class III subjects were already higher at the beginning of the period evaluated (P1). A worsening trend for the Class II and III malocclusions was identified during the period evaluated.
Finally, changes in the McNamara cephalometric triangle values were markedly different in the three normodivergent skeletal malocclusion classes. In these Latin American subjects the pubertal growth spurt occurred at different times with respect to the Caucasian and Asian norms.

Key words: *Cephalometry; malocclusion; Angle class II; malocclusion; Angle class III.*

ESTUDIO SEMI-LONGITUDINAL DEL TRIÁNGULO CEFALOMÉTRICO DE MCNAMARA EN SUJETOS DE CLASE II Y CLASE III AGRUPADOS POR ESTADIO DE MADURACIÓN DE VERTEBRAS CERVICALES

RESUMEN
El objetivo del estudio fue comparar los valores del triángulo cefalométrico de McNamara en sujetos normodivergentes no tratados con maloclusión de Clase II y Clase III de origen latinoamericano agrupados por estadío de maduración de vértebras cervicales, en comparación con un grupo control sin tratamiento, normodivergentes y con maloclusión de Clase I.
El estudio se realizó sobre una muestra de seiscientos diez cefalogramas laterales de cabeza pretratamiento (250 hombres, 360 mujeres) que fueron agrupados de acuerdo a su relación esquelética sagital (Clase I, II ó III), estadío de maduración vértebras cervicales (pre pico puberal P1 = CS1 y CS2, pico puberal P2 = CS3 y CS4, y post pico puberal P3 = CS5 y CS6) y sexo. Se midió en cada cefalograma el triángulo cefalométrico de McNamara Co-A, Co-Gn y ENA-Me. Se realizaron las pruebas de ANOVA y post-hoc Tukey HSD para determinar las diferencias entre grupos.Los resultados en hombres muestran que los mayores aumentos maxilares y mandibulares se produjeron durante la etapa P3 (CS5 para CS6), mientras que en las mujeres se produjeron en la etapa P2 (CS3 para CS4). Las distancias Co-A y Co-Gn muestran diferencias significativas entre las diferentes clases ($p<0,05$). La longitud maxilar de los sujetos de Clase II y la longitud mandibular de los sujetos de Clase III estuvieron aumentadas al inicio del período evaluado (P1). Se identificó una tendencia al empeoramiento de las maloclusiones de Clase II y III durante el período evaluado.
Finalmente los cambios en los valores del triángulo cefalométrico de McNamara fueron marcadamente diferentes en las tres clases de maloclusión en sujetos normodivergentes. En estos sujetos latinoamericanos el estirón puberal se produjo en momentos diferentes con respecto a la raza caucásica y las normas asiáticas.

Palabras clave: *Cefalometría; maloclusión; clase II de Angle; maloclusión; clase III de Angle.*

INTRODUCTION

The variation in the amount and direction of craniofacial growth influences the development of the individual's facial form [1-3]. Facial skeletal pattern plays a key role in the prognosis of orthodontic treatment [4]. Therefore, orthodontists should take these factors into account during diagnosis and treatment planning.

The McNamara cephalometric triangle[5] is part of a proposed cephalometric analysis that evaluates three fundamental craniofacial measurements: lower anterior face height, midfacial length, and mandibular length. These measurements allow the clinician to contrast the size of the jaws and know if they are adequate, increased or decreased.

A few cross-sectional studies [6-9] and only a handful of longitudinal studies [10-12] have compared these craniofacial dimensions in subjects with Class I, II and III malocclusion. Some of these studies [10-13] did consider the skeletal maturation as determined by CVM,[14] but none of them specifically considered how the skeletal pattern influences these dimensions grouped by stage of cervical vertebrae maturation. Additionally, some studies were limited in terms of sample size, absence of control of skeletal maturation, and appropriate selection criteria.

For cases of Class III Caucasian malocclusion, craniofacial growth trends towards an accentuated Class III profile with increased vertical facial dimension [13]. In contrast, craniofacial growth in Caucasian subjects with untreated Class II malocclusion is essentially similar to that of untreated subjects with normal occlusion at all developmental intervals, with the exception of significantly smaller increments in mandibular length at the pubertal growth spurt (interval CS3-CS4).[11,12] It is considered that Class II dentoskeletal disharmonies do not tend to self-correct through future facial growth. Actually a worsening of the sagittal and vertical deficiency in the total mandibular length and mandibular ramus height has been identified [12].

Almost all of the published articles [6,8-14] that have evaluated the sagittal and vertical growth of the mandible and maxilla have been conducted in Caucasian groups of European and North American ancestry, and Asian populations [7,15-17], but studies on Latin American samples are scarce.[18] With the growing proportion of the US population with Latin American ancestry, the importance of a better understanding of any potential differences in their craniofacial growth should not be minimized.

Longitudinal studies are ideal for evaluating craniofacial growth; however the availability of large samples and the ethical consideration for annual or semi-annual radiographic records currently makes them more difficult to execute. The use of longitudinal samples taken more than 50 years ago has also been questioned because of the so-called secular trends [9,13]. The next best alternative is to use large cross-sectional studies with subjects matched by skeletal maturation, sex, sagittal facial pattern, facial divergence and malocclusion complexity degree.

Considering the above information, the purpose of this study was to compare the values for the McNamara cephalometric triangle grouped by stage of cervical vertebrae maturation in untreated normodivergent Class II and Class III malocclusion subjects of Latin American origin as compared to an untreated Class I malocclusion control group.

MATERIALS AND METHODS

The study protocol was approved by the ethics committee of the School of Dentistry, Universidad Científica del Sur - UCSUR, Lima, Perú. The sample included 610 pretreatment lateral cephalograms (250 male, 360 female) from a diagnostic imaging center, grouped according to their skeletal relationship (Class I, II or III) [19], stage of cervical vertebrae maturation[14] (P1= CS1 and CS2, P2= CS3 and CS4, P3= CS5 and CS6) and sex (male and female).

A minimum sample size was calculated considering a clinically relevant mean difference of 3mm in mandibular length (Co-Gn) at CG stage between Class I and III malocclusion groups, with a standard deviation of 3.8mm (obtained from a pilot study). With a one-sided significance level of 0.05 and a power of 80%, a minimum of 20 patients per skeletal group, cervical vertebrae maturation stage and sex was required.

All the subjects considered in the sample were Peruvian. Their Latin American origin was determined by their last names (Hispanic American), community sociodemographic characteristics corresponding to indigenous populations, living in Lima, Perú, Spanish speaking, coppery skin color, and black hair.

All the cephalograms were taken at maximum intercuspation. Subjects with previous or current orthodontic treatment at the time of image acquisition and/or with congenitally missing or extracted teeth were not considered. Imaging was performed with digital cephalometric panoramic equipment (ProMax®, Planmeca, Finland). Device settings were set at 16mA, 72 kV and 9.9 seconds.

Group classification

Skeletal pattern

The study sample consisted of three groups categorized according to their sagittal skeletal facial growth pattern. Only normodivergent patterns were considered. (Tables 1 and 2)

- The Class I control group included subjects with ANB 2° ± 2°, bilateral Class I molar relations (Angle Class I malocclusion), Frankfort to mandibular plane angle within 25°± 3°, overjet between 2 and 3mm, overbite between 1 and 4mm and with complete permanent dentition (including third molars).
- The Class II group included subjects with ANB>5°, bilateral Class II molar relations of at least one half cusp (Angle Class II-1 malocclusion), overjet greater than 5mm, Frankfort to mandibular plane angle within 25°± 3°, overbite between 1 and 4mm and with complete permanent dentition (including third molars).
- The Class III group included subjects with ANB<0°, bilateral Class III molar relations of at least one half cusp (Angle Class III malocclusion), overjet lower than -1mm, Frankfort to mandibular plane angle within 25°± 3°, overbite between 1 and 4 mm and with complete permanent dentition (including third molars).

Stage of cervical vertebrae maturation

The sample consisted of three groups categorized according to CVM method.[18] In this study the sample was grouped according to three stages.

- The pre-pubertal growth spurt group (P1) included subjects in the interval CS1 and CS2.
- The pubertal growth spurt group (P2) included subjects in the interval CS3 and CS4.
- The post-pubertal growth spurt group (P3) included subjects in the interval CS5 and CS6.

Table 1: Characteristics of the sample by growth pattern and cervical vertebrae stage.

Group		n	Mean	S.D.	Min	Max
Class I (control group)						
P1	ANB	45	3.45	1.03	1.41	4.50
	F-GoMe	45	26.14	2.73	23.00	29.00
P2	ANB	88	2.71	1.09	0.10	4.60
	F-GoMe	88	26.51	2.74	22.94	29.05
P3	ANB	85	3.12	0.80	1.30	4.11
	F-GoMe	85	26.04	2.34	23.07	29.15
Skeletal Class II group						
P1	ANB	40	6.17	1.05	5.30	9.40
	F-GoMe	40	27.03	3.58	22.78	29.12
P2	ANB	90	6.15	1.02	5.20	10.00
	F-GoMe	90	27.21	3.92	22.15	29.19
P3	ANB	115	6.47	1.19	5.10	9.32
	F-GoMe	115	27.36	4.73	22.34	29.72
Skeletal Class III group						
P1	ANB	41	-1.66	0.82	-3.70	-0.80
	F-GoMe	41	25.96	2.55	22.79	29.44
P2	ANB	49	-0.85	0.54	-2.80	-0.10
	F-GoMe	49	26.20	2.56	22.42	29.00
P3	ANB	57	-1.15	0.59	-4.10	-0.22
	F-GoMe	57	26.75	2.61	23.13	29.28

P1 = Pre-Pubertal Growth, P2 = Pubertal Peak Growth, P3 = Post-Pubertal Growth
Comparisons of the ANB and F-GoMe angles between skeletal patterns at each cervical vertebrae stage
ANB angle: P1, P2, P3 (I,II,III = p<0.001)* (p<0.001, I and II)+ (p<0.001, I and III)+ (p<0.001, II and III)+
F-GoMe: P1 (I,II,III p= 0.453)*, P2 (I,II,III p=0.321)*, P3 (I,II,III p= 0.310)*
Comparisons of the ANB and F-GoMe angles between cervical vertebrae stages by skeletal pattern
ANB angle: Class I (P1, P2, P3 p= 0.334)*, Class II (P1, P2, P3 p= 0.269)*, Class III (P1, P2, P3 p= 0.660)*
F-GoMe: Class I (P1, P2, P3 p= 0.742)*, Class II (P1, P2, P3 p= 0.917)*, Class III (P1, P2, P3 p= 0.852)*
(*) ANOVA test; (+) Tukey test

Sex

The sample was divided according to sex into males and females.

Cephalometric analysis (all in mm)

A McNamara cephalometric triangle analysis was performed including measurements of Co-A, Co-Gn and ENA-Me on each tracing. Cephalometric measurements were performed digitally by two calibrated researchers with the MicroDicom viewer 0.8.1 software (Simeon Antonov Stoykov), without magnification, at a scale of 1:1.

Statistical analysis

All statistical analyses were performed using SPSS ver.20 for Windows (IBM SPSS, Chicago, Illinois, USA). The normality and homogeneity of variance assumptions were satisfied as determined by Shapiro Wilk tests. One-way analysis of variance (ANOVA) test and Tukey HSD post-hoc test were performed to determine whether there were differences between the different groupings (according to their skeletal relationship, cervical vertebrae maturation stage, and sex) with regard to midfacial length, mandibular length and lower anterior face height. Statistical significance was set at $p<0.05$ for all tests.

Table 2: Distribution of the sample by skeletal pattern and sex.

Group	Sex		Total
	Female	Male	
Class I (control group)			
P1	20	25	45
P2	53	35	88
P3	53	32	85
Skeletal Class II group			
P1	20	20	40
P2	54	36	90
P3	78	37	115
Skeletal Class III group			
P1	21	20	41
P2	24	25	49
P3	37	20	57
Total	360	250	610

P1 = Pre-Pubertal Growth, P2 = Pubertal Peak Growth, P3 = Post-Pubertal Growth

RESULTS

Reliability

The intra-examiner and inter-examiner reliability was assessed with the Intraclass Correlation Coefficient (ICC), which was greater than 0.90 for all the cephalometric measurements. The Dahlberg method error was less than 1 mm for linear measurements and 1° for angular measurements. All the lateral cephalograms were traced twice by both researchers with a two-month interval between tracings.

The concordance in the stages of cervical vertebrae was evaluated with Kappa statistic. The intra-examiner and inter-examiner concordance values were all greater than 0.80.

Cephalometric measurements (Table 3)

The cephalometric measurements (lower anterior face height, midfacial length, and mandibular length) showed differences between the male and female subjects in both timing and dimensions during the pubertal growth spurt ($p<0.001$).

Midfacial length (Co-A)

In males, the measurements differed significantly for the Class II group, where an increase of approximately 4mm in comparison with the control group for the phases P1 and P2 ($p<0.001$) was identified. For the final stage of growth, the differences decreased to 1mm. Among Class I and Class III groups, the Co-A distance was very similar in all the tested stages ($p>0.05$) (Tables 3-5).

In females, the distance Co-A showed significant differences among the three classes in all cervical vertebrae maturation stages ($p<0.05$). Class II was larger than the control group by approximately 4mm in stage P1, 2mm in stage P2 and 1.5mm in stage P3 ($p<0.001$). The Co-A distance did not differ significantly between Class I and Class III (Tables 4-6).

Mandibular length (Co-Gn)

In males, the rate of growth differed significantly among the three classes throughout craniofacial growth ($p<0.001$). In Class III it was greater by approximately 5 mm at stage P1, 7mm at stage P2 and 3mm at stage P3 in comparison to the control group. Class II had shorter values for distance Co-Gn than Class III ($p< 0.05$), but with respect to Class I they were shorter only in the final stage (P3) ($p=0.013$) (Tables 3-5).

Table 3: McNamara triangle assessment by stage of cervical vertebrae and skeletal pattern in males.

McNamara Measures	Skeletal pattern	P1				P2				P3			
		n	Mean	SD	p	n	Mean	SD	p	n	Mean	SD	p
Co-A	Class I	25	79.32	3.97		35	81.26	4.22		32	84.42	4.64	
	Class II	20	84.34	3.33	<0.001	36	84.94	4.13	<0.001	37	85.08	4.19	0.381
	Class III	20	78.44	4.56		25	81.38	3.20		20	83.32	4.87	
Co-Gn	Class I	25	99.37	3.78		35	101.75	5.77		32	112.54	8.55	
	Class II	20	100.32	4.15	0.003	36	102.43	5.28	<0.001	37	107.33	6.76	<0.001
	Class III	20	104.13	4.80		25	108.69	7.18		20	115.34	6.48	
ENA-Me	Class I	25	58.81	3.30		35	60.70	4.58		32	66.36	6.83	
	Class II	20	60.56	4.17	0.260	36	63.21	5.34	0.141	37	67.00	6.93	0.877
	Class III	20	58.66	3.64		25	62.56	5.64		20	66.06	7.74	

P1 = Pre-Pubertal Growth, P2 = Pubertal Peak Growth, P3 = Post-Pubertal Growth
ANOVA Test
Tukey multiple comparisons

Co-A P1 = (I,II p=0.002) (I,III p=0.782) (II,III p<0.001)
P2 = (I,II p=0.001) (I,III p=0.994) (II,III p=0.009)
P3 = (I,II p=0.819) (I,III p=0.673) (II,III p=0.347)

Co-Gn P1 = (I,II p=0.797) (I,III p=0.004) (II,III p=0.029)
P2 = (I,II p=0.885) (I,III p=0.001) (II,III p=0.002)
P3 = (I,II p=0.013) (I,III p=0.384) (II,III p=0.001)

ENA-Me P1 = (I,II p=0.360) (I,III p=0.992) (II,III p=0.286)
P2 = (I,II p=0.819) (I,III p=0.673) (II,III p=0.347)
P3 = (I,II p=0.928) (I,III p=0.988) (II,III p=0.883)

Table 4: McNamara triangle assessment by stage of cervical vertebrae and skeletal pattern in females.

McNamara Measures	Skeletal pattern	P1				P2				P3			
		n	Mean	SD	p	n	Mean	SD	p	n	Mean	SD	p
Co-A	Class I	20	76.19	3.84		53	79.69	3.22		53	80.06	3.51	
	Class II	20	80.13	3.56	0.026	54	81.50	4.22	0.002	78	81.49	3.67	<0.001
	Class III	21	77.12	4.50		24	77.77	3.87		37	78.39	3.48	
Co-Gn	Class I	20	95.07	5.28		53	101.50	4.51		53	103.72	5.82	
	Class II	20	97.41	4.44	0.024	54	99.85	6.12	0.196	78	101.22	5.24	<0.001
	Class III	21	101.19	5.47		24	102.03	5.37		37	105.74	4.53	
ENA-Me	Class I	20	55.06	3.65		53	58.99	4.31		53	61.25	4.78	
	Class II	20	60.05	4.30	0.009	54	60.76	4.60	0.328	78	61.99	4.83	0.075
	Class III	21	57.35	4.06		24	59.65	13.42		37	59.81	4.46	

P1 = Pre-Pubertal Growth, P2 = Pubertal Peak Growth, P3 = Post-Pubertal Growth
ANOVA Test
Tukey multiple comparisons

Co-A P1 = (I,II p=0.029) (I,III p=0.843) (II,III p=0.143)
P2 = (I,II p=0.039) (I,III p=0.213) (II,III p=0.004)
P3 = (I,II p=0.068) (I,III p=0.077) (II,III p<0.001)

Co-Gn P1 = (I,II p=0.435) (I,III p<0.018) (II,III p=0.152)
P2 = (I,II p=0.257) (I,III p=0.942) (II,III p=0.370)
P3 = (I,II p=0.024) (I,III p=0.177) (II,III p<0.001)

ENA-Me P1 = (I,II p=0.007) (I,III p=0.393) (II,III p=0.230)
P2 = (I,II p=0.297) (I,III p=0.931) (II,III p=0.819)
P3 = (I,II p=0.659) (I,III p=0.335) (II,III p=0.059)

Table 5: Growth assessment McNamara triangle by stage of cervical vertebrae and skeletal pattern in males.

Skeletal pattern	McNamara Measures	P1			P2			P3			p
		n	Mean	SD	n	Mean	SD	n	Mean	SD	
Class I	Co-A	25	79.32	3.97	35	81.26	4.22	32	84.42	4.64	<0.001
	Co-Gn	25	99.37	3.78	35	101.75	5.77	32	112.54	8.55	<0.001
	ENA-Me	25	58.81	3.30	35	60.70	4.58	32	66.36	6.83	<0.001
Class II	Co-A	20	84.34	3.33	36	84.94	4.13	37	85.08	4.19	0.825
	Co-Gn	20	100.32	4.15	36	102.43	5.28	37	107.33	6.76	<0.001
	ENA-Me	20	60.56	4.17	36	63.21	5.34	37	67.00	6.93	0.001
Class III	Co-A	20	78.44	4.56	25	81.38	3.20	20	83.32	4.87	0.003
	Co-Gn	20	104.13	4.80	25	108.69	7.18	20	115.34	6.48	<0.001
	ENA-Me	20	58.66	3.64	25	62.56	5.64	20	66.06	7.74	0.001

P1 = Pre-Pubertal Growth, P2 = Pubertal Peak Growth, P3 = Post-Pubertal Growth
ANOVA Test
Tukey multiple comparisons

Class I
Co-A = (P1, P2 p=0.300) (P1, P3 p<0.001) (P2, P3 p=0.015)
Co-Gn = (P1, P2 p=0.466) (P1, P3 p<0.001) (P2, P3 p<0.001)
ENA-Me = (P1, P2 p=0.471) (P1, P3 p<0.001) (P2, P3 p<0.001)

Class II
Co-A = (P1, P2 p=0.872) (P1, P3 p=0.814) (P2, P3 p=0.989)
Co-Gn = (P1, P2 p=0.449) (P1, P3 p<0.001) (P2, P3 p=0.001)
ENA-Me = (P1, P2 p=0.299) (P1, P3 p=0.001) (P2, P3 p=0.020)

Class III
Co-A = (P1, P2 p=0.108) (P1, P3 p=0.002) (P2, P3 p=0.369)
Co-Gn = (P1, P2 p=0.074) (P1, P3 p<0.001) (P2, P3 p=0.005)
ENA-Me = (P1, P2 p=0.124) (P1, P3 p=0.001) (P2, P3 p=0.183)

Table 6: Growth assessment McNamara triangle by stage of cervical vertebrae and skeletal pattern in females.

Skeletal pattern	McNamara Measures	P1			P2			P3			p
		n	Mean	SD	n	Mean	SD	n	Mean	SD	
Class I	Co-A	20	76.19	3.84	53	79.69	3.22	53	80.06	3.51	0.002
	Co-Gn	20	95.07	5.28	53	101.50	4.51	53	103.72	5.82	<0.001
	ENA-Me	20	55.06	3.65	53	58.99	4.31	53	61.25	4.78	<0.001
Class II	Co-A	20	80.13	3.56	54	81.50	4.22	78	81.49	3.67	0.398
	Co-Gn	20	97.41	4.44	54	99.85	6.12	78	101.22	5.24	0.030
	ENA-Me	20	60.05	4.30	54	60.76	4.60	78	61.99	4.83	0.170
Class III	Co-A	21	77.12	4.50	24	77.77	3.87	37	78.39	3.48	0.612
	Co-Gn	21	101.19	5.47	24	102.03	5.37	37	105.74	4.53	0.009
	ENA-Me	21	57.35	4.06	24	59.65	13.42	37	59.81	4.46	0.643

P1 = Pre-Pubertal Growth, P2 = Pubertal Peak Growth, P3 = Post-Pubertal Growth
ANOVA Test
Tukey multiple comparisons

Class I
Co-A = (P1, P2 p=0.005) (P1, P3 p=0.002) (P2, P3 p=0.840)
Co-Gn = (P1, P2 p=0.001) (P1, P3 p<0.001) (P2, P3 p=0.077)
ENA-Me = (P1, P2 p=0.019) (P1, P3 p<0.001) (P2, P3 p=0.028)

Class II
Co-A = (P1, P2 p=0.417) (P1, P3 p=0.392) (P2, P3 p=1.000)
Co-Gn = (P1, P2 p=0.252) (P1, P3 p=0.029) (P2, P3 p=0.341)
ENA-Me = (P1, P2 p=0.850) (P1, P3 p=0.275) (P2, P3 p=0.305)

Class III
Co-A = (P1, P2 p=0.908) (P1, P3 p=0.612) (P2, P3 p=0.860)
Co-Gn = (P1, P2 p=0.909) (P1, P3 p=0.030) (P2, P3 p=0.048)
ENA-Me = (P1, P2 p=0.737) (P1, P3 p=0.624) (P2, P3 p=0.997)

In females, the distance Co-Gn also showed significant differences between Classes I, II and III at stages P1 and P3 ($p<0.05$). The Class III dimension was larger than the control group by approximately 6mm at stage P1 and 2mm at stage P3 ($p < 0.05$). There was no significant difference between Class 1 and II at stages P1 and P2 ($p>0.05$), but there was a difference in the final stage (P3) of approximately 2.5mm ($p=0.024$) (Tables 4-6).

Lower anterior face height (ENA-Me)

In males, the anterior facial height did not differ significantly between the grouped classes at any of the growth stages ($p>0.05$) (Tables 3-5).

In females, anterior facial height differed significantly between Class I and II only at stage P1 ($p=0.007$), where it was larger than the control group by about 5mm. Numerically, the anterior facial height during all growth stages was larger in Class II than in Classes I and III (Tables 4-6).

DISCUSSION

Craniofacial growth is influenced by genetic and environmental factors. Environmental factors vary over time because of constant changes in nutrition and lifestyle [1-4,20]. For this reason, updated craniofacial growth studies maybe necessary from time to time. In addition, the proportion of the population with different racial backgrounds keeps changing due to the increased mobility of human populations. The USA does not differ in this regard. The proportion of the US population of Latin American origin keeps increasing; therefore, it is important to have thorough understanding of the craniofacial growth pattern in the Latin American population. The amount of remaining mandibular and maxillary growth is also an important variable for determining the craniofacial growth potential that any given orthodontic patient may have.

Ideally, the amount of growth remaining should be determined over time in the same subject using longitudinal studies [12,13] but due to ethical concerns, longitudinal studies are not a viable option nowadays and cross-sectional studies with adequately matched samples gain relevance. In our study, the groups were matched according to sagittal and vertical skeletal facial pattern, malocclusion, skeletal maturation and sex. This was done with the aim of reducing the possibility of selection bias. Moreover, we compared only the growth of normodivergent subjects in order to avoid the influence of the vertical pattern. This could be taken into account in future studies. Furthermore, it is known that skeletal growth curves differ slightly between longitudinal and cross-sectional studies. Cross-sectional data slightly overestimates midfacial growth, but closely agrees with mandibular length measurements [9,10,13].

In this study, the values for the McNamara cephalometric triangle[8] changed between sagittal skeletal patterns in the different craniofacial development stages. The current results indicate sexual dimorphism in the three measurements. This has been reported previously [9,13]. Our study is also consistent with the suggestion [9,10] that in Class II and III malocclusion with a normodivergent facial growth pattern, the remaining craniofacial growth tends to worsen the facial profile.

Previously published results [9,13] report differential growth in untreated subjects with Class III malocclusion. For Caucasian females with Class III malocclusion, the annualized growth was about 1mm for midfacial length, 3mm for mandibular length and 1mm for lower anterior face height. For males, the differences during the same time interval were 0.5mm greater than for the females. These values were 0.5 to 1mm lower in subjects with Class I malocclusion [20]. In subjects with Class II malocclusion, the craniofacial growth was very similar to that of untreated subjects with normal occlusion with the exception of significantly smaller increases in the mandibular length [10-12]. Our results differ from these reported studies [6,,7, 9, 11, 13, 14] in two situations.

The first difference is related to the time of appearance of peak pubertal growth as related to McNamara measurements. In males, the greatest lengths in maxillary growth occurred mainly during stage P3, while in females, greatest growth occurred at stage P2. This may indicate that craniofacial growth rate in the evaluated Latin American group differs significantly in some aspects from that reported in Asian and Caucasian groups, probably because of the influence of racial, environmental and nutritional factors.

The second difference in our study is related to the amount of maxillary and mandibular growth during the growth stages evaluated. The Class I group had a total increase in the maxilla of 5mm and 4mm in males and females respectively, these differences being lower than those reported by other

authors [9,11,13,14]. We can only assume that this may be due to racial factors. In the Class III group the values were 1mm and 2mm smaller at each stage of growth in males and females respectively compared to the Class I group. In the Class II group the increase during the evaluated growth period was also small, but it should be noted that the initial dimensions were larger than for Class I and III malocclusion groups. Our Class II sample had what most would consider a maxillary protrusion at stage P1, which indicates that the differential maxillary growth may occur in earlier skeletal maturation stages in this sample (before stage P1) in Classes II and III. These results appear to be unique based on previous literature [6, 9, 11, 14].

Previous reports on samples [9,10,13] of Caucasian subjects with Class III found the largest average annual increments or the mandibular length during the adolescent growth spurt were 3.0 and 3.7mm for females and males respectively, with total growth of approximately 18 to 20mm between 8 and 17 years of age [9,13]. In our study, total increase for the Class I group was approximately 12mm. For the Class II group the total increase was 7mm while for the Class III group it was 11mm. It should be noted that this distance was greater in Class III at baseline by about 4mm compared to Class I or II. This also seems to indicate that in this sample the mandibular growth difference largely starts before stage P1 [14].

The growth of the lower anterior face height was similar in all three skeletal classes, showing sexual dimorphism and was slightly higher in the Class II group at the beginning and end of craniofacial growth. Comparing only the length of the lower anterior height with other studies,[9,13] our reported values are lower. It should be kept in mind that only normodivergent cases were considered in this study.

CONCLUSIONS

Changes in the McNamara cephalometric triangle differed markedly among the three distinct skeletal normodivergent classes grouped by cervical vertebrae maturation stage. In these Latin American subjects, the pubertal growth spurt occurred at different times with respect to the Caucasian and Asian norms.

In males, the pubertal growth spurt in the maxilla and mandible dimensions occurred during stage P3, while in females they occurred in stage P2.

The maxillary and mandibular lengths were already increased in Class II and Class III subjects, respectively, at the beginning of the evaluated growth periods (before stage P1) compared to Class I subjects.

CORRESPONDENCE
Dr. Luis Ernesto Arriola-Guillén
Calle Los Girasoles # 194, Dpto. # 302, Urb.
Residencial Los Ingenieros de Valle Hermoso,
Santiago de Surco, Lima, Perú.
e-mail: luchoarriola@gmail.com

REFERENCES

1. Enlow DH. A morphogenetic analysis of facial growth. Am J Orthod. 1966; 52:283-299.
2. Enlow DH, Kuroda T, Lewis AB. The morphological and morphogenetic basis for craniofacial form and pattern. Angle Orthod. 1971; 41:161-188.
3. Björk A. Variations in the growth pattern of the human mandible: longitudinal radiographic study by the implant method. J Dent Res. 1963; 42:400-411.
4. Merrifield LL. Differential diagnosis. Semin Orthod. 1996; 2:241-253.
5. McNamara JA Jr. A method of cephalometric evaluation. Am J Orthod. 1984; 86:449-469.
6. Baccetti T, Reyes BC, McNamara JA Jr. Craniofacial changes in Class III malocclusion as related to skeletal and dental maturation. Am J Orthod Dentofacial Orthop. 2007; 132: 171.e1-171.e12.
7. Miyajima K, McNamara JA Jr, Kimura T, Murata S, Iizuka T. An estimation of craniofacial growth in the untreated Class III female with anterior crossbite. Am J Orthod Dentofacial Orthop. 1997;112:425-434.
8. Battagel JM. The etiological factors in Class III malocclusion. Eur J Orthod. 1993; 15:347-370.
9. Baccetti T, Reyes BC, McNamara JA Jr. Gender differences in Class III malocclusion. Angle Orthod. 2005; 75:510-520.
10. Baccetti T, Franchi L, McNamara JA Jr. Growth in the untreated Class III subject. Semin Orthod. 2007; 13: 130-142.
11. Baccetti T, Stahl F, McNamara JA Jr. Dentofacial growth changes in subjects with untreated Class II malocclusion

from late puberty through young adulthood. Am J Orthod Dentofacial Orthop. 2009 ;135:148-154.
12. Stahl F, Baccetti T, Franchi L, McNamara JA Jr. Longitudinal growth changes in untreated subjects with Class II Division 1 malocclusion. Am J Orthod Dentofacial Orthop. 2008; 134:125-137.
13. Alexander AE, McNamara JA Jr, Franchi L, Baccetti T. Semilongitudinal cephalometric study of craniofacial growth in untreated Class III malocclusion. Am J Orthod Dentofacial Orthop. 2009; 135:700.e1-14;
14. Baccetti T, Franchi L, McNamara J. The Cervical Vertebral Maturation (CVM) Method for the assessment of optimal treatment timing in dentofacial orthopedics. Semin Orthod. 2005; 11:119-129.
15. Sugawara J, Tsuchikawa T, Soya T, Mitani H. Late adolescent growth of skeletal Class III craniofaces in Japanese girls: average growth from 14-17 years of age based on longitudinal data. Jpn J Orthod Soc. 1983;42:399-408.
16. Mitani H, Sato K, Sugawara J. Growth of mandibular prognathism after pubertal growth peak. Am J Orthod Dentofacial Orthop. 1993; 104:330-336.
17. Sakamoto E, Sugawara J, Umemori M, Mitani H. Craniofacial growth of mandibular prognathism during pubertal growth period in Japanese boys: longitudinal study from 10-15 years of age. Jpn J Orthod Soc. 1996; 55:372-86.
18. Generoso R, Sadoco EC, Armond MC, Gameiro GH. Evaluation of mandibular length in subjects with Class I and Class II skeletal patterns using the cervical vertebrae maturation. Braz Oral Res. 2010 ; 24:46-51.
19. Steiner C. Cephalometric for you and me. Am J Orthod Dentofacial Orthop. 1953; 39: 729-755.
20. Ricketts RM, Bench RW, Gugino CF, Hilgers JJ, Schulhof RJ. Bioprogressive therapy. Denver, Colorado: Rocky Mountain® Orthodontics; 1979. Visual treatment objective or V.T.O; p. 35-54.

USE OF HUMAN FASCIA LATA IN RAT CALVARIAL BONE DEFECTS

Mariano A.R. Amer[1], Pablo A. Rodríguez[2], Sandra J. Renou[3], María B. Guglielmotti[3,4]

[1] Department of Anatomy, School of Dentistry, University of Buenos Aires, Argentina.
[2] Department of Endodontics, School of Dentistry, University of Buenos Aires, Argentina.
[3] Department of Oral Pathology, School of Dentistry, University of Buenos Aires, Argentina.
[4] National Research Council (CONICET), Argentina.

ABSTRACT

Tooth loss leads to a decrease in alveolar bone volume, and consequently to the need for guided bone regeneration (GBR) techniques to restore bone anatomy, and the adequate choice of therapy. Fascia lata membrane (FLM) has been used in surgical procedures in neurology, orthopedics, otorhinolaryngology, cardiology, vascular surgery, gynecology, and dentistry for guided tissue regeneration.

The aim of the present preliminary study was to evaluate bone tissue response in rat calvarial bone defects covered with human fascia lata membrane (FLM).

Eight Wistar rats, 230g body weight, were subjected to bone surgery to create a 5x5mm long/ 1mm deep calvarial bone defect on either side of the median suture, using a piezoelectric scalpel and irrigation. The animals were treated according to the following protocol: Group I (GI): placement of a single layer of FLM (Biotar, Rosario, Prov. de Santa Fe, Argentina) to cover the defects; Group II (GII): double layer of FLM to cover the defects; Group III: no membrane; Group IV: control. All the animals were euthanized 60 days post-surgery; the heads were resected, radiographed, decalcified, and processed for embedding in paraffin and Hematoxylin-Eosin and Masson's trichrome staining.

All bone defects covered with a single or double layer of FLM showed adequate osteogenesis, and none exhibited an inflammatory response. Groups III and IV Control showed scant osteogenesis and no alterations in soft tissues.

The results obtained with this experimental model show biocompatibility of FML with the surrounding tissues at the studied time points. No alterations were observed in osteocytic lacunae or osteocytes in the bone after osteotomy using a piezoelectric scalpel.

Further studies need to be conducted to assess bone tissue response to FLM in combination with bone substitutes.

Key words: *fascia lata; skull; bone regeneration; piezosurgery; rats.*

FASCIA LATA HUMANA EN DEFECTOS ÓSEOS DE CALOTA DE RATA

RESUMEN

La pérdida de piezas dentarias conlleva la disminución volumétrica del hueso alveolar y la necesidad de recurrir a técnicas de regeneración ósea guiada (ROG) para restablecer las condiciones anatómicas y aplicar las terapéuticas adecuadas.

La membrana de fascia lata (MFL) ha sido utilizada en intervenciones quirúrgicas del área neurológica, ortopédica, otorrinolaringológica, cardiológica, vascular, ginecológica y odontológica para regeneración tisular guiada.

El objetivo de este trabajo preliminar fue evaluar la respuesta tisular de defectos óseos en calota de rata recubiertos con MFL.

Se utilizaron 8 ratas Wistar de 230 gr de peso, a las que se les realizó en la calota 2 defectos óseos de 5 x 5 mm de lado por 1 mm de profundidad, con bisturí piezoeléctrico e irrigación, a ambos lados de la línea media, según técnica estandarizada.

Se realizó el siguiente protocolo: Grupo I (G I): colocación de una sola capa de MFL (Biotar, Rosario, Prov. de Santa Fe, Argentina) para cubrir el defecto; Grupo II (G II): colocación de MFL en doble capa para cubrir el defecto; Grupo III (G III): sin membrana; Grupo IV (G IV): control. Se les provocó la eutanasia a los 60 días. Las calotas fueron resecadas, radiografiadas y procesadas, previa descalcificación, para su inclusión en parafina y coloración con Hematoxilina-Eosina y Tricrómico de Masson.

En todos los defectos óseos recubiertos con MFL simple o doble se evidenció una adecuada osteogénesis y ausencia de respuesta inflamatoria y macrófagos. El G III y el G Sham evidenciaron escasa osteogénesis y no mostraron alteraciones en el tejido blando. La MFL en el modelo experimental utilizado evidenció una respuesta compatible con el tejido circundante en los períodos estudiados. El tejido óseo remanente a la osteotomía con bisturí piezoeléctrico presentó las lagunas osteocíticas ocupadas con osteocitos y sin alteraciones.

En estudios futuros se evaluará la respuesta tisular con MFL y utilizando un sustituto óseo.

Palabras clave: *fascia lata; calota; regeneración ósea; piezocirugía; ratas.*

INTRODUCTION

The use of autologous bone, and heterologous and alloplastic bone substitutes is widespread in surgery rooms and dental offices at present. According to estimates, 2.2 million bone grafts were placed in 2013, including procedures to repair defects in maxillofacial surgery, neurosurgery, orthopedics, and dentistry[1]. Nevertheless, autogenous bone harvesting

is associated with complications such as hematoma and infection, a second surgical site where the graft is harvested, and potential damage to neighboring anatomic structures[3]. Interestingly, the use of a heterologous or alloplastic graft avoids the consequences associated with autologous bone harvesting, as regards morbidity of the donor site and the limited volume of bone available for grafting.

Barrier membranes used in guided tissue regeneration procedures can be either resorbable or non-resorbable. The membrane forms a barrier that prevents migration of soft tissue into the graft site, and thus enhances bone healing[3]. Fascia lata membranes (FLM) have been used as a resorbable biomaterial[4] in a number of surgical specialties, including dentistry. The structure of the membrane fibers, its ease of harvesting, and clinical manageability have made FLM a predictable biomaterial, which has been successfully used in numerous surgical fields.

Traditionally, experimental models of bone defects involved the use of conventional rotating burs to create the defect. However, these traditional methods pose disadvantages, such as overheating and damage to bone tissue. Piezosurgery avoids the complications associated with conventional rotary drilling methods, and has become a method of choice for cutting bone in a number of procedures, including maxillary sinus floor augmentation, crestal bone splitting, autogenous bone harvesting, and orthognathic procedures [5].

Clinical and preclinical studies combined with *in vitro* studies have shown that piezosurgery produces clean and precise osteotomies, and decreases bleeding[6]. Studies evaluating micromorphological differences after using a rotary tool, an oscillating saw, and piezoelectric osteotomy, showed that piezosurgery preserved the original structure of the bone[7], resulting in a lower number of inflammatory cells and lower expression of pro-inflammatory cytokines, 7 and 14 days after surgery[8].

The aim of the present study was to perform a qualitative evaluation of bone tissue response in rat calvarial bone defects made using a piezoelectric device and treated with guided bone regeneration with and without placement of FLM, using histological studies.

MATERIALS AND METHODS

Eight adult male Wistar rats, 230 g body weight, were randomly divided into four groups. The animals were housed in galvanized wire cages, four animals per cage, at 22°C to 24°C temperature and 52% to 56% humidity, under 12 hour-light/dark cycles.

All experiments were performed following the Guide for the Care and Use of Laboratory Animals of the National Research Council (US)[9], and the protocols were approved by the Ethics Committee of the School of Dentistry of the University of Buenos Aires.

On day 0, all the animals were anesthetized by intraperitoneal injection of 2% xylazine hydrochloride (5mg/kg body weight) and 5% ketamine hydrochloride (50 mg/kg body weight). The surgical area was infiltrated with 0.2 ml lidocaine (1:200.000). Using a scalpel (Bard-Parker scalpel, 15C blade, Swam Norton, England), a 15 mm sagittal incision was made in the parietal region, from the frontal bone to the occipital protuberance. The calvarium was exposed by blunt dissection with a Molt curette with atraumatic non-sharp edges (Hu-Friedy, USA). Bone defects measuring 5x5mm and 1mm deep were performed on both sides of the median suture, avoiding perforation of the inner bone plate. Bone surgery was performed using a piezosurgery device (Variosurg, NSK, NAKANISHI INC., Japan) and a SG3 scaler (NSK, NAKANISHI INC., Japan). Animals in Group I (GI) underwent surgery and placement of a single layer of human fascia lata membrane (FLM) (Biotar, Rosario, Prov. de Santa Fe, Argentina) on both defects (Fig. 1). Those in Group II (GII) were subjected to surgery and placement of a double

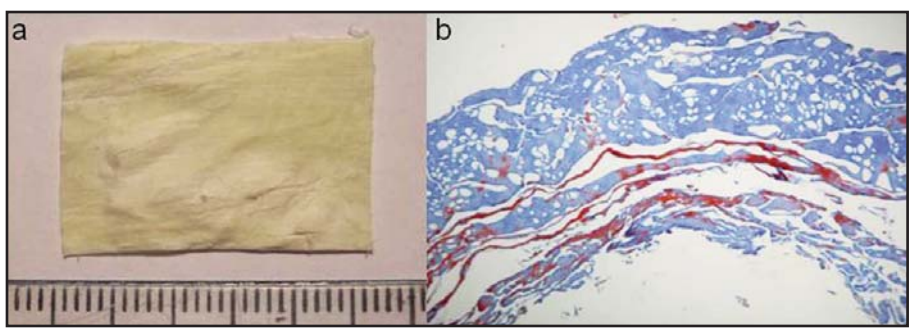


Fig. 1: Fascia Lata Membrane; a: Macroscopic appearance of FLM; b: Light microscopy of FLM stained with Masson's Trichrome. Note the fibrous tissue. Orig. Mag. X 2.5.

layer of FLM on both defects. Animals in Group III underwent surgery without placement of the barrier membrane, and Group IV served as control.

The wound was closed with single knot sutures using 5-0 nylon (Ethicon.USA). The rats were maintained on standard chow and tap water *ad libitum*, and were administered antibiotic therapy and a daily subcutaneous dose of 2 mm^3 of Dipenisol. In addition, they received a 1ml dose of Dexamethazone the first two days post-surgery.

All the animals were euthanized at 60 days; the heads were resected and fixed in 10% formalin solution. The skulls were radiographed, decalcified in formic acid, and processed for embedding in paraffin. Cross-sections oriented sagittally to the bone defect were obtained and stained with H-E and Masson's Trichrome.

RESULTS

None of the bone defects covered with a single (G I) (Fig. 2) or double (G II) (Fig. 3) layer of FLM showed an inflammatory response or macrophages, and all exhibited adequate osteogenesis with lamellar bone formation.

It is of note that no alterations were observed in the osteocyte lacunae containing osteocytes or in the

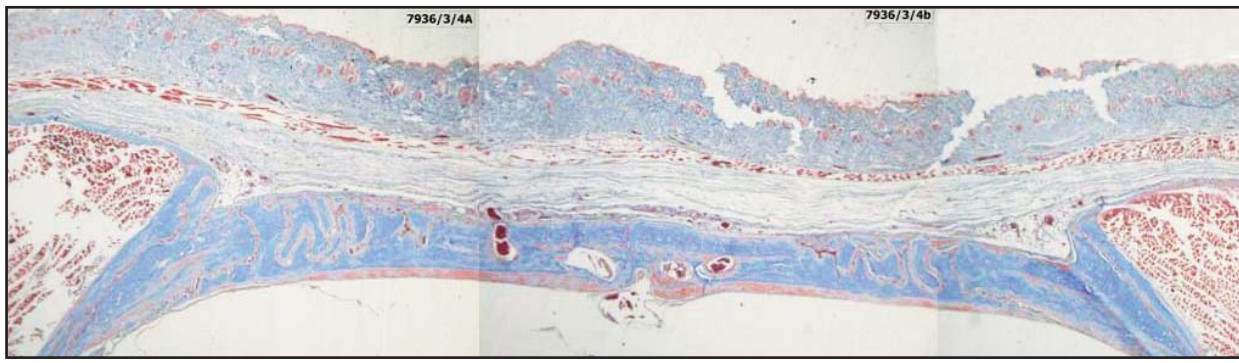


Fig. 2: Microphotograph of a histological section corresponding to Group I (60 days post surgery). The defect was covered with a single layer of human fascia lata membrane. Note the presence of lamellar bone tissue in the bone defect. Also note that the bone of the surgical bed is of adequate quality, and contains an adequate number of osteocytes. Orig. Mag. X 2.5; Masson's Trichrome stain.

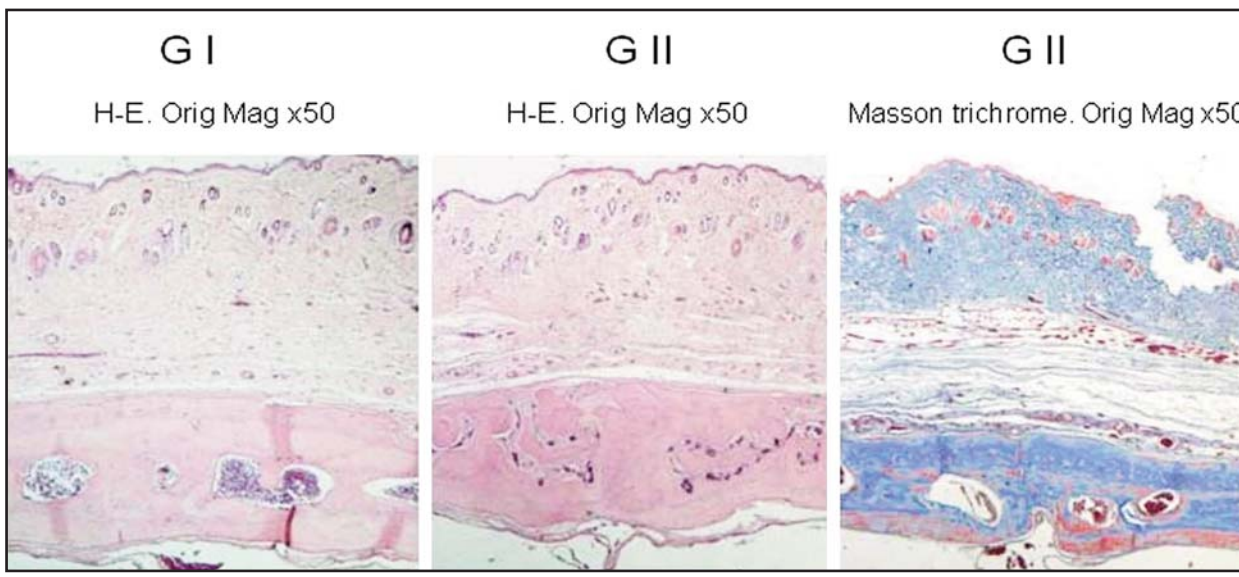


Fig. 3: Microphotographs of histological sections corresponding to Groups I and II (60 days post surgery). The defects were covered with a single layer of human fascia lata membrane, and G II defects were covered with a double layer of human fascia lata membrane. Both groups showed newly formed bone tissue in the bone defect. The fascia lata membranes exhibited no alterations in their structure, which consisted mainly of a collagen matrix. Orig. Mag. X 50; H-E stain.

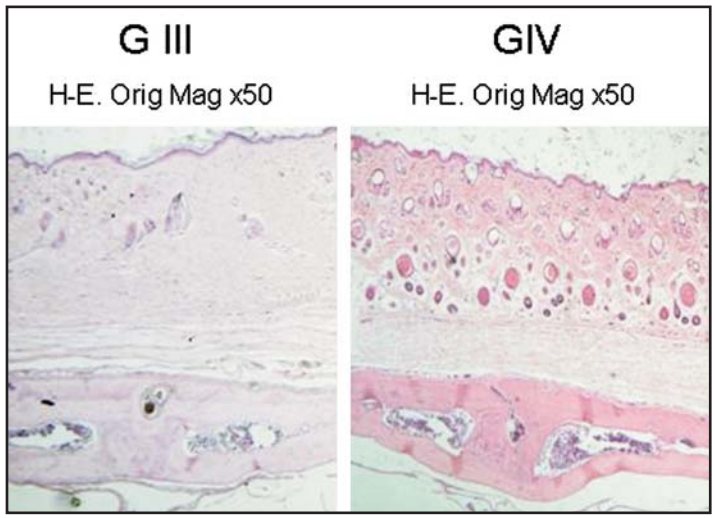


Fig. 4: Microphotographs of histological sections corresponding to Groups III and IV (60 days post surgery). No membrane was placed in G III defects, and GIV served as control. No alterations and scant osteogenesis were observed in both these groups. Orig. Mag. X 50; H-E stain.

surgical bed, where the bone defect was created by osteotomy using a piezoelectric scalpel.

No alterations and scant osteogenesis were observed in G III and G IV (Fig. 4).

The fascia lata membranes showed no evidence of alterations in their structure, which consisted mainly of a collagen matrix. No sign of inflammation and no macrophages or giant multinucleated cells were observed at the periphery of the membranes.

DISCUSSION

Treatment of bone defects created by trauma, infection, tumor resection, and skeletal abnormalities poses a great challenge[4]. Although there are numerous methods for bone reconstruction, each has specific indications and limitations. Well established methods include bone grafting and placement of bone substitutes or growth factors, among others [10-17].

The concept of GBR has been used in reconstructive surgery since the mid-1950s.

In 1964, P.J. Boyne published a study on the regeneration of alveolar bone beneath cellulose acetate filter implants[18].

As stated by Retzepi M et al (2010)[19], bioresorbable membranes were developed to avoid the need for surgical removal.

The results of the present study showed adequate wound healing of bone defects treated_with a single or double layer of human fascia lata membrane (FLM; Biotar, Rosario, Prov. de Santa Fe, Argentina) (Group I and Group II respectively), as shown by the finding of osteogenesis with lamellar bone formation. The defects in Group III, which were not treated with the FLM, exhibited less lamellar bone at the surgical defect. Attention must be drawn to the biocompatibility of FLM, as evidenced by the absence of inflammation, macrophages, and giant multinucleated cells.

The bone of the surgical bed created by osteotomy using a piezoelectric scalpel showed lamellar bone tissue of adequate quality, and contained an adequate number of osteocytes, demonstrating that piezosurgery does not alter the structure of the bone at the surgical site.

The results obtained with the experimental model used here, involving osteotomy with a piezoelectric scalpel to create a bone defect in rat calvaria and treatment of the defect with GBR using FLM, showed adequate osteogenic repair and adequate quality of the bone at the surgical site, both when using a single layer and a double layer of FLM.

CONCLUSION

Tissue response to FLM observed 60 days post-surgery using the experimental model presented here, provides further evidence of the biocompatibility of human fascia lata, as well as of its enhancing effect on osteogenesis during the repair process of experimentally created bone defects.

Further studies need to be conducted to assess bone tissue response to FLM in combination with bone substitutes.

ACKNOWLEDGEMENTS

The authors acknowledge the collaboration of Marcela Álvarez and Ricardo Orzusa, technical assistants at the Departments of Oral Pathology and Biochemistry respectively, of the School of Dentistry, University of Buenos Aires, Argentina.

This work was supported by research Grants UBACYT 2014- 2017 20020130100332 and PIP CONICET 2014-2016 11220130100091.

CORRESPONDENCE

Dr. Mariano Axel Ramón Amer.
Department of Anatomy.
School of Dentistry. University of Buenos Aires.
Marcelo T. de Alvear 2142 1B, (C1122AAH),
Buenos Aires, Argentina.
e-mail: marianoamer@gmail.com

REFERENCES

1. Cunha MJ, Esper LA, Sbrana MC, et al. Effect of low-level laser on bone defects treated with bovine or autogenous bone grafts: in vivo study in rat calvaria. Biomed Res Int 2014; 2014:104-230.
2. Prolo DJ, Rodrigo JJ. Contemporary bone graft physiology and surgery. Clin Orthop Relat Res 1985; 200:322-342. Review.
3. Herford AS, Dean JS. Complications in bone grafting. Oral Maxillofac Surg Clin North Am 2011; 23:433-442.
4. Dimitriou R, Mataliotakis GI, Calori GM, Giannoudis PV. The role of barrier membranes for guided bone regeneration and restoration of large bone defects: current experimental and clinical evidence. BMC Med 2012; 26:81-105. Review.
5. Esteves JC, Marcantonio E Jr, de Souza Faloni AP, et al. Dynamics of bone healing after osteotomy with piezosurgery or conventional drilling - histomorphometrical, immunohistochemical, and molecular analysis. J Transl Med 2013; 23: 221-234.
6. Claire S, Lea SC, Walmsley AD. Characterization of bone following ultrasonic cutting. Clin Oral Investig 2013; 17:905-912.
7. Maurer P, Kriwalsky MS, Block Veras R, et al. Micromorphometrical analysis of conventional osteotomy techniques and ultrasonic osteotomy at the rabbit skull. Clin Oral Implants Res 2008; 19:570-575.
8. Preti G, Martinasso G, Peirone B, et al. Cytokines and growth factors involved in the osseointegration of oral titanium implants positioned using piezoelectric bone surgery versus a drill technique: a pilot study in minipigs. J Periodontol 2007; 78:716-722.
9. National Research Council (US) Committee for the Update of the Guide for the Care and Use of Laboratory Animals. Guide for the Care and Use of Laboratory Animals. 8th edition. Washington (DC): National Academies Press (US); 2011.
10. Bauer TW, Muschler GF. Bone graft materials. An overview of the basic science. Clin Othop Relat Res 2000; 371;10-27
11. Giannoudis PV, Dinopoulos H, Tsiridis E. Bone substitutes: an update. Injury 2005; 36:S20-27. Review.
12. Pederson WC, Person DW. Long bone reconstruction with vascularized bone grafts. Othoped Clin North Am 2007; 38:23-35
13. Gorustovich A, Rosenbusch M, Guglielmotti MB. Characterization of bone around titanium implants and bioactive glass particles: An experimental study in rats. Int J Oral Maxillofac Implants 2002; 17:644-650.
14. Stvrtecky R, Gorustovich A, Perio C, Guglielmotti MB. A histologic study of bone response to bioactive glass particles used before implant placement: A clinical report. J Prost Dent 2003; 90:424-428.
15. Gorustovich A, Veinsten F, Costa O, Guglielmotti MB. Histomorphometric evaluation of the effect of bovine collagen granules on bone healing. An experimental study in rats. Acta Odontol Latinoamer 2004; 17:9-13.
16. Gorustovich AA, López JM, Guglielmotti MB, Cabrini RL. Biological performance of boron-modified bioactive glass particles implanted in rat tibia bone marrow. Biomed Mater 2006; 1:100-105.
17. Redondo MA, Renou SJ, Puia SA, Costa OR, Guglielmotti MB. Use of anorganic bovine bone matrix in an experimental model of bone healing. Acta Odontol Latinoam 2012; 25:306-311.
18. Boyne PJ. Regeneration of alveolar bone beneath cellulose acetate filter implants. J Dent Res 1964; 43:827-829.
19. Retzepi M, Donos N. Guided bone regeneration: biological principle and therapeutic applications. Clin Oral Implants Res 2010; 21:567-576.

Resultado. Simple y Original.

Micromotores

MT6000F • MT3000F • MT2000F

Avios

Primera cirugía • Segunda cirugía • Expansión ósea

Implantes

IA • HC • IPC • IPTB

Supraestructuras

Cementables • Cementables antirrotacional

Angulada • Ball-Attach • Ucla calcinable

Ucla calcinable antirrotacional

Ucla de titanio antirrotacional

* Próximo lanzamiento.

desde 1988

Marcelo T. de Alvear 2149 4°B (1122) Buenos Aires - Argentina / Tel: 4825.6581 - Fax: 4824.5640
implvel@fibertel.com.ar / **www.implantvel.com.ar**

ASSESSMENT OF MANDIBULAR MOVEMENTS IN 10 TO 15 YEAR-OLD PATIENTS WITH AND WITHOUT TEMPOROMANDIBULAR DISORDERS

Silvina G. Cortese, Ana M. Biondi, Diana E. Fridman, Ingrid Guitelman, Catalina L. Farah

Department of Comprehensive Pediatric Dentistry, School of Dentistry, Buenos Aires University, Argentina

ABSTRACT

The aim of this study was to establish reference values for mandibular movements in 10- to 15-year-olds without dysfunction and compare these values to those in patients of the same age with tempromandibular disorders (TMD) and those found previously in a group of children younger than 11 years old without TMD. Children of both genders who visited the Department of Comprehensive Pediatric Dentistry at Buenos Aires University in 2013 and whose parents or guardians provided consent were evaluated using TMD/RDC by standardized pediatric dentists (Kappa 0.88). Three groups were formed according to diagnostic summary: Group C, without TMD; Group Ia, with myofascial pain, and Group Ib, pain with limited mouth opening. The following variables were analyzed: age, gender and mandibular movements. The sample included 169 patients aged 12.5±1.76 years, of whom 62.36% did not have TMD (C) while 37.27% were diagnosed with muscle disorder (29.58% Ia and 7.69% Ib). For Group C, the following values (in mm) were recorded: maximal unassisted opening: 48.28±6.14; right lateral movement 8.78±2.50; left lateral movement: 9.60±2.64; protrusion: 4.94±2.58 and overbite: 2.98 ± 2.5, with no variation associated to sex, but with differences in the values recorded for all movements compared to those obtained for mixed dentition (p= 0.0001). Analysis of mean values for mandibular movements in all 3 groups only revealed differences for maximal unassisted opening (p= 0.0317). With relation to gender, TMD was more frequent in females, with significant differences between Groups C and Ia (p=0.019). In males without dysfunction, average maximal opening was 48.28±6.14mm, with lower values in patients with TMD. Mandibular movements in pediatric patients without TMD showed significant differences according to dentition type and age.

Key words: *Temporomandibular joint disorders; Facial pain; Children.*

VALORACIÓN DE MOVIMIENTOS MANDIBULARES EN PACIENTES DE 10 A 15 AÑOS CON Y SIN TRASTORNOS TÉMPORO-MANDIBULARES

RESUMEN

El objetivo del presente trabajo fue establecer valores de referencia de movimientos mandibulares en niños de 10-15 años sin disfunción; compararlos con los de pacientes de la misma edad con trastornos temporomandibulares (TTM) y con los hallados anteriormente en un grupo menor de 11 años sin TTM. Niños de ambos sexos que acudieron a la Cátedra Odontología Integral Niños de UBA en 2013 y cuyos responsables brindaron consentimiento fueron evaluados con CDI/TTM por odontopediatras estandarizados (Kappa 0.88) conformándose 3 grupos en función del resumen diagnóstico; C: sin TTM, Ia: con dolor miofacial e Ib: dolor con limitación de la apertura bucal, para el análisis de las siguientes variables: edad, sexo y movimientos mandibulares. La muestra quedó constituida por 169 pacientes de 12.5±1.76 años. El 62.36% no presentó TTM (C) y en el 37.27% se estableció un diagnóstico de trastorno muscular (29.58% Ia y 7.69% Ib). En C se registraron los siguientes valores en mm: Apertura máxima no asistida: 48.28±6.14; Lateralidad derecha 8.78±2.50; izquierda: 9.60±2.64; Protrusión: 4.94±2.58 y Sobremordida: 2.98 ± 2.5 sin variaciones en relación al sexo, pero con diferencias en los registros de todos los movimientos comparados con los obtenidos en dentición mixta. (p=0.0001). El análisis de los valores medios de los movimientos mandibulares entre los 3 grupos sólo reveló diferencias para la apertura máxima no asistida (p= 0.0317). En relación al sexo, los TTM fueron más frecuentes en niñas siendo significativa la diferencia entre C e Ia (p=0.019). En los niños sin disfunción se estableció una apertura máxima promedio de 48.28±6.14mm, observándose valores inferiores en los pacientes con TTM. Los movimientos mandibulares en pacientes pediátricos sin TTM mostraron diferencias significativas en función del tipo de dentición y la edad.

Palabras clave: *articulación temporomandibular; lesiones-dolor facial- niños.*

INTRODUCTION

Mandibular movement measurements are a simple but important parameter for evaluating and monitoring dysfunctions of the masticatory system known as temporomandibular disorders (TMD)[1]. In pediatric patients, multifactorial etiology for muscle dysfunction

is accepted; nevertheless, and less frequently, mandibular mobility can also be affected by causes such as severe trauma (fractures), developmental alterations, tumors and rheumatoid arthritis, among others.[2] The temporomandibular joint (TMJ) is affected in half of the children who have juvenile idiopathic arthritis, causing limited opening; thus, these measurements are also useful to rheumatologists[3,4].

TMJ development begins during the eighth week of intrauterine life and finishes in the second decade of life. Postnatal development is closely related to and interacts with maturation of masticatory and swallowing functions; growth of mandibular, masseter and temporal muscles; and development of dentition[5]. Monitoring TMJ physiological changes is relevant in pediatric patients. Condylar growth, like growth in general, slows during childhood, accelerates during adolescence and slows again after the pubertal growth spurt. Between the ages of 10 and 15 years, males have greater growth potential than females[6].

During mouth opening there are simultaneously rotational and translational movements of the two condyles and their disks along the articular eminence. The articular eminence and the temporomandibular ligament limit mouth opening. Mandibular closing movement should not be considered the inverse of opening because it is not limited by the same anatomical structures, but rather, the condyle moves against the anterior wall of the articular fossa through the muscles of mastication. During protrusion, the two condyles move forward and downward, making contact with the articular eminence. Lateral movements are more complex than opening or protrusion because they are asymmetrical, with the muscles on each side acting in different ways[1].

Very large range of movement (hypermobility) or limited range (hypomobility) are considered signs of dysfunction; therefore simple, objective recording methods and reference values are needed for each age group in order to facilitate TMD diagnosis[7].

There is little information in this regard for children, and the values reported are not consistent and have been gathered by means of different sampling and data recording methods[8].

In view of the difficulties caused by methodological differences for diagnosis, the International Association for Dental Research (IADR) adopted Research Diagnostic Criteria for Temporomandibular Disorders (RDC/TMD) (índex prepared by Dworkin and LeResche), which provide a standardized system for examining, diagnosing and classifying the most common subtypes of TMD[9]. These criteria, currently used by many research groups, have been validated in 18 languages for children as from 10 years of age, and were used for our study. The system has two components for evaluation. "Axis I" is a questionnaire and clinical evaluation designed to distinguish myofascial pain, disk displacement, arthralgia, arthritis and arthrosis[9].

The aims of this study were to establish reference values for mandibular movements in 10- to 15-year-olds without dysfunction and compare them to those of patients of the same age with TMD and to those found in a previous study on a group of children younger than 11 years old without TMD[10].

MATERIALS AND METHODS

Design, subject selection and methods

We designed an observational, prospective, cross-sectional study, which was approved by the Ethics Committee of the School of Dentistry at Buenos Aires University (code number 26/09/2012 – 27). We evaluated all 10- to 15-year-olds of both genders who sought care at any of the four different shifts at the Department of Comprehensive Pediatric Dentistry at Buenos Aires University from March to August 2013, whose parents or guardians provided consent for participation in this study. We excluded children with medical compromise; developmental, neurological and/or psychiatric disorders, and/or undergoing orthodontic treatment. Patients were evaluated using RDC/TMD Axis I by standardized pediatric dentists (Kappa 0.88). Data were entered into a spreadsheet (MS Excel 2010, version 14) for subsequent statistical analysis.

Three groups were formed according to the results of the RDC/TMD diagnostic summary: without TMD (C), with myofascial pain (Ia) and with limited mouth opening (Ib).

The following variables were analyzed: gender, age, overbite in mm, maximal unassisted and assisted mouth opening, right and left lateral movement and protrusion. Frequencies were estimated using percentages with confidence intervals (95%), and the rest of the quantitative variables were analyzed using means, standard deviation, Student and Welch test, ANOVA analysis of variance and Chi squared

with 95% significance. The values recorded for C were compared to previous findings for mixed dentition and to findings for Ia and Ib
Reference values for opening were calculated by subtracting overbite from maximal opening.

Clinical recording
Patients sat in a dental chair and were measured using a millimeter ruler.
Maximal Unassisted Opening. The patient was asked to open his/her mouth as wide as possible, even if it caused discomfort. The edge of the millimeter ruler was placed at the incisal edge of the upper central incisor and the distance between it and the incisal edge of the lower incisor (interincisal distance) was measured and recorded (Fig. 1).
Right and Left Lateral Movements. The patient was asked to open his/her mouth a little and move the mandible as far as possible to one side (right or left). With teeth slightly apart, the distance between the interdental space between upper central incisors and interdental space between lower incisors was measured and recorded, for both sides (Fig. 2).
Protrusion. The patient was asked to open his/her mouth slightly and protrude the mandible without interference from the incisors. The distance on the midline between upper and lower incisor edges was measured and recorded (Fig. 3).
Table 1 shows diagnostic criteria used[11].

Fig. 1: Recording Maximal Opening.

Fig. 2: Recording Lateral Excursion.

Fig. 3: Recording Protrusion.

RESULTS
The sample comprised 169 patients; mean age was 12.5 years, SD 1.76. Of these, 62.36% (54.59-69.67) did not have TMD (C), while 37.27% (CI 30-45,05) were diagnosed with muscle disorders: 29.58% (CI 22.80-37.09) in Group Ia and 7.69% (CI 4.14-12.80) in Group Ib.

Analysis of Group C: without TMD
Group C comprised 106 children, mean age 12.37± 1.6 years, of whom 41.50% (31.99-51.51) were female. Table 2 shows the average values for final maximal comfortable opening, with compensation for overbite, broken down according to age.
Comparison for maximal opening according to gender shows that males (age 12.5±1.55) had 48.38±6.47 mm and females (age 12.2±1.66) had 48.12±5.70 mm (p=0.83).
Values in mm were: maximal unassisted opening 48.28±6.14 (CI 47.1-49.46), right lateral movement 8.78±2.50 (8.30-9.26), left lateral movement 9.60±2.64 (9.09-10.11), protrusion 4.94±2.58 (4.44-5.44) and overbite 2.98 ± 2.5 (1.61-2.38). Values in mm found previously in mixed dentition in a group of 107 children, mean age 6.9±1.65 were:

Table 1: Summary of diagnostic criteria for TMD.

	RDC/TMD Diagnostic Criteria Axis I [11]
Muscle Disorders	Ia Myofascial pain: • Pain at 3 or more muscle points evaluated. • At least one of them matches the questionnaire report.
	Ib Myofascial pain with limited opening: • Pain similar to Ia • Unassisted opening < 40 mm • 5 mm or more difference with maximal assisted opening
Disc displacements	IIa Disc displacement with reduction • Reciprocal click 2 out of 3 consecutive times, or • Reproducible click on vertical movement 2 out of 3 consecutive times and reproducible click during lateral or protrusive excursion on 2 out of 3 consecutive times.
	IIb Disc displacement without reduction with limited opening • Significant history of limited opening • Maximal unassisted opening ≤35 mm • Increase in maximal opening by unassisted passive stretch ≤ 4 mm • Contralateral excursion <7 mm and/or uncorrected deviation to ipsilateral side during opening • Absence of noise or presence of noises that do not meet the criteria for IIa.
	IIc Disc displacement without reduction without limited opening • Significant history of limited opening • Maximal unassisted opening >35 mm • Increase in maximal opening by unassisted passive stretch ≥5 mm • Contralateral excursion ≥7 mm • Presence of noises that do not meet the criteria for IIa.
Arthralgia, osteoarthritis, osteoarthrosis	IIIa Arthralgia • Pain in one or both joints during palpation • One or more instances of self reported joint pain during maximal assisted and unassisted opening and during lateral excursion • Absence of crepitus.
	IIIb Osteoarthritis • Arthralgia similar to IIIa • Crepitus or radiographic evidence of arthrosis.
	IIIc Osteoarthrosis • Absence of signs of arthralgia • Gross crepitus or radiographic or radiographic evidence of arthrosis.

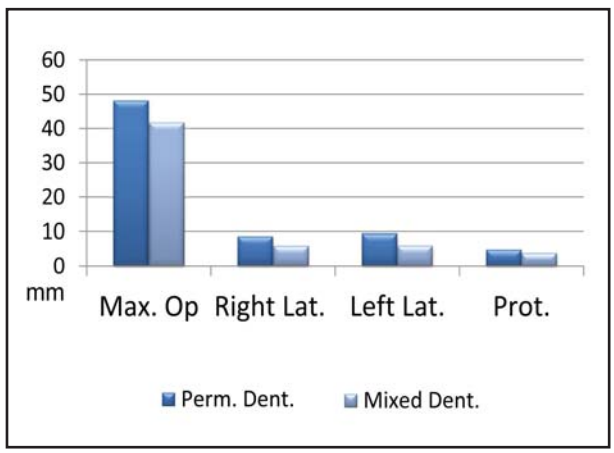


Fig. 4: Comparison of mandibular movements in permanent and mixed dentition.

maximal opening 41.9 ± 5.27 (40.96-42.98), right lateral movement 6.05±1.99 (5.67-6.43), left lateral movement 6.13±2.21 (5.75-6.51) and protrusion 3.96±1.92 (3.59-4.33)[10]. The differences recorded for all movements between the two groups were significant (p=0.0001, Fig. 4).

Analysis of Groups Ia and Ib

A total 63 patients had muscle disorders, 2 patients from Group Ia also had disc displacement with reduction (IIa) and 6 patients from Group Ib had disc displacement without reduction (IIb). Table 3 shows the composition both groups. The values in mm were: maximal unassisted opening 45.8±4.37, 33.38±5.63; right lateral movement 7.98±2.76,

8.53±2.25; left lateral movement 8.98±2.86, 9.30±2.78; protrusion 4.62±2.42, 4.92±2.49 and overbite 2.67±2.12, 3.38±1.93 for Groups Ia and Ib, respectively.

In Group Ia, 17 patients had lower values for maximal opening than those found for Group C, considered as reference values (42.14-54.42).

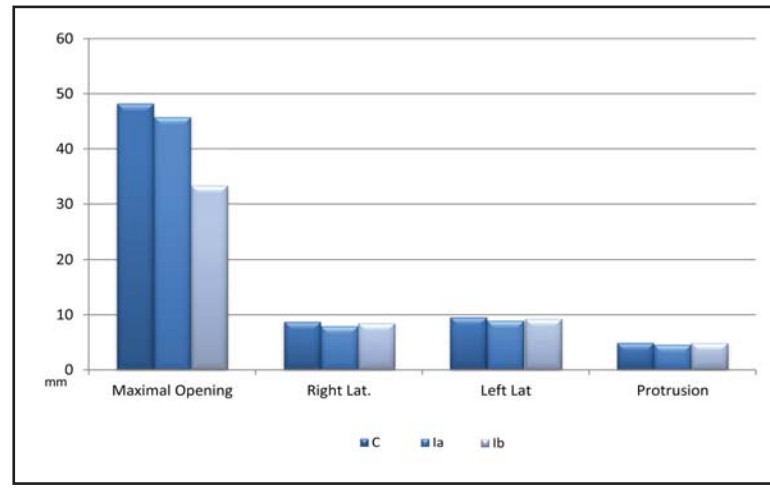


Fig. 5: Comparison of mandibular movements with and without TMD in permanent dentition.

Comparison of Groups C, Ia and Ib

Fig. 5 shows mean values for mandibular movements in all 3 groups.

TMD was more frequent in females, with significant difference between Groups C and Ia (p=0.019) (C-Ib p=0.100; Ia-Ib p=0.499).

Comparison of all 3 groups revealed significant differences for maximal unassisted opening (p= 0.0317), and no significant difference for the rest of the variables analyzed. Table 4 shows the p values.

DISCUSSION

There is little available information on mandibular movements in children, and results are inconsistent, possibly due to differences in recording methodology and inclusion criteria[12-16]. In a previous paper, reference values were established for primary and mixed dentition without signs of TMD[10].

Table 2: Distribution of maximal opening according to age.

Age	N	Maximal Opening (mm)	SD
10	15	44.0	3.5
11	22	46.9	6.3
12	21	49.7	5.7
13	17	47.3	6.6
14	19	51.9	6.1
15	12	49.3	5.2

Table 3: Composition of Groups Ia and Ib.

	Ia	Ib
N=	50	13
Age	12.57±2.05	13.30±1.47
Female	68% (53.26-80.51)	61.5% (31.49-6.25)

Reference values for maximal opening in a group of 4- to 17-year-olds have recently been published, though without prior evaluation of the TMJ; therefore the results reported with percentiles include people with dysfunction[3].

Cases have been evaluated with different methods, ranging from the very simple and imprecise, such as thickness of fingers, to the sophisticated, such as the system used by Hayasaki et al. on a small sample, which analyzes simultaneous movements in three dimensions[17-19]. In addition, pantographic and ultrasound systems have been designed and placed on the market, though there has been little application of them in epidemiological research and clinical practice[20].

The International Association for Dental Research (IADR) recommends the use of the RDC/TMD[21]. Their diagnostic parameters, which were followed

Table 4: Comparison of C, Ia and Ib (ANOVA).

	p=
Age	0.1828
Maximal opening	0.0317
• C –Ia	<0.05
• C-Ib y Ia-Ib	<0.001
Right lateral excursion	0.1788
Left lateral excursion	0.4114
Protrusion	0.7571
Overbite	0.5701

in this research protocol, do not discriminate variations in limited opening according to age.

Several studies have shown that opening increases progressively from birth to the end of adolescence and then decreases progressively with age[12, 22, 24]. Our study considered opening less than 40 mm to be limited, as provided in the RDC/TMD. Nevertheless, the group without TMD had a mean value of 48.28±6.14mm, therefore in this age range, openings lower than 43 mm should be considered limited.

The most extreme opening movements are observed at puberty, which is believed to be a consequence of the hyperlaxitude characteristic of this stage[25, 26]. The increase in values for mandibular movement with increasing age may be due to anatomical changes, maturation of the central nervous system, skeletal growth and maturation of the occlusal function[10,14,15,27,28]. Ingervall suggests that by 10 years of age, children have attained adult mandibular motion range[12]. Thus, statistically significant differences were found between values for Group C and those found previously in a group of children younger than 11 years for all variables in the mandibular motion ranges.

The values for protrusion movement that we found for Group C were noticeably lower than those reported by other authors[5,12,14,16,18] , which may be because they added overjet, which our study did not take into account[8,12,15,16,27]. For Group C lateral movements, our study found higher values than those reported by other authors[15,16,27].

Our results for patients without TMD according to gender are consistent with those published by Müller, who reported no significant difference in opening between genders up to the age of 13 years[3]. Female joints are usually more flexible and laxer than male joints, which would explain the greater incidence of temporomandibular dysfunction in females[1,8].

TMD frequency was 37.27%, with 29.58% in Group Ia and 7.69% in Group Ib. Hirsch reported for a group with mean age 13 years a frequency of 10.2 %, with 7.9% corresponding to IIa and 2.3 % to Ia, III a/b [8]. Our study only found 1.18% IIa and 3.55% IIb and high frequency of muscle disorders without movement limitation (Ia).

Pizolato et al. report 26.3% TMD in 8- to 12-year-old patients with no difference according to gender[29]. In contrast, other authors found prevalences of over 70% in older adolescent patients[30,31]. According to the RDC/TMD, Group Ia has no limitation of movement; nevertheless, in our study and applying those criteria, we found significantly lower maximal opening values compared to Group C.

Our sample is made up of patients from a pediatric dental care facility, where pathologies may be found more often than in the general population. It would be interesting to conduct a similar study on samples of the population not demanding care.

The results of this study may contribute to providing professionals with more precise indicators for early diagnosis of TMD in patients in an age range at which visits due to this problem are frequent and information is scarce and controversial.

CONCLUSIONS

Mandibular movements in pediatric patients without TMD showed significant differences according to age and dentition type. Average maximal opening was established at 48.28±6.14mm, with no difference according to gender.

In the 37.27% with TMD, maximal opening was significantly lower, at 45.8±4.37mm for the group with myofascial pain and 33.38±5.63 mm for the group with myofascial pain and limited opening.

ACKNOWLEDGMENTS

This work was supported by a grant from the Buenos Aires University (Program UBACyT, nº 20720120200008BA).

The authors wish to thank Od.Alicia Estikle for help with data collection.

CORRESPONDENCE

Dra. Silvina G Cortese
Cátedra de Odontología Integral Niños
Facultad de Odontología, UBA
M.T. de Alvear 2142, 15A, (C1122AAH),
Buenos Aires, Argentina
sgcortese@hotmail.com

REFERENCES

1. Okeson JP. Management of temporomandibular disorders and occlusion. 6th Ed. St. Louis, Missouri. Mosby, 2007.
2. American Academy of Pediatric Dentistry (AAPD). Guideline on acquired temporomandibular disorders in infants, childrenand adolescents. Chicago (IL): Pediatr Dent. 2015; 37:78-84.
3. Muller L, Kellenberger CJ, Cannizzaro E, Ettlin D, Schraner T, Bolt IB, Peltomaki T, Saurenmann RK: Early diagnosis of temporomandibular joint involvement in

juvenile idiopathic arthritis: a pilot study comparing clinical examination and ultrasound to magnetic resonance imaging. Rheumatology (Oxford) 2009; 48:680-685.
4. Stoll ML, Sharpe T, Beukelman T, Good J, Young D, Cron RQ: Risk factors for temporomandibular joint arthritis in children with juvenile idiopathic arthritis. J Rheumatol 2012, 39:1880-1887.
5. Gómez de Ferraris M, Campos Muñoz A. Histología y embriología bucodental. 2da. ed. Madrid, Ed Médica Panamericana; 2004 Fa.
6. Buschang PH, Gandini LG. Mandibular skeletal growth and modelling between 10 and 15 years of age. Eur J Orthod. 2002; 24:69-79.
7. Padamsee M, Ahlin JH, Ko CM, Tsamtsouris A. Functional disorders of the stomatognathic system: part II. A review. J Pedod 1985; 10:1-21.
8. Hirsch C, John MT, Lautenschläger C, List T. Mandibular jaw movement capacity in 10-17-yr-old children and adolescents: normative values and the influence of gender, age and temporomandibular disorders. Eur J Oral Sci 2006; 114: 465-470.
9. Dworkin S, LeResche L Research Diagnostic Criteria for Temporomandibular Disorders. Journal of Craniomandibular Disorders 1992. Validación al español: González Y. y col. Criterios Diagnósticos para la Investigación de los Trastornos Témporomandibulares. URL: http://www.rdc-tmdinternational.org.
10. Cortese,SG; OliverLM; Biondi AM. Determination of range of mandibular movements in children without temporomandibular disorders.Cranio 2007; 25:200-205.
11. Manfredini D, Guarda-Nardini L, Winocur E, Piccotti F, Ahlberg J, Lobbezoo F. Research diagnostic criteria for temporomandibular disorders: a systematic review of axis I epidemiologic findings. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2011; 112:453-462.
12. Ingervall B. Range of movement of mandible in children. Scand J Dent Res 1970; 78:311-322.
13. Könönem, M. Nyström M, Kleemola-Kujala E, Kataja M, Evälahti M, Laine P, Peck L. Signs and symptoms of craniomandibular disorders in a series of Finnish children. Acta Odontol Scand 1987; 45:109-114.
14. Kumar A, Mehta R, Goel M, Dutta S, Hooda A. Maximal mouth opening in Indian children using a new method. J Cranio Max Dis 2012; 1:79-86.
15. Machado BCZ, Medeiros APM, Felício CM. Mandibular movement range in children. Pró-Fono 2009; 21:189-194.
16. Al-Omary W. The Impact of Chronological Age on Mandibular Movements. Al-Rafidain Dent J 2013; 13:29-36. No aparece en Pubmed.
17. Zawawi KH, Al-Badawi EA, Lobo SL, Melis M, Mehta NR. An index for the measurement of normal maximum mouth opening. J Can Dent Assoc 2003; 69:737-741.
18. Abou-Atme YS, Chedid N, Melis M, Zawawi K. Clinical measurement of normal maximal mouth opening in children. Cranio 2008; 26:191-196*The journal of craniomandibular practice.* 2008; 26. URL: http://www.kau.edu.sa/Files/0003432/Researches/49279_20082.pdf.
19. Hayasaki H, Yamasaki Y, Nishijima N, Naruse K, Nakata M. Characteristics of protrusive and lateral excursions of the mandible in children with the primary dentition. J Oral Rehabil. 1998; 25:311-320.
20. Furtado D, Pereira A, Olivera A, Peres D, Ribeiro M. A specialized motion capture system for real-time analysis of mandibular movements using infrared cameras. Bio Med Eng On Line 2013; 12-17.
21. Dworkin, SF. Research Diagnostic Criteria for Temporomandibular Disorders: current status & future relevance. J Oral Rehabil 2010; 37:734-743.
22. Mezitis M, Rallis G, Zachariades N. The normal range of mouth opening. J Oral Maxilofac Surg 1989; 47:1028-1029.
23. Yao K, Lin C, Hung C. (2009) Maximum mouth opening of ethnic Chinese in Taiwan. J Dent Sci DOI: 10.1016/S1991-7902(09)60007.
24. De Cunto C, Maroldo M, Liberatore D, Imach E. Hiperlaxitud articular: estimación de su prevalencia en niños en edad escolar. Arch Argent Pediat 2001; 99:105-110.
25. Szentpétery A. Clinical utility of mandibular movement ranges. J Orofac Pain 1993; 7:163-168.
26. De Sousa L, Nagamine H, Chaves T, Grossi D, Regalo S, Oliveira A. Evaluation of mandibular range of motion in Brazilian children and its correlation to age, height, weight and gender. Braz Oral Res 2008; 22:61-66.
27. Bonjardim LR, Gavião MB, Pereira LJ, Castelo PM. Mandibular movements in children with and without signs and symptoms of temporomandibular disorders. J Appl Oral Sci 2004; 12:39-44.
28. Pizolato RA, Fernandes F, Gavião MB. Speech evaluation in children with temporomandibular disorders. J Appl Oral Sci 2011; 19:493-499.
29. Kitsoulis P, Marini A, Iliou K, Galani V, Zimpis A, Kanavaros P, Paraskevas G. Signs and Symptoms of Temporomandibular Joint Disorders Related to the Degree of Mouth Opening and Hearing Loss. BMC Ear, Nose Throat Disord2011; 11:5 doi: 10.1186/1472-6815-11-5.
30. Lauriti L[1], Motta LJ, Silva PF, Leal de Godoy CH, Alfaya TA, Fernandes KP, Mesquita-Ferrari RA, Bussadori SK.Are occlusal characteristics, headache, parafunctional habits and clicking sounds associated with the signs and symptoms of temporomandibular disorder in adolescents. J Phys Ther Sci 2013; 25:1331-1334.

FiS

Federa implante Switch

El sistema FiS fue concebido para restauraciones implanto-odontólogicas de alta exigencia estética donde se requiere de una gran flexibilización de las opciones protéticas, tanto iniciales como sus posteriores reemplazos (switch) para adaptarse a la natural evolución de los tejidos.

Presentado por

Implantes Dentales
FEDERA

FEDERA S.R.L. Av. Córdoba 1856, 4°Piso. Bs As.
Tel/Fax: 011-4815-4467
info@implantesfedera.com www.federa.com.ar

EFFICACY OF CORE NEEDLE BIOPSY TECHNIQUE FOR JAWBONE DISEASES

Federico Stolbizer[1,2], Romulo L. Cabrini[3], Alicia Keszler[3]

[1] Emergency Department.
[2] Department of Oral-maxillofacial Surgery.
[3] Oral Pathology Department, School of Dentistry University of Buenos Aires. Argentina.

ABSTRACT

Core needle biopsy (CNB) has been proven useful for diagnosing bone lesions, although it is not often used for jawbone lesions. The aim of this study was to evaluate the efficacy of the CNB method in a series of cases of intramaxillary lesions.

CNB was performed on 85 patients with intraosseous lesions which were grouped according to radiographic appearance as: radiopaque lesions (RO, n=13), radiolucent lesions (RL, n=39) and mixed lesions with both radiolucent and radiopaque areas (RL-RO, n=33). The technique enabled us to obtain several tissue cylinders from each lesion (average 2.5 cylinders), which were processed following routine histopathological technique and H&E stain, plus special techniques when necessary. The histopathological analysis together with clinical data enabled accurate diagnosis (AD) in 81% of the cases and descriptive diagnosis (DD) in 14%. The material obtained in 5% of the cases was not appropriate for study (ND). The difference between successful (AD) and unsuccessful (DD+ND) CNB cases is statistically significant.

The highest percentage of successful CBNs was for RO and RL-RO lesions (85% and 100% respectively). RL lesions were more difficult because most of them were cystic lesions with fluid content.

Key words: *Core needle biopsy; jaw diseases.*

EFICACIA DE LA TÉCNICA DE PUNCIÓN ÓSEA PARA BIOPSIA DE LESIONES DE MAXILARES

RESUMEN

La biopsia-punción ósea (Core needle biopsy, CNB) es un procedimiento de probada utilidad en el diagnóstico de lesiones óseas. Sin embargo, no es una técnica de uso frecuente en las lesiones de los maxilares. La finalidad de este trabajo fue evaluar la eficacia del método de CNB en una serie de casos de lesiones intramaxilares.

Se realizaron CNB en 85 pacientes con lesiones intraóseas, las cuales fueron agrupadas según su aspecto radiográfico en lesiones radiopacas (RO, n=13), lesiones radiolucidas (RL, n=39) y lesiones mixtas con sectores radiolúcidos y radiopacos (RL-RO, n=33). La técnica permitió obtener varios cilindros de tejido de cada lesión (promedio: 2.5 cilindros) los cuales fueron procesados según técnica histopatológica de rutina con tinción de H&E y técnicas especiales en los casos en que fueron necesarias. El análisis de los cuadros histopatológicos conjuntamente con los datos clínicos, permitió realizar un un diagnóstico de certeza (AD) en el 81% de los casos y un diagnostico descriptivo (DD) en el 14 % . En el 5% de los casos el material obtenido no fue adecuado para su estudio (ND) La diferencia entre los casos de CNB exitosa y no exitosa (DD+ND) es estadisticamente significativa.

El mayor porcentaje de CBN exitosas correspondió a las lesiones RO y RL-RO (85% y 100% respectivamente) Las lesiones RL presentaron mayor dificultad debido a que, en su mayoría, eran lesiones quísticas con contenido líquido.

Palabras clave: *biopsia punción ósea; lesiones de los maxilares.*

INTRODUCTION

Biopsy sampling is a surgical procedure for obtaining material from a lesion on which to perform histopathological studies for use together with all the clinical data to reach a diagnosis and provide appropriate treatment. The choice of technique and instruments for taking the biopsy depends on the nature and location of the lesion. For intraosseous lesions, a tissue sample can be obtained by performing either surgical biopsy (open biopsy) or puncture biopsy (closed biopsy).

Since 1901, when the first instrument for performing puncture biopsy was registered [1] great efforts have been made to develop techniques and instruments to avoid the complications that can arise with an open biopsy, particularly when the lesions are located deep inside the tissue or are in contact with vital structures [1-11]. Progress in these devices has generated great interest among physicians. A number of case series published have shown the efficacy and advantages of using core needle biopsy (CNB), reporting success rates

ranging from 20 to 95% [1,6,12-19]. The published data demonstrate how the technique has developed and improved over the years.

In contrast to the widespread use of CNB in the field of medicine, and in spite of the frequency and variety of lesions that affect the jaws, the procedure has not had significant application in the field of dentistry. The aim of this study was to assess the efficacy of the CNB method in a series of intramaxillary lesion cases.

MATERIALS AND METHODS

Core needle biopsies were performed on eighty-five patients, 45 female and 40 male, age range 6 to 80 years, with intramaxillary lesions and indication for biopsy. All patients provided informed consent. The project was approved by the Ethics Committee of the School of Dentistry, University of Buenos Aires.

The radiographic appearance of biopsied lesions was radiolucent (RL) in 39 cases, mixed radiolucent-radiopaque (RL-RO) in 33 cases, and radiopaque (RO) in 13 cases.

The CNB technique was performed on outpatients under local anesthesia with 4% Carticaine Chlorhydrate with L-Adrenaline 1:1000,000 (Bernabó Laboratory, Buenos Aires, Argentina), using 11 gauge/10cm-long needles, originally designed for taking bone marrow samples from the iliac crest, (Gallini medical devices, BM 11G-10cm, Italy). A transmucosal puncture was performed without prior incision. Several samples were taken using the same puncture site and positioning the needle in different directions. The number of samples taken depended in each case on the size of the lesion and the degree of homogeneity in the X-ray image. The operation does not require subsequent suture. The tissue cylinders obtained were fixed in 10% formalin and demineralized in 7% nitric acid for 12-24 h, depending on their degree of mineralization. Following routine embedding in paraffin, sections were obtained and stained with H-E. When special techniques were needed as a diagnostic aid, histochemical and immunohistochemical stains were also performed on adjacent sections. The following techniques were used: Periodic acid Schiff, Gomori's Trichrome stain, and immunohistochemical reactions for the expression of the antigens CK7/20, CD3, CD20, CD1a, S100, Vimentin and kappa and lambda light chains.

After correlating histopathological findings with the individual patients´ histories, clinical and image data, final diagnoses were grouped in three categories as follows: 1- Accurate diagnosis (AD), when it was possible to arrive at the diagnosis of a defined pathological entity, 2- Descriptive diagnosis (DD), when the histopathological diagnosis was descriptive but not conclusive, and together with clinical and radiographic data enabled a defined pathology to be suggested, though not identified; 3- No diagnosis (ND), when the quantity or quality of the material obtained from the biopsy was not sufficient to establish a diagnosis.

Final AD diagnoses of biopsy samples obtained by CNB were then confirmed with the study and diagnosis of the surgical specimen, when the treatment of the biopsied lesion was surgery. In cases not requiring surgery, (e.g. fibrous dysplasia) the diagnosis was confirmed through clinical and radiographic follow-up of the patient for at least two years. For the purpose of this study, we did not change the final DD and ND of the CNBs, even if subsequent studies enabled improvement in the diagnosis.

To evaluate the efficacy of the CNB method, the percentage of AD (successful CNBs) was compared to the percentage of DD+ND (unsuccessful CNBs) using the One-Sample Proportion Test.

We also evaluated the percentage of AD, DD and ND with relation to the radiographic appearance of the lesions to determine whether lesion type conditions the success of the CNB. Pearson´s Chi-squared test was used for this purpose.

RESULTS

The technique enabled material to be obtained in all cases (100%), with an average of 2.5 tissue cylinders per patient.

CNB diagnosis of a defined entity or accurate diagnosis (AD), subsequently confirmed by the study of the surgically removed tissue or patient follow-up, was achieved in 81% of the cases. Descriptive diagnoses (DD) were made for 14% of the cases and no adequate material for diagnosis (ND) was obtained in 5%. The percentage of successful CNBs (AD) differed statistically from DD + ND (One-Sample Proportion Test, $p= 0.005$).

Fig. 1 shows an example of the material studied in one case in which accurate diagnosis of ossifying fibroma was made. Table 1 shows final diagnoses of all the study cases.

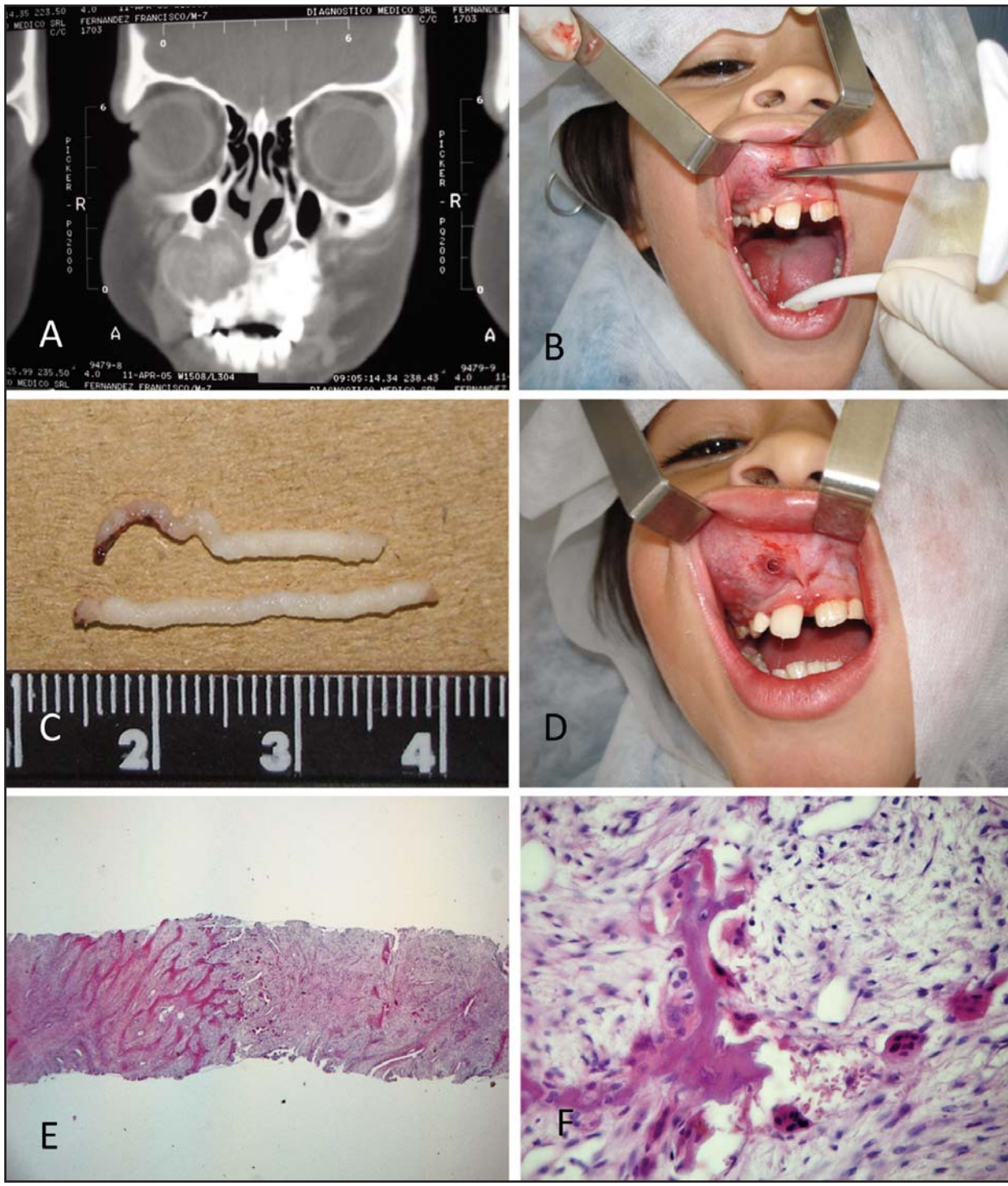


Fig. 1: Lesion in the upper jawbone in which core needle biopsy led to the diagnosis of ossifying fibroma. A) CT image, B) Puncture technique C) Tissue cylinders obtained from the lesion, C) Lesion in the mucosa after puncture, - D-F) Microscopic features . HE. Original magnification X 5 and X 40.

Table 2 shows the distribution of percentages of CNB final diagnoses with relation to the different radiographic appearances. The CNB was effective for all radiologic types of the biopsied lesions. However, radiolucent lesions were more difficult. The percentage of successful cases is significantly higher in RO and RL-RO lesions (85% and 100% respectively) than in the group of RO lesions

Table 1: Lesions under study and diagnoses of core needle biopsies.

Radiopaque lesions		Radiolucent-Radiopaque lesions		Radiolucent lesions	
	n CNB		n CNB		n CNB
Osteomyelitis	5AD 2DD	Fibrous dysplasia	15AD	Adenocarcinoma Metastasis	3AD 1DD
Chronic Periostitis	2AD	Ossifying fibroma Fibroma osificante	6AD	Lymphoma	4AD
Condensing Osteitis	2AD	Paget's disease	3AD	Squamous Cell Carcinoma	3AD
Odontoma	1AD	Adenocarcinoma Metastasis	2AD	Central Giant cell Granuloma	3 AD
Paget's disease	1AD	Cystic fibrous osteitis (primary HPT)	2AD	Keratocystic Odontog.Tumor	1AD 2DD
		Cherubism	1AD	Langerhans Cell Histiocytosis	2AD
		Gnathodiaphyseal dysplasia	1AD	Hemangioma	1AD 2DD
		Renal osteodystrophy (secondry HPT)	1AD	Radicular Cys	2ND
		Calcifying Epithelial Odontog. Tumor	1AD	Radicular Granuloma	1ND 1DD
		Bone undergoing repair	1AD	Dentigerous Cyst	2DD
				Ameloblastoma	1AD 2DD
				Adenocarcinoma	1AD
				Neuroectodermal Tumor	1AD
				Plasmacytoma	1AD
				Fibromatosis	1DD
				Desmoplastic Fibrosarcoma	1AD
				Malignant Schwannoma	1AD
				Ossifying Fibroma	1AD
				Inverted Papilloma	1AD
				Nasal palatal Cyst	1ND
				Hemorrhagic Cyst	1ND

n: number of cases; CNB diagnoses from core needle biopsy
AD: accurate diagnosis; DD: descriptive diagnosis; ND: no diagnosis

Table 2: Distribution of percentages of CNB final diagnoses for different radiographic appearances.

	AD	DD	ND	n
RO	85%	15%	-	13
RL-RO	100%	-	-	33
RL	64%*	26%	10%	39

RO: radiopaque lesions; RL-RO mixed radiolucent-radiopaque lesions; RL: radiolucent lesions, AD: accurate diagnoses; DD: descriptive diagnoses; ND: no diagnoses, n: number of cases. * different from RO and RL-RO groups. Pearson´s Chi-squared test, p=0.002.

(Pearson´s Chi-squared test, p=0.002). In RO lesions group diagnosis was conclusive in cases of periostitis, condensing focal osteitis, odontoma, Paget's disease, and in 5 out of the 7 cases of osteomyelitis. The group of RL-RO lesions included a wide range of pathologies of very different nature. The group of RL lesions, in which 64% of CNBs were successful, included several cystic lesions (Table 2).

DISCUSSION

Core needle biopsy has several advantages over open biopsies. It allows tissue to be obtained from the depth of the lesion, whereas surgical open biopsy is often limited to the periphery of the lesion. This is relevant for large lesions. For smaller lesions, it may be possible to obtain a tissue cylinder for the entire diameter of the lesion. By placing the needle at different angles through a single puncture site, several representative cylinders can be obtained from different zones of the lesion.

This is particularly important when the lesion is large and/or has a heterogeneous radiographic appearance. CNB does not require incisions or sutures, thus shortening the surgical times and being less traumatic, less invasive, and better tolerated by the patient. Moreover, it usually causes less hemorrhage and less contamination of the surgical site and often reduces the cost of surgery.

However, the use of puncture biopsies in the field of maxillofacial surgery is not yet widespread. This may be due partly to the fact that there is easy access to the oral cavity for conventional surgical biopsies. However, once minimal necessary training has been acquired, a specialist surgeon has all the advantages described above.

Some authors have evaluated the use of fine needle aspiration biopsy in intramaxillary lesions, and have found it to have significant diagnostic value [20-22] in spite of the fact that by using this technique, material is only obtained for the study of isolated cells. The few papers that refer to the application of CNB in jaw pathologies describe the methodology and report single clinical cases [23-26], but we have not found any reports of series of cases evaluating the usefulness of the method. The CNB technique proved effective in radiopaque lesions and radiologically inhomogeneous lesions. The lower efficacy of CNB in radiolucent lesions was due to the fact that this group included six cystic lesions containing fluid, and three keratocystic odontogenic tumors for which a conclusive diagnosis was only achieved in one case, in which a sample of the cystic membrane was obtained.

Assessing the efficacy of CNB according to the radiographic appearance of a lesion is clinically relevant, and would serve in clinical practice as a basis to determine the fastest, simplest, and most suitable biopsy technique in each specific case.

CORRESPONDENCE
Dr. Federico STOLBIZER.
Servicio de Urgencias Facultad Odontología.
Universidad de Buenos Aires
M.T. de Alvear 2146. C1122AAH C.A.B.A. Argentina
e- mail: fstolbizer@hotmail.com

REFERENCES

1. Ackermann W. Application of the trephine for bone biopsy. Result in 635 cases. JAMA 1963; 184:11-17.
2. Turkel H, Bethell FH. Biopsy of bone marrow performed by a new and simple instrument. J Lab Clin Med 1943; 28: 1246-1251.
3. McFarland W, Dameshek W. Biopsy of bone marrow with the Vin- Silverman needle. JAMA 1958; 166:1464-1466.
4. Silverman I. A new biopsy needle. Am J Surg 1938; 40:671-672.
5. Conrad ME, Crosby WH. Bone marrow biopsy: modification of the Vim- Silverman needle. J Lab Clin Med 1961; 57: 642-654.
6. Ellis LD, Jensen WN, Westerman MP. Needle biopsy of bone marrow. An experience with 1445 biopsies. Arch Intern Med 1964; 114:213-221.
7. Miller GC, Dennis DT. Bone and marrow biopsy with saw-toothed modification of Vin- Silverman needle. Lancet 1968; 14:1278-1279.
8. Ackermann W. Vertebral trephine biopsy. Ann Surg 1956; 143:373.
9. Jamshidi K, Swaim WR. Bone marrow biopsy with unaltered architecture: a new biopsy device. J Lab Clin Med 1971; 77: 335-342.
10. Inwood MJ. Jamshidi bone marrow needle modification. J Lab Clin Med 1975; 86:535-537.
11. Islam A. A new bone marrow biopsy needle with core securing device. J Clin Pathol 1981; 35:359-364.
12. Schajowicz F, Derqui JC. Puncture biopsy in lesions of the locomotor system. Review of results in 4050 cases, including 941 vertebral punctures. Cancer 1968; 21: 531-548.
13. Ayala AG, Ro JY, Fanning CV, Flores JP, Yasko A. Core needle biopsy and fine needle aspiration in the diagnosis of bone and soft tissue lesions. Hematol Oncol Clin North Am 1995; 9:633-651.
14. Ayala AG, Zornosa J. Primary bone tumors: percutaneous needle biopsy. Radiologic-Pathologic study of 222 biopsies. Radiology 1983; 149:675-679.
15. Bishop PW, Mc Nally K, Harris M. Audit of bone marrow trephines. J Clin Pathol 1992; 45:1105-1108.
16. Mink J. Percutaneous bone biopsy in the patient with known or suspected osseous metastases. Radiology 1986; 161:191-194.
17. Mitsuyoshi G, Naito N, Kawai A, Kunisada T, Yoshida A, Yanai H, Dendo S, Yoshino T, Kanazawa S, Ozaki T. Accurate diagnosis of musculoskeletal lesions by core needle biopsy. J Surg Oncol 2006; 94:21-27.
18. Altuntas AO, Slavin J, Smith PJ, Schlict SM, Powell GJ, Ngan S, Toner G, Choong PF. Accuracy of computed tomography guided core needle biopsy of musculoskeletal tumours. ANZ J Surg 2005; 75:187-191.

19. Santini-Araujo E, Olvi LG, Muscolo DL, Velan O, González ML, Cabrini RL. Technical Aspects of Core Needle Biopsy and Fine Needle Aspiration in the Diagnosis of Bone Lesions. Acta Cytologica 2011; 55: 100-105.
20. Fernández LR, Carraro JJ. Punción aspirativa en los maxilares. Rev Asoc Odontol Argent 1972; 60:375-377.
21. Platt JC, Rodgers SF, Davidson D, Nelson CL. Fine needle aspiration biopsy in oral and maxillofacial surgery. Oral Surg Oral Med Oral Pathol 1993; 75:152-55.
22. Friedlander AH. Use of a needle biopsy in oral and maxillofacial surgery. Oral Surg Oral Med Oral Pathol 1976; 41:411-415.
23. Najjar TA, Gaston GW. Biopsy technique for fibro-osseous and osteolytic lesions of the jaws. Oral Surg Oral Med Oral Pathol 1977; 44:177-182.
24. Yeh CH[1], Yeow KM, Chu SY, Pan KT, Hung CF, Hsueh S, Su IH. Imaging findings in mandibular primitive neuroectodermal tumour: a report of a rare case and review of the literature. Dentomaxillofac Radiol. 2011;40:451-6
25. Larheim TA, Westesson P, Hicks DG, Eriksson L, Brown DA. Osteonecrosis of the temporomandibular joint: correlation of magnetic resonance imaging and histology. J Oral Maxillofac Surg 1999; 57:888-898.
26. Kerawala, CJ. Endoscopically-guided core biopsy of the condylar head. Br J Oral Maxillofac Surg 2008; 46:306-307.

FAMILY IMPACT SCALE (FIS): CROSS-CULTURAL ADAPTATION AND PSYCHOMETRIC PROPERTIES FOR THE PERUVIAN SPANISH LANGUAGE

Jenny Abanto[1], Ursula Albites[2], Marcelo Bönecker[1], Saul M. Paiva[3], Jorge L. Castillo[4], Denisse Aguilar-Gálvez[2]

[1] Department of Pediatric Dentistry and Orthodontics, School of Dentistry, University of São Paulo, Brazil.

[2] Department of Pediatric Dentistry, Dental School, Universidad Científica del Sur, Peru.

[3] Department of Pediatric Dentistry and Orthodontics, School of Dentistry, Federal University of Minas Gerais, Brazil.

[4] Department of Pediatric Dentistry, Dental School, Universidad Peruana Cayetano Heredia, Peru.

ABSTRACT

The lack of a Family Impact Scale (FIS) in Spanish language limits its use as an indicator in Spanish-speaking countries and precludes comparisons with data from other cultural and ethnic groups. The purpose of this study was therefore to adapt the FIS cross-culturally to the Peruvian Spanish language and assess its reliability and validity. In order to translate and adapt the FIS cross-culturally, it was answered by 60 parents in two pilot tests, after which it was tested on 200 parents of children aged 11 to 14 years who were clinically examined for dental caries experience and malocclusions. Internal consistency was assessed by Cronbach's alpha coefficient while repeat administration of the FIS on the same 200 parents enabled the test-retest reliability to be assessed via intraclass correlation coefficient (ICC). Construct and discriminant validity were based on associations of the FIS with global ratings of oral health and clinical groups, respectively. Mean (standard deviation) FIS total score was 5.20 (5.86). Internal consistency was confirmed by Cronbach's alpha 0.84. Test-retest reliability revealed excellent reproducibility (ICC = 0.96). Construct validity was good, demonstrating statistically significant associations between total FIS score and global ratings of oral health ($p=0.007$) and overall wellbeing ($p=0.002$), as well as for the subscale scores ($p<0.05$) with exception of the financial burden subscale. The FIS was also able to discriminate between children with and without dental caries experience and malocclusions ($p<0.05$). Satisfactory psychometric results for the Peruvian Spanish FIS confirm it as a reliable, valid instrument for assessing the impact on the family caused by children's oral conditions.

Key words: *oral health; quality of life; family.*

ESCALA DE IMPACTO FAMILIAR (FIS): ADAPTACIÓN TRANSCULTURAL Y PROPIEDADES PSICOMÉTRICAS AL IDIOMA ESPAÑOL DE PERÚ

RESUMEN

La falta de la Escala de Impacto Familiar (FIS) en el idioma español limita su uso como indicador en países hispanohablantes, así como impide las comparaciones con diferentes grupos culturales y étnicos. Por lo tanto, el objetivo de este estudio fue adaptar transculturalmente el FIS al idioma español de Perú y evaluar su validad y confiabilidad. Para traducir y adaptar transculturalmente el FIS, 60 padres respondieron al instrumento en dos pruebas piloto. Posteriormente, el FIS fue probado en 200 padres de niños de 11 a 14 años de edad, quienes fueron clínicamente evaluados para la experiencia de caries dental y maloclusiones. La consistencia interna fue evaluada por el coeficiente alfa de Cronbach, mientras que la re-aplicación del FIS en los mismos 200 padres permitió la evaluación de la confiabilidad test-retest por medio del coeficiente de correlación intraclase (CCI). La validez de constructo y discriminante se basaron en las asociaciones del FIS con las puntuaciones globales de salud bucal y grupos clínicos, respectivamente. La media (desviación estándar) de la puntuación total del FIS fue 5,20 (5,86). La consistencia interna fue confirmada por el alfa de Cronbach de 0,84. La confiabilidad test-retest reveló una excelente reproducibilidad (CCI=0,96). La validez de constructo fue buena, demostrando una asociación estadísticamente significativa entre la puntuación total del FIS y las puntuaciones globales de salud bucal ($p=0,007$), bienestar ($p=0,002$), así como para las puntuaciones de las sub-escalas ($p<0,05$), con excepción de la sub-escala de carga financiera. El FIS fue capaz de discriminar niños con y sin caries dental y maloclusiones ($p<0,05$). Los resultados psicométricos satisfactorios de la versión peruana del FIS confirman que es un instrumento válido y confiable para evaluar el impacto en la familia causado por las condiciones bucales de los niños.

Palabras clave: *salud bucal; calidad de vida; familia.*

INTRODUCTION

Oral diseases and disorders are common during childhood and have a negative impact on children's oral health-related quality of life (OHRQoL)[1,2]. The American Academy of Pediatrics defines child health as 'the social, physical and emotional functioning of the child and, when indicated, his or her family… therefore, measurement of health-related quality of life must be from the perspective of the child and the family'[3]. However, there are few instruments that evaluate the impact of a child's oral condition on the family's quality of life (QoL). The only instrument available for this purpose is the Family Impact Scale (FIS)[4].

To date, the validity and reliability of the FIS have been demonstrated in English-speaking parents in Canada[5], United Kingdom[6], China[7] and Brazil[8]. Nevertheless, although it has been validated in some languages, to the best of our knowledge, it has not been cross-culturally adapted and validated in Spanish. The lack of the FIS in Spanish language limits its use in Spanish-speaking countries, such as Peru, and precludes comparisons with data from other cultural and ethnic groups[9]. In addition, the FIS has a potential role considering that a parent's reports of the child's oral health or OHRQoL may be influenced by the degree to which the parent is physically or psychological affected by the child's condition[5,10].

Therefore, the aim of the present study was to carry out the cross-cultural adaptation of the FIS to the Peruvian Spanish language and to test its reliability and validity.

MATERIAL AND METHODS

The study was approved by the Human Research Ethics Committee of the Científica del Sur University, Peru. The participants' legal guardians signed an informed consent form.

Description of the Family Impact Scale (FIS)

The FIS consists of 14 items divided into three subscales[5]: parental / family activity (PA), parental emotions (PE), family conflict (FC) and financial burden (FB). The items refer only to the frequency of events in the previous 3 months. The items have five Likert response options: 'never = 0', 'once or twice = 1', 'sometimes = 2', 'often = 3', and 'every day or almost every day = 4'. The number of 'don't know' responses was counted, but excluded from the total FIS score for each patient.

Total FIS scores and scores for individual subscales are calculated as a simple sum of the response codes. Since there are 14 items, the final score can range from 0 to 56, where a higher score denotes greater impact of a child's oral condition on family QoL.

Following the validation process of the original FIS, answers were obtained to two questions asking the parents for global ratings of their children's oral health and the extent to which their oral health affected their overall well-being[5]. These global ratings had a five-point response format. The responses were scored as follows: "excellent"=0, "very good"=1, "good"=2, "average"=3, "poor"=4 for oral health, and not at all=0, very little=1, somewhat=2, a lot=3 and very much=4 for general wellbeing.

Translation and Adaptation of the FIS

The original FIS was translated and adapted to Spanish for Peru following standard guidelines[11-13]. Based on these guidelines, two initial translations into Spanish were made independently by two native Spanish translators. Both translations were reviewed in a consensus meeting in Peru. The Review Panel for this meeting consisted of four postgraduate professors, fluent in both Spanish and English, who knew the objectives of the study and had experience in OHRQoL studies[13]. The Review Panel evaluated the translations and determined the conceptual and item equivalence in order to retain content similarity in the different cultures. A consensus-translated version of the FIS was developed as a result of this process and then pilot-tested on a convenience sample of 40 parents of children aged 11–14 years old. Modifications were made according to parents' suggestions, in order to clarify the content of the instrument. The panel developed a first Peruvian version of the FIS, which was translated back into English by two native English-speaking translators. The back-translated English consensus version was compared to the original English version to determine semantic equivalence.

Finally, the draft of the first version of the FIS was pilot-tested for a second time on a different convenience sample of twenty parents of 11-to-14-year-old children. There was no change in terms of new suggestions or difficulties in comprehension, and the Review Panel wrote the final Peruvian version of the FIS.

The Peruvian version of the FIS during pilot tests and assessment of psychometric properties was administered in face-to-face independent interviews. Structures, instructions, mode of administration and measurement methods of the instrument were similar to the original English version of the FIS[5].

Assessment of validity and reliability

Validity is usually assessed on a sample size of 50 to 200 people in a cross-sectional design, while test-retest reliability is assessed on a sub-sample (of about 10% or 30 people)[11-13]. In line with this requirement, the Peruvian version of the FIS was administered in face-to-face independent interviews with 200 parents of 11- to 14-year-olds from four schools – two public schools in a deprived area and two private schools in a wealthy area. All schools were located in the city of Lima, capital of Peru. Children were randomly selected from official school registries. All parents were invited to participate in the study according to the following inclusion criteria: parents who have children with no systemic and / or neurological diseases, with children who could be examined intra-orally and who had not received dental treatment during the study.

Interviews were carried out before the clinical oral examinations by three trained interviewers who were blind to the oral screening examination findings. The interviewers were trained in the administration and intonation of each item of the Peruvian FIS. They were also clearly instructed to avoid suggesting responses or showing the answer options while reading them.

The children's oral examinations looked at dental caries and malocclusions and were carried out by a single specialist in pediatric dentistry who was previously trained and calibrated (Kappa intra-agreement = 0.92 and 0.89 for dental caries and malocclusions, respectively). Dental caries experience was assessed as number of decayed, missed and filled teeth (DMFT)[14]. Then, children were divided into two clinical groups[15]: those with no dental caries experience (DMFT=0) vs. those with dental caries experience in one or more teeth (DMFT ^{3}1). Malocclusions were classified using the Dental Aesthetic Index (DAI)[16], and the children were divided into two clinical groups: children with malocclusions and without malocclusions.

Data analysis

The SPSS software program (version 17.0 SPSS Inc., Chicago, IL, USA) was used for data analysis. Descriptive analyses were performed initially to assess the prevalence of oral impacts and measures of central tendency (means and standard deviations) of total and individual domain scores of the Peruvian FIS.

Internal consistency of the FIS was assessed using Cronbach's alpha, inter-item and item-total correlation coefficients. The test-retest reliability was assessed by calculating the Intraclass Correlation Coefficient (ICC) for the FIS score using the data from the same 200 parents who were interviewed for a second time, 7–14 days after the first interview, by the same interviewers.

To test construct validity, correlations between the scores of each subscale, total scale and global ratings were analyzed using Spearman's correlation coefficient. Discriminant validity was tested by comparing the mean FIS scores between children with caries experience/malocclusions and those without. As the FIS scores were not normally distributed, the nonparametric Mann-Whitney test was used to evaluate the difference in mean scores between clinical groups. The level of significance was set at 0.05.

RESULTS

A total 243 parents were invited to participate in the validation study, of whom 43 were not included because they did not conform to the study criteria. All 200 eligible parents provided signed parental informed consent, resulting in a response rate of 82.3%.

Of the 200 parents interviewed, 85.0% were mothers and 15.0% fathers. The mean (standard deviation) age of children was 12.5 (1.12), of whom 95 (47.5%) attended public schools and 105 (52.5%) attended private schools. Of these, 54.0% were girls and 46.0% boys, and a total 108 (54.0%) and 148 (74.0%) had dental caries experience and presence of malocclusions, respectively.

All questionnaires were completed. The scores for the total scale in the study population ranged from 0 to 28, with a mean (standard deviation) of 5.20 (5.86). Overall, 64.5% of parents reported oral impact (total FIS scores >0). Of these, 121 parents (60.5%) reported experiencing impact on parental/ family activity; 110 (55.0%) reported impact on

parental emotions; 98 (49.0%) reported impact on family conflict and 46 (23.0%) reported financial burden impacts.

Reliability

Cronbach's alpha coefficient was 0.84 for the total scale and ranged from 0.23 for financial burden subscale to 0.77 for parental/family activity subscale (Table 1). Test-retest reliability was assessed using the ICC, which was 0.96 for the total scale ranging from 0.78 for financial burden subscale to 0.97 for parental/family activity subscale (Table 1).

Construct validity

The correlations between global ratings (oral health and overall well-being) and the full scale (r= 0.190 and r= 0.214), parental/family activity subscale (r= 0.195 and r= 0.241) and family conflict subscale (r= 0.158 and r= 0.140) were not strong but statistically significant (Table 2). The financial burden subscale was not significantly associated to global ratings.

Discriminant validity

There was a significant difference in total scale and subscales scores of the FIS between children without dental caries experience and those with dental caries experience in one or more teeth (Table 3). This result was similar for malocclusion groups (Table 4).

DISCUSSION

This study adapted and validated the FIS cross-culturally for use among Peruvian parents. To the best of our knowledge, this is the first study that has adapted and evaluated the psychometric properties of this measure in a Latin American language such as Spanish after its original validation in English.

Table 1: Reliability statistics for total FIS scale and subscales (n = 200).

Variable	Number of items	Cronbach's alpha	Intraclass correlation coefficient (95% CI)*
Total scale	14	0.84	0.96 (0.90-0.98)
Subscales			
Parental/family activity	5	0.77	0.97 (0.94-0.94)
Parental emotions	4	0.70	0.96 (0.93-0.98)
Family conflict	4	0.68	0.96 (0.91-0.98)
Financial burden	1	0.23	0.78 (0.67-0.85)

* Two-way random effects model: $p < 0.001$ for all values

Table 2: Construct validity: rank correlations between total FIS scale and subscale scores, and global rating of oral health and overall wellbeing (n = 200).

	Global ratings			
	Oral health		Overall wellbeing	
	r*	p-value	r*	p-value
Total scale	0.190	0.007	0.214	0.002
Subscales				
Parental/family activity	0.195	0.006	0.241	0.001
Parental emotions	0.191	0.007	0.221	0.002
Family conflict	0.158	0.026	0.140	0.049
Financial burden	-0.064	0.369	0.047	0.511

*Spearman's rank correlation coefficient

Table 3: Discriminant validity: total FIS scale and subscales scores for children without and with caries experience.

	Without caries experience (n=92)		With caries experience (n=108)		p-value*
	Mean(SD)	Median	Mean(SD)	Median	
Total scale	1.49 (2.51)	0.00	8.36 (6.06)	8.00	<0.001
Subscales					
Parental/family activity	0.65 (1.15)	0.00	3.04 (2.39)	2.00	<0.001
Parental emotions	0.53 (1.03)	0.00	2.61 (1.96)	3.00	<0.001
Family conflict	0.23 (0.54)	0.00	2.19 (2.13)	2.00	<0.001
Financial burden	0.08 (0.30)	0.00	0.52 (0.77)	0.00	<0.001

*Mann-Withney test

Table 4: Discriminant validity: total FIS scale and subscale scores for children without malocclusion and with malocclusion.

	Without malocclusion (n=52)		With malocclusion (n=148)		p-value*
	Mean(SD)	Median	Mean(SD)	Median	
Total scale	2.96 (4.40)	0.00	5.99 (6.11)	4.00	<0.0001
Subscales					
Parental/family activity	1.31 (2.01)	0.00	2.16 (2.31)	1.50	0.007
Parental emotions	0.98 (1.54)	0.00	1.89 (1.97)	2.00	0.002
Family conflict	0.56 (1.06)	0.00	1.55 (2.04)	1.00	<0.0001
Financial burden	0.12 (0.58)	0.00	0.39 (0.65)	0.00	0.001

*Mann-Withney test

When a QoL instrument is translated for use in a context and country different from those it was created in, the cross-cultural adaptation should be evaluated, considering that it will be influenced by the wider social context including family environment, friends, schools and cultural customs in different countries[17]. By adapting the QoL instrument, its validity and reliability will be similar to those in the original version[18]. Our study meticulously applies standard guidelines for the translation and cross-cultural adaptation of QoL measures[11-13] and conducts pilot tests to identify any potential problems in its content, such as misunderstandings of the intended meaning of the items, clarity and cultural relevance. The results showed semantic equivalence between the English and Peruvian Spanish language versions of the FIS. The Peruvian version of the FIS for the Spanish language also exhibited good psychometric properties and provided acceptable support for its validity and reliability. Test-retest reliability was confirmed by the ICC, which showed excellent correlations between the first and second total FIS scale and subscales scores. Cronbach's alpha coefficient for the full scale and subscales indicates good internal consistency, as values of 0.5 or above are considered acceptable[19]. Similar results were obtained in the Canadian, Chinese and Brazilian validation studies[5,7,8].

Concerning construct validity, our findings on the associations of the full FIS scale and subscale scores with global ratings on oral health and overall well-being proved the validity of the measure, except between the financial burden (FB) subscale and global ratings. In agreement with our study, the Brazilian FIS version also found that the FB subscale did not correlate with global ratings[8]. In contrast, in the Chinese version, the FB subscale was only associated with the overall wellbeing rating[7]. In Canada, the FB subscale was associated

with both global indicators[5]. This may be because the FB subscale comprises a single item and addresses economic rather than psychosocial or behavioral impact[5,7]. It has therefore been recommended that the full scale score be used as a primary outcome, as there is stronger support for its validity than for the subscales alone[7,8].

The ability of the Peruvian FIS to discriminate significantly between different clinical groups according to caries experience and presence of malocclusions was also demonstrated. Similar results were found in the Chinese validation[7]. However, the Canadian and Brazilian FIS versions did not find significant differences between clinical groups in total scores[5,8]. Considering the mean values found for both clinical groups in our study, children with dental caries experience achieved higher scores on the total scale and subscales than the malocclusion group, indicating that dental caries could have higher negative impact than malocclusion at this sample age. This may be because dental caries commonly causes toothache and discomfort, demanding more parental attention and concern than malocclusions, which do not usually produce oral symptoms. Parents may thus choose to treat malocclusions or not, sometimes also resulting in higher treatment costs.

Since the FIS is a short instrument, it can be used in epidemiological surveys and also as an indicator for purposes such as political, research, public health and clinical actions[4].

CONCLUSION

The Peruvian Spanish version of the FIS proved to be valid and reliable for assessing the impact of a child's oral condition on the family's quality of life.

ACKNOWLEDGMENTS

This study was financially supported by Fundação de Amparo à Pesquisa do Estado de Minas Gerais – FAPEMIG.

CORRESPONDENCE

Dr. Jenny Abanto
Department of Pediatric Dentistry and Orthodontics
Faculty of Dentistry, University of São Paulo.
Av. Lineu Prestes, 2227, São Paulo
SP, 05508-000, Brazil.
jennyaa@usp.br

REFERENCES

1. Abanto J, Carvalho TS, Mendes FM, Wanderley MT, Bönecker M, Raggio DP. Impact of oral diseases and disorders on oral health-related quality of life of preschool children. Community Dent Oral Epidemiol 2011; 39: 105-114.
2. Abanto J, Tsakos G, Paiva SM, Carvalho TS, Raggio DP, Bönecker M. Impact of dental caries and trauma on quality of life among 5- to 6-year-old children: perceptions of parents and children. Community Dent Oral Epidemiol 2014; 42:385-394.
3. Fink R. Issues and problems in measuring children's health status in community health research. Soc Sci Med 1989; 29:715-719.
4. Abanto J, Paiva SM, Raggio DP, Celiberti P, Aldrigui JM, Bönecker M. The impact of dental caries and trauma in children on family quality of life. Community Dent Oral Epidemiol 2012; 40:323-331.
5. Locker D, Jokovic A, Stephens M, Kenny D, Tompson B, Guyatt G. Family impact of child oral and orofacial conditions. Community Dent Oral Epidemiol 2002; 30: 438-448.
6. Marshman Z, Rodd H, Stem M, Mitchell C, Robinson PG. Evaluation of the Parental Perceptions Questionnaire, a component of the COHQoL, for use in the UK. Community Dent Health 2007; 24:198-204.
7. McGrath C, Pang HN, Lo EC, King NM, Hägg U, Samman N. Translation and evaluation of a Chinese version of the Child Oral Health-related Quality of Life measure. Int J Paediatr Dent 2008; 18:267-274.
8. Goursand D, Paiva SM, Zarzar PM, Pordeus IA, Allison PJ. Family Impact Scale (FIS): psychometric properties of the Brazilian Portuguese language version. Eur J Paediatr Dent 2009; 10:141-146.
9. Abanto J, Albites U, Bönecker M, Martins-Paiva S, Castillo JL, Aguilar-Gálvez D. Cross-cultural adaptation and psychometric properties of the Child Perceptions Questionnaire 11-14 (CPQ11-14) for the Peruvian Spanish language. Med Oral Patol Oral Cir Bucal 2013; 18: e832-e838.
10. Rothman ML, Hendrick SC, Blucroft KA, Hickman DH, Rubenstein LZ. The validity of proxy generated scores as measures of patient health status. Med Care 1991; 29: 115-124.
11. Guillermin F, Bombardier C, Beaton D. Cross-cultural adaptation of health-related quality of life measures: literature review and proposed guidelines. J Clin Epidemiol 1993; 46:1417-1432.
12. Herdman M, Fox-Rushby J, Badia X. A model of equivalence in the cultural adaptation of HRQoL instruments: the universalist approach. Qual Life Res 1998; 7:323-335.

13. Van Widenfelt BMV, Treffers PDA, Beurs E, Siebelink BM, Koudijs E. Translation and cross-cultural adaptation of assessment instruments used in psychological research with children and families. Clin Child Fam Psychol Rev 2005; 8:135-147.
14. WHO. WHOQOL – measuring quality of life. The World Health Organization quality of life instruments. World Health Organization, Geneva. 1997.
15. Knutson JW. An index of the prevalence of dental caries in school children. Public Health Rep 1944; 59: 253-263.
16. Cons NC, Jenny J, Kohout FJ: DAI: the Dental Aesthetic Index. Iowa, USA: College of Dentistry, University of Iowa, 1986.
17. Cox MJ, Paley B. Families as systems. Annu Rev Psychol 1997; 48:243-267.
18. Albites U, Abanto J, Bönecker M, Paiva SM, Aguilar-Gálvez D, Castillo JL. Parental-caregiver perceptions of child oral health-related quality of life (P-CPQ): Psychometric properties for the Peruvian Spanish language. Med Oral Patol Oral Cir Bucal 2014; 19:e220-e224.
19. Cronbach LJ. Coefficient alpha and the internal structure of tests. Psychometrika 1951; 16:297-334.

LINEAR DIMENSIONAL STABILITY OF IRREVERSIBLE HYDROCOLLOID MATERIALS OVER TIME

Analía B. Garrofé, Beatriz A. Ferrari, Mariana Picca, Andrea E. Kaplan

Department of Dental Materials, School of Dentistry, University of Buenos Aires, Argentina.

ABSTRACT

The aim of this study was to evaluate the linear dimensional stability of different irreversible hydrocolloid materials over time. A metal mold was designed with custom trays made of thermoplastic sheets (Sabilex, sheets 0.125 mm thick). Perforations were made in order to improve retention of the material. Five impressions were taken with each of the following: Kromopan 100 (LASCOD) [AlKr], which has dimensional stability of 100 hours, and Phase Plus (ZHERMACK) [AlPh], which has dimensional stability of 48 hours. Standardized digital photographs were taken at different time intervals (0, 15, 30, 45, 60, 120 minutes; 12, 24 and 96 hours), using an "ad-hoc" device. The images were analyzed with software (UTHSCSA Image Tool) by measuring the distance between intersection of the lines previously made at the top of the mold. The results were analyzed by ANOVA for repeated measures. Initial and final values were (mean and standard deviation): AlKr: 16.44 (0.22) and 16.34 (0.11), AlPh: 16.40 (0.06) and 16.18 (0.06). Statistical evaluation showed significant effect of material and time factors. Under the conditions in this study, time significantly affects the linear dimensional stability of irreversible hydrocolloid materials.

Key words: *Dimensional measurement accuracy; alginates.*

DETERMINACIÓN DE LA ESTABILIDAD DIMENSIONAL LINEAL DE HIDROCOLOIDES IRREVERSIBLES

RESUMEN

El objetivo de este estudio fue evaluar la estabilidad dimensional lineal de diferentes hidrocoloides irreversibles en función del tiempo. Se confeccionó una matriz metálica con sus correspondientes cubetas individuales realizadas con láminas termoplásticas (Sabilex, de 0.125 mm de espesor). Se le realizaron perforaciones para la retención del material. Se tomaron cinco impresiones con cada material a esta matriz, utilizando Kromopan 100 (LASCOD) [AlKr], que presenta una estabilidad dimensional de 100 horas, y Phase Plus (ZHERMACK) [AlPh], que tiene una estabilidad dimensional de 48 horas. Luego se tomaron fotografías estandarizadas a diferentes intervalos de tiempo (0, 15, 30, 45, 60, 120 minutos; 12, 24 y 96 horas), usando un dispositivo ad-hoc. Las imágenes se analizaron con software de procesamiento de imágenes (UTHSCSA Image Tool) realizando la medición de la distancia entre las intersecciones de surcos previamente realizados en la porción superior de la matriz. Los resultados obtenidos fueron analizados mediante Análisis de Varianza para mediciones repetidas. Se detallan los datos iniciales y finales obtenidos (media y DS): AlKr: 16,44 (0,22) y 16,34 (0,11), AlPh: 16,40 (0,06) y 16,18 (0,06). El análisis estadístico ha mostrado el efecto significativo para las variables material y tiempo. Bajo las condiciones de este estudio podemos concluir que tiempo afectaría significativamente la estabilidad dimensional lineal de los hidrocoloides irreversibles.

Palabras clave: *exactitud de medida de dimensiones; alginatos.*

INTRODUCTION

Impressions of oral structures to be used for making casts are needed for various purposes, including diagnoses, rigid restorations and dental prostheses. They should be made from a material which is easy to manipulate and provides accurate replication, adequate dimensional stability and biological compatibility.

The choice of material for an impression is one of the most important steps in the process. Among the various materials available, alginate is undoubtedly one of the most frequently used, thanks to its elasticity, simple manipulation technique and low cost, among other properties.

Studies on the dimensional stability of alginate[1-5], have given special attention to syneresis and imbibition[3,6,7]. Syneresis is the loss of water by evaporation from the surface or exudation, which makes the gel to contract. Imbibition, in contrast, is the absorption of water during immersion. Both these processes alter the original dimensions of the gel and thereby its dimensional stability[6,7]. It is important to take syneresis and imbibition into account because any dimensional change that

occurs after removing the impression from the mouth will produce inaccurate pours and casts.

Manufacturers add fillers and small amounts of certain components in order to control consistency, setting time, elasticity, resistance and dimensional stability. Some alginates which have recently appeared on the market promise dimensional accuracy and stability for up to 5 days.

The literature describes different storage methods for alginate impressions during the time until the cast is made, including waiting times and standardized temperature and moisture conditions[2-5].

The aim of this study was to determine and compare linear dimensional stability of two irreversible hydrocolloids which are sold as materials with improved dimensional stability over time. We did not standardize the temperature and storage conditions of the impression until the cast was made, in order to replicate the conditions to which the materials are normally exposed during everyday use.

MATERIALS AND METHODS

Individual trays were made from 0.125 mm thick thermoplastic sheets (Sabilex) on a metal mold, following a standardized insertion path when the impression was taken (Figs.1 and 2). Perforations were made to improve retention of the material. The impressions were made with two irreversible hydrocolloid commercial brands: Kromopan 100 (Lascod, batch 161.301.119.201, expiry date 03/2014) [AlKr], for which the instructions specify that the maximum time to making the cast is 100 hours; and Phase Plus, (Zhermack batch 93131, expiry date 09/2014) [AlPh], for which the instructions specify that maximum time is 48 hours. We defined n=5 for both groups. In order to ensure precise quantities, the powders were weighed on a digital scale (Ohaus Analytical Standard) with a precision of 4 decimals, and the water was measured with a measuring cylinder. Both materials were prepared according to the manufacturers' instructions for mixing, working and gelling times. The impressions were washed for 30 seconds and stored in a hermetically sealed rigid container with cotton soaked in water apart from the samples to prevent any direct contact with the material.

A piece of metal 5 mm long was placed in each impression to calibrate the software used to measure the distances between marks. Photographs were taken of the impressions with a Sony® digital camera (model DSC-W1), using an *ad-hoc* device to standardize the impression-camera position (Figs. 3 y 4). Photographs were taken at the following time intervals: 0, 15, 30, 45, 60, 120 minutes; 12, 24 and 96 hours. For each picture, the impression was removed from the container, placed in the device and photographed within 45 seconds. The pictures were analyzed with image analysis software (UTHSCSA ImageTool Version 3.0) to measure the distance between the intersections of the grooves formed by the upper part of the mold (Fig.5). Data were analyzed statistically by ANOVA (Analysis of Variance for repeated measures for the time factor) using SPSS software version 9.0. Significance was established as $\alpha = 5\%$.

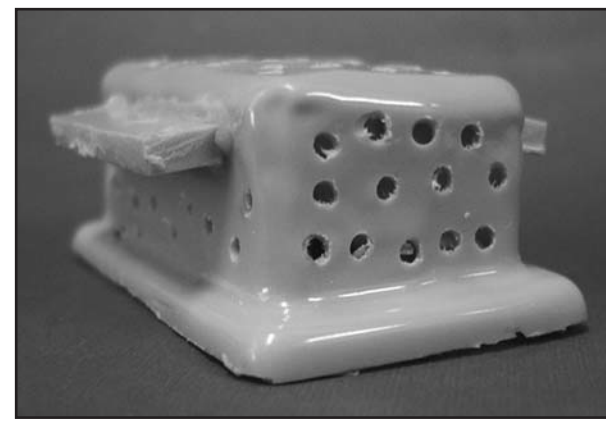


Fig. 1: Individual tray made of thermoplastic sheet.

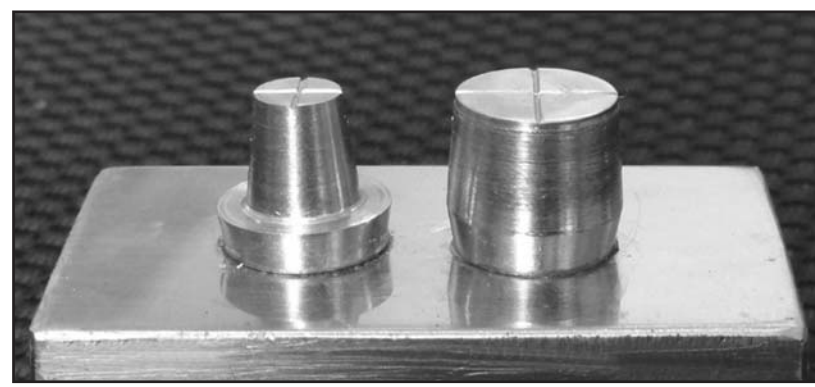


Fig. 2: Lateral view of the metal mold used as a model for taking impressions.

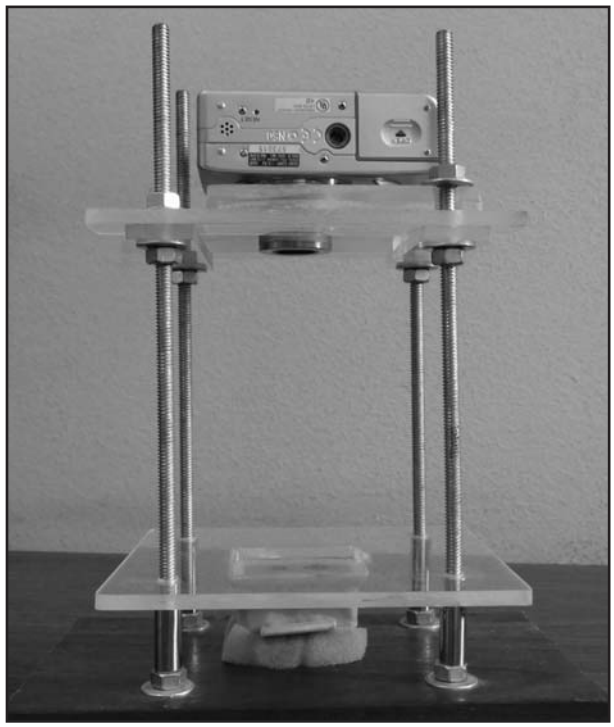


Fig. 3: Ad-hoc device for taking photographs.

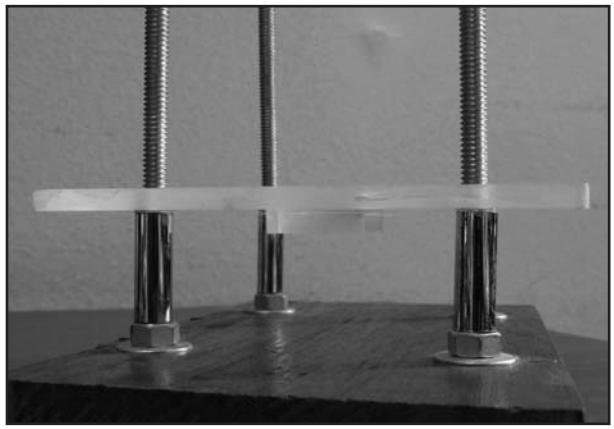


Fig. 4: Lateral view of the impression positioner in the ad-hoc device.

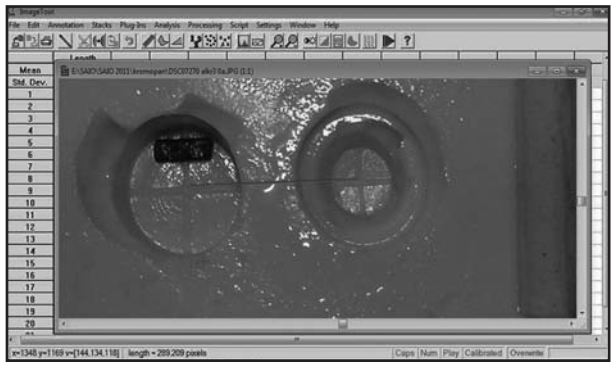


Fig. 5: Measuring with Image Tool image processing software.

RESULTS

Table 1 shows arithmetic means, standard deviations and percentage of dimensional variation for the different times compared to the first measurement taken (0 minutes). For Phase Plus, change over time was more constant (Fig. 6). Its greatest stability was observed at 15 minutes from time 0 and lowest at 96 hours. At 24 hours there was already a difference of 0.91% compared to the original measurement. However, the difference was never statistically significant ($p>0.05$).

For Kromopan 100, variation was not constant. It was greatest at 24 hours (0.04%) and lowest at 12 hours. At 96 hours dimensional change was 0.61% with respect to the original measurement. The difference was not statistically significant ($p>0.05$). Table 2 provides the result of the statistical analysis, showing that there was a significant effect for the time factor ($p=0.026$), and no significant effect for the brand variable ($p=0.403$) or for interaction between variables ($p=0.264$).

DISCUSSION

The literature we reviewed revealed two ways of evaluating dimensional stability of impression materials: on the impression material itself[2,3] or on the models made from an impression[1,4,5,8]. Our study recorded dimensional stability directly from the impression at different times: 0, 15, 30, 45, 60, 120 minutes; 12, 24 and 96 hours, in order to avoid any changes which might occur as a result of using other materials.

The papers by Chen[1], Bayindir[2] and Lapria Faria[8] all evaluated time intervals not longer than 24 hours, probably due to the possible dimensional changes in viscoelastic materials over long periods of time. Bayindir et al.[2] evaluated changes in

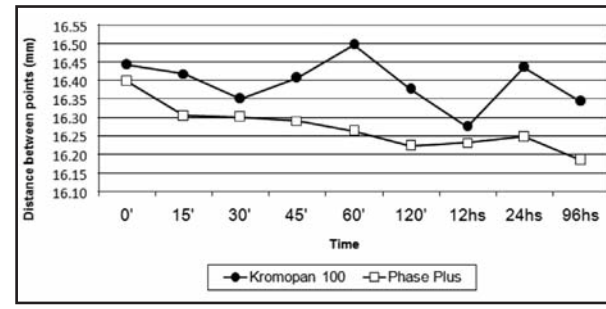


Fig. 6: Dimensional changes (in millimeters compared to baseline) recorded for each impression material (Kromopan and Phase Plus).

Table 1: Variation in size (mean and standard deviation) expressed as difference between initial and final value at each time evaluated.

MATERIAL		0 min	15 min	30 min	45 min	60 min	120 min	12 h	24 h	96 h
Kromopan 100	Mean	16.4415	16.4173	16.3488	16.4058	16.4960	16.3758	16.2733	16.4350	16.3420
	SD	0.2229	0.1468	0.0749	0.3441	0.2205	0.2353	0.2676	0.2839	0.1080
	dimensional change (%)	0%	-0.15%	-0.56%	-0.21%	+0.33%	-0.40%	-1.02%	-0.04%	-0.61%
Phase Plus	Mean	16.3972	16.3028	16.2994	16.2888	16.2620	16.2210	16.2300	16.2458	16.1834
	SD	0.0567	0.0259	0.0719	0.1269	0.1144	0.1646	0.1046	0.0810	0.0598
	dimensional change (%)	0%	-0.58%	-0.60%	-0.66%	-0.82%	-1.07%	-1.02%	-0.92%	-1.30%

Table 2: Analysis of variance. Test of Within-Subjects Contrasts.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Time	.063	1	.063	11.792	.026
material	.234	1	.234	.874	.403
Time* material	.009	1	.009	1.682	.264

temperature and pH, and variations in dimensional stability for 4 alginate brands by measuring directly under a microscope for a maximum 2 hours at 10-minute intervals, concluding that the linear dimensional changes in the four irreversible hydrocolloids differed significantly for the variable time and for the variable material.

In contrast, our study, in agreement with Walker[3], Imbery[4], and Sedda[5], evaluated dimensional accuracy over longer periods of time. Walker et al.[3] evaluated dimensional changes in three alginate brands at 30 minutes, 48 and 100 hours, showing that all materials contracted after being stored for 30 minutes, and that conventional alginate continued to contract over a longer time, and was thus most accurate at 30 minutes. In contrast, alginates with longer pouring times, Alginmax and Kromopan 100, underwent minimal dimensional changes at all storage times. Nevertheless, Kromopan 100 had the best accuracy at 100 hours. Imbery et al.[4] studied the effects of extended storage time on the dimensional accuracy of irreversible hydrocolloids (one conventional and another with extended pouring time). Their results indicate that when the manufacturer's instructions are followed, either by pouring immediately or at 5 days, both materials produced plaster casts with no significant difference compared to the master model. The material with extended pouring time was less accurate when poured immediately and at 24 hours, whereas conventional alginate was less accurate when poured at 3 and 4 days. They conclude that extended pour alginate tends to produce smaller models compared to the mold used; and that this type of alginate did not change dimensionally by more than 0.5% at any of the times at which measurements were taken. Sedda[5] et al. studied the dimensional stability of five alginate brands, over different times, on models obtained from impressions stored at constant temperature and moisture. Their results showed that the dimensional stability of the alginate impressions were material- and time-dependent.

Another variable considered in our study, which is not often found in the literature, was exposing impressions to different temperature ranges. Although this variable was not fully controlled, all impressions were taken and evaluated at the same time, so they all underwent the same conditions. These temperature changes also contribute to dimensional change, and it should be considered, for example, that a decrease in temperature produces slight contraction[6]. Our study did not control for the temperature at which the impressions were stored, because we intended to replicate usual manipulation

at a dental office or laboratory. It is important to take this into account when attempting to replicate environmental conditions to which they are usually exposed while being moved to the laboratory, in order to create protocols for alginate impression manipulation and ensure that more accurate plaster casts are obtained.

Nevertheless, the lack of standardization in the techniques for evaluating hydrocolloid dimensional stability and the variability in the results found in this and other studies[9] do not enable manipulation protocols to be established. Thus, according to the type of cast that needs to be made, it is important to determine the waiting time between taking the impression and making the cast. When a cast is needed for diagnosis, small dimensional changes may not have great impact on its clinical application. However, when more accurate casts – working casts – are needed, and the storage time and/or transportation conditions and temperatures that the impression will undergo are unknown, it is advisable to pour it immediately.

CONCLUSION

Under the conditions in this study, we conclude that time may significantly affect the linear dimensional stability of alginates sold as materials with greater dimensional stability.

ACKNOWLEDGMENTS

This study was supported by University of Buenos Aires, UBA-CyT Program. (number20020090100178).

CORRESPONDENCE

Dr. Analía Garrofé
Catedra de Materiales Dentales,
Facultad de Odontología, UBA.
M.T. de Alvear 2142
(1122) CABA, Argentina
agarrofe@odon.uba.ar

REFERENCES

1. Chen SY, Liang WM, Chen FN. Factors affecting the accuracy of elastomeric impression materials. J Dent 2004; 32:603-609.
2. Bayindir F, Yanikoglu N, Duymus Z. Thermal and pH changes, and dimensional stability in irreversible hydrocolloid impression material during setting. Dent Mat J 2002; 21:200-209.
3. Walker M, Burckhard J, Mitos D, Williams K. Dimensional change over time of extended-storage alginate impression materials. Angle Orthod. 2010; 80:1110-1115.
4. Imbery T, Nehring J, Janus C, Moon P. Accuracy and dimensional stability of extended-pour and conventional alginate impression materials. Jour Amer Dent Assoc 2010; 141:32-39.
5. Sedda M, Casarotto A, Raustia A, Borracchini A. Effect of Storage Time on the Accuracy of Casts Made from Different Irreversible Hydrocolloids. J Contemp Dent Pract 2008 4:059-066.
6. Anusavice K. Phillips Ciencia de los Materiales Dentales. Parte II, Capítulo 9: Materiales de Impresión (Chiayi Shen). 11º Edición. Editorial Elsevier. 2004.
7. Macchi R. Materiales Dentales. Sección III, Capítulo 19: Hidrocoloides para impresión. 4º Edición. Buenos Aires. Editorial Panamericana. 2007.
8. Lapria Faria AC, Silveira Rodriguez RC, Macedo AP, Chiarello M, Faria Ribeiro R. Accuracy of stone casts obtained by different impression materials. Braz Oral Res 2008; 22:293-298.
9. Nassar U, Aziz T, Flores-Mir C. Dimensional stability of irreversible hydrocolloid impression materials as a function of pouring time: a systematic review. J Prosthet Dent. 2011 106:126-33.

QUANTITATIVE SENSORY TESTING OF THE EFFECT OF DESENSITIZING TREATMENT AFTER DENTAL BLEACHING

Vanessa Rahal[1], Marjorie O. Gallinari[1], Jorge Perdigão[2], Luciano T. A. Cintra[1], Paulo H. dos Santos[3], André L. F. Briso[1]

[1] Department of Restorative Dentistry, Araçatuba Dental School, UNESP, Univ. Estadual Paulista, Araçatuba, São Paulo, Brazil.

[2] Department of Restorative Sciences, University of Minnesota, School of Dentistry, Minneapolis, Minnesota, United States of America.

[3] Department of Dental Materials and Prosthodontics, Araçatuba Dental School, UNESP, Univ. Estadual Paulista, Araçatuba, São Paulo, Brazil.

ABSTRACT

The aim of this study was to quantify tooth sensitivity during bleaching and after a desensitizing treatment. Sensitivity was measured with a new device, TSA-II, which uses thermal stimuli for Quantitative Sensory Testing (QST). Ten patients underwent bleaching treatment using Whiteness HP Maxx (FGM Produtos Odontológicos Ltda) containing 35% hydrogen peroxide. After the bleaching session, the teeth were cleaned with air/water spray and the product Desensibilize KF 2% (FGM Produtos Odontológicos Ltda) was applied to the upper left teeth. Saline solution at room temperature was applied in the upper right teeth. QST was performed before bleaching, immediately after bleaching, and immediately after desensitizing treatment. In order to standardize tooth analysis, a 100% ethylene copolymer and vinyl acetate tray with circular perforations was used during measurements. Analysis of variance and the Student's t-test were used (a=0.05). Mean temperatures (SD) of cold sensation threshold for the upper right quadrant were: BB-13.898 (4.81), AB-19.241 (3.68), AD-20.646 (3.72) and for the upper left quadrant they were: BB-14.102 (3.22), AB-19.646 (4.82), AD-13.835 (3.63). Dental bleaching with highly concentrated peroxides changed dental cold sensation thresholds, but the topical desensitizer changed the immediate cold sensation thresholds produced by the cold stimulus.

Key words: *Clinical Trial; Hydrogen Peroxide; Tooth Bleaching; Dentine Sensitivity.*

TESTE SENSORIAL QUANTITATIVO DO EFEITO DE UM TRATAMENTO DESSENSIBILIZANTE APÓS O CLAREAMENTO DENTAL

RESUMO

O objetivo do presente estudo foi quantificar a sensibilidade dentária durante o tratamento clareador e após a aplicação de um dessensibilizante utilizando um equipamento de análise neurosensorial, o TSA II, que utiliza estímulos térmicos para a realização do Teste Sensorial Quantitativo (QST). Assim, dez pacientes (n=10) receberam o tratamento clareador com Whiteness HP Maxx (FGM Produtos Odontológicos Ltda.) contendo peróxido de hidrogênio a 35%. Após a sessão clareadora, os dentes foram limpos com jatos de ar/água e foi realizada a aplicação do Desensibilize KF 2% (FGM Produtos Odontológicos Ltda.), um dessensibilizante tópico a base de nitrato de potássio a 5% e fluoreto de sódio a 2%, apenas no hemi-arco esquerdo da maxila, utilizando o método da boca dividida. No hemi-arco direito foi aplicada uma solução salina a temperatura ambiente (controle). O QST foi realizado antes do clareamento (AC), imediatamente depois do clareamento (DC) e imediatamente após a aplicação do dessensibilizante (DD). Para padronizar o local do estímulo, uma moldeira de acetato com perfurações circulares foi utilizada durante as mensurações. A análise estatística foi realizada por meio do teste t de Student (α=0,05). As temperaturas médias (DP) do limiar de sensação ao frio para o hemi-arco direito foram: AC-13,898 (4,81), DC-19,241 (3,68), DD-20,646 (3,72) e para o hemi-arco esquerdo foram: AC-14,102 (3,22), DC-19,646 (4,82), DD-13,835 (3,63). Clareamento dental com peróxidos de alta concentração exacerbaram a sensibilidade dental ao estímulo térmico e o uso do dessensibilizante foi efetivo para reverter esta situação.

Palavras-chave: *Ensaio Clínico; Peróxido de Hidrogênio; Clareamento Dental; Sensibilidade Dentária.*

INTRODUCTION

The in-office dental bleaching technique with highly concentrated hydrogen peroxide has been widely used because it provides quick results without the use of trays.[1,2] Dental professionals usually apply products containing 30% to 38% hydrogen peroxide while protecting soft tissues against deleterious effects.[3] Despite the effectiveness of dental bleaching, current studies reveal that patients who undergo it

report different frequency and intensity of dental sensitivity,[3] a negative treatment outcome that reduces patient satisfaction.

The current literature does not explain the origin of dental sensitivity. Knowing the cause would be crucial in establishing an effective bleaching technique that would cause minimum patient discomfort. Desensitizers have been applied to reduce discomfort with no apparent influence on bleaching efficacy.[4,5]

Clinical studies have evaluated the frequency and intensity of dental sensitivity and presented subjective data based on personal reports and pain questionnaires according to the Visual Analogue Scale (VAS).[6] However, misunderstanding of data may have affected the reliability and reproducibility of results, leading to the search for a more reliable quantification method of patient discomfort concomitant with the dental bleaching process.

Devices such as the TSA II (Medoc Advanced Medical Systems Ltd., Ramat Yishai, Israel) enable the quantification of the neurosensory response related to major and minor nerve fibers[7] through Quantitative Sensory Testing (QST) using thermal or mechanical stimuli.[8-11] Because QST is a fast and useful method resulting from accurate standardized analyses,[9,11] it is used in several fields, including anesthesiology,[10] acupuncture,[11] endocrinology,[12] neurology,[13] and dermatology.[14] In dentistry, it was initially employed to quantify maxillofacial surgery and orofacial pain[8,9,15] for evaluation of neurological responses after third molar exodontia and orthognathic surgery. However, to date, research has not evaluated dental sensitivity using QST, a method that might improve the accuracy of these studies.

The aim of this study was to use neurosensory analysis to quantify dental thermal sensitivity before and after dental bleaching and to compare patient cold sensations with and without desensitization methods. More accurate knowledge of patient symptomology in the dental bleaching process may help the development of safe and comfortable bleaching therapies.

As a null hypothesis, it was assumed that dental bleaching with highly concentrated peroxides would not influence cold sensation thresholds and that the use of topical desensitizer containing sodium fluoride and potassium nitrate would not change the immediate cold sensation thresholds produced by a specific cold stimulus.

Table 1: Inclusion and exclusion criteria for selection of patients.

Inclusion Criteria
Dental bleaching using in-office technique indicated
Healthy and vital upper teeth
No decayed teeth
No visible enamel defects
No orthodontic brackets
Overall good systemic health
Healthy oral soft tissue
Non-smoking
Exclusion Criteria
Direct and indirect restorations in the upper anterior region
Adverse reaction to peroxide
Use of opioids or medications influencing neurosensory response
Use of pacemaker
Presence of dental staining (tetracycline, trauma, fluorosis, and unknown etiology)
Neurological diseases
Chronic or acute diseases
Dentine exposure

Table 2: Treatment according to the split-mouth design method.

	Treatment	Posology
Right maxillary quadrant (n=10)	35% hydrogen peroxide*	3 15-minute applications every 7 days
	Saline	1 10-minute application immediately after each bleaching session
Left maxillary quadrant (n=10)	35% hydrogen peroxide*	3 15-minute applications every 7 days
	5% potassium nitrate + 2% sodium fluoride*	1 10-minute application immediately after each bleaching session

* FGM – Dental products

MATERIALS AND METHODS

After approval by the Committee of Ethics in Research (00278/2011), 10 patients aged 18 to 25 years were selected according to the criteria shown in Table 1. The maxillary arch was divided into right and left quadrants, which would undergo different desensitizing treatments (Table 2). When two interventions are applied in the same patient, in a split-mouth approach, there are paired observations and the required sample size is smaller because of the reduced variability. The split-mouth

design resembles the crossover design, more often encountered in clinical trials, like this study[16].

Sample size was calculated considering the type-I error rate (α) - the probability of finding a difference when a difference does not exist. Most medical literature uses an alpha cut-off of 5% (0.05), indicating a 5% chance that a significant difference is actually due to chance and is not a true difference; type-II error rate (β) - the probability of not detecting a difference when one actually exists. Beta is directly related to study power (Power = 1 - β). A beta cut-off of 20% (0.2) is usually used, indicating a 20% chance that a significant difference is missed. It was also considered a 5% level of significance, which means that the chance of the finding being true is 95%, and power of the test 80%, which means that, if indeed there is any difference, the probability of detecting it will be 80%.[16,17]

After the patients provided informed consent, they underwent careful evaluation, anamnesis and appropriate clinical and radiographic exams before allocation (Fig. 1).

This study is a factorial, blinded clinical trial with equal randomization, and includes the factors: (1) desensitizing treatment at 2 levels (5% potassium nitrate associated with 2% sodium fluoride or saline) and (2) 3 periods of evaluation: before bleaching (BB), immediately after bleaching (AB), and immediately after desensitizing (AD). Dental sensitivity to thermal stimuli was evaluated, and the split-mouth design method was used.

Quantitative Sensory Testing (QST)

Quantitative Sensory Testing (QST) for cold sensation thresholds was conducted during the first bleaching session from upper right canine to upper left canine. The evaluation was performed in the following sequence: upper right canine, upper left canine, upper right lateral incisor, upper left lateral incisor, upper right central incisor, and upper left

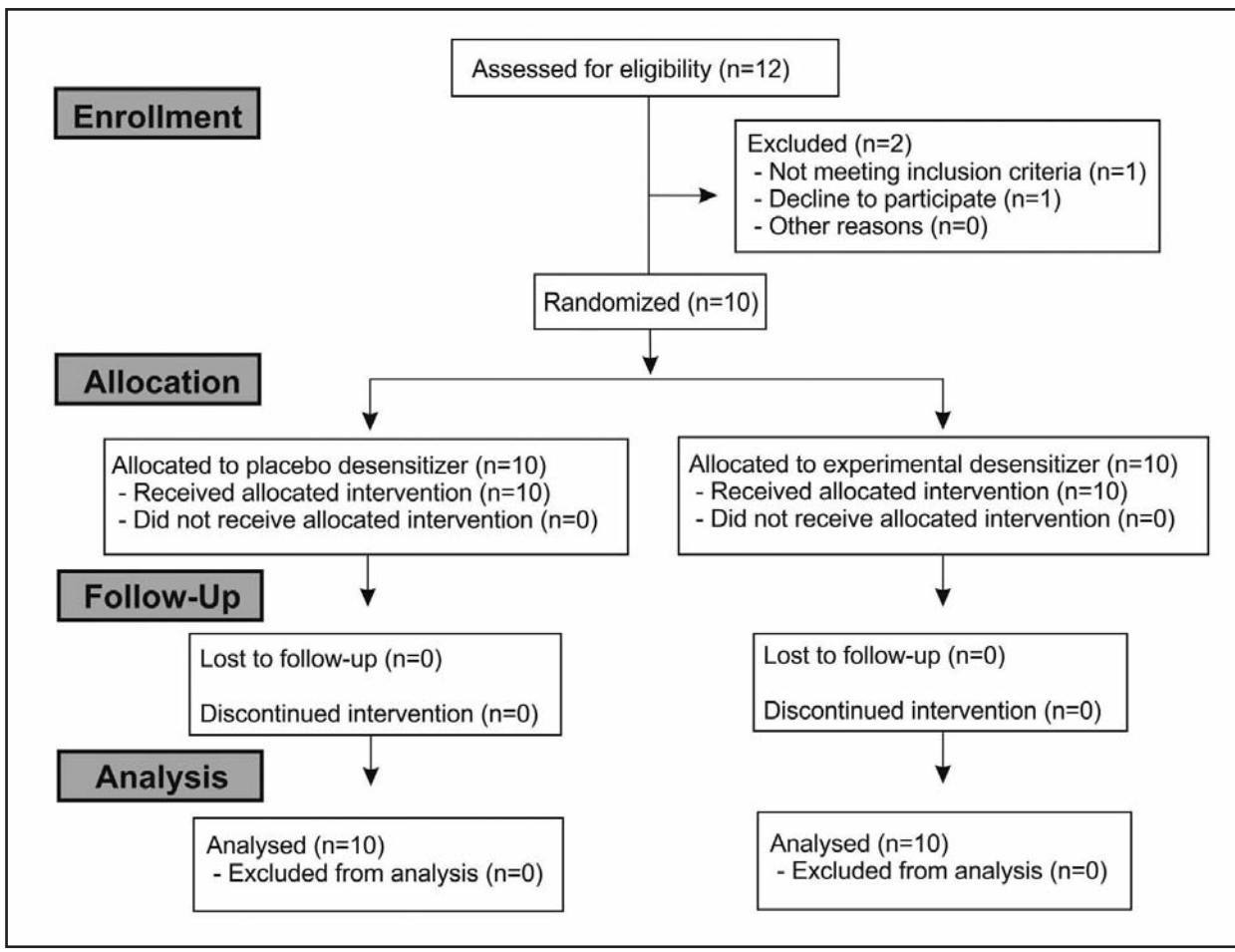


Fig. 1: Flow chart diagram showing enrollment, allocation, follow-up and analysis during the study (CONSORT Statement).

central incisor. Analyses were conducted before bleaching (BB), immediately after bleaching (AB), and immediately after application of either desensitizer or saline (AD).

For standardization of tooth analysis, a 100% ethylene copolymer and vinyl acetate tray was fabricated for each patient. The tray had circular perforations with a diameter similar to the active tip of the intraoral thermode. The perforations were created on the buccal surface and 2 mm below the cervical, incisal, and proximal margins of the upper teeth, except the canine position, which was perforated 3 mm below the distal margin (Fig. 2).

Before measurement, each tooth was covered with 0.05 ml of thermal conductive paste containing silver oxide (IPT - Pasta Térmica Implastec, Implastec Eletroquímica Ltd., Votorantim, São Paulo, Brazil) to optimize thermal conduction. The tests were performed at a dental clinic in a silent environment with a constant temperature of 26º C. The sessions were conducted from 8:00 a.m. to 10:00 a.m.

Fig. 2: Individual tray fabricated with circular perforations for Quantitative Sensory Testing.

The neurosensory analyzer TSA II (Medoc TSA II Neurosensory Analyzer, Ramat Yishai, Israel) was used to test cold sensation thresholds employing CST. TSA II consists of a central unit that generates thermal signals, controlled by software and connected to another device held by the patient. The patient is able to stop cooling of the intraoral thermode at any time (Fig. 3).

The TSA II was configured using the "Limits" function, and three descending temperature tests were performed on each tooth. The test began at 36º C, and the thermode cooling speed was 0.5º C per second, resulting in slow temperature variation that allowed even transference of the thermal stimulus to the dentine-pulp complex. After patient perception of temperature alteration, the patient paused the stimulus, and the measurement was repeated for two times, 30 seconds after the previous one.

The values obtained during the first test were discarded, and the mean of the following tests was used as the initial temperature for cold sensation threshold testing in each tooth. Subsequently, the cold sensation thresholds for all teeth from the same quadrant were grouped for comparison between treatments.

Bleaching Treatment

The in-office bleaching technique used 35% hydrogen peroxide (Whiteness HP Maxx, FGM Produtos Odontológicos – Ltda., Joinville, Santa Catarina, Brazil) (Table 3) and no physical activation. The product is commercially available as a two-bottle system (one bottle contains the peroxide and the other contains the activator, and the substances are mixed in a peroxide/activator ratio of 3:1 drops.

After tooth prophylaxis and soft tissue isolation using a light cured resin gingival barrier (Top Dam

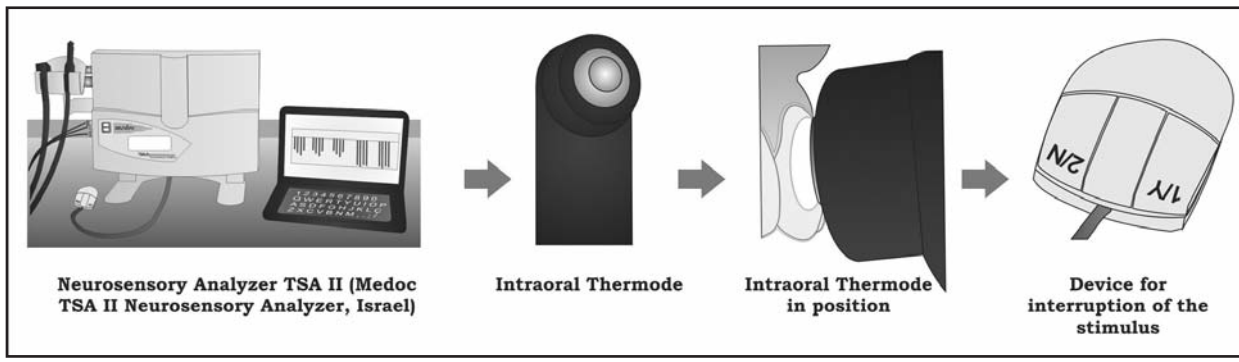


Fig. 3: Diagram of TSA II equipment used for neurosensory analysis.

– FGM Produtos Odontológicos Ltda., Joinville, Santa Catarina, Brazil), the bleaching agent was inserted into a graduated syringe, and 0.06 mL of the bleaching product was applied on the buccal surface of the teeth for 15 minutes. After the first application, the teeth were cleaned and dried with gauze, and the procedure was repeated twice, totalizing 45 minutes of contact between the bleaching product and enamel (Fig. 4).

Desensitizing Treatment

After the bleaching session and immediately after the second cold sensation measurement, the teeth were cleaned with an air/water spray and 5% potassium nitrate with 2% sodium fluoride (Desensibilize KF 2%, FGM Produtos Odontológicos Ltda., Joinville, Santa Catarina, Brazil) (Table 3) was applied to the teeth of the maxillary left quadrant, according to the manufacturer's instructions. In the right quadrant, saline at room temperature was applied as described above. After 10 minutes, the tooth surfaces were rinsed thoroughly (Fig. 5).

Statistical Analysis

The mean values obtained were grouped according to the period of evaluation: before bleaching (BB), after bleaching (AB), and after application of either desensitizer or saline (AD). The data for each quadrant was submitted to Student's t-test at a 5% level of significance using Pacotico 5.1 statistics software.

RESULTS

This study applied one treatment to the right quadrant (saline solution) and another to the left (desensitizing). Table 4 shows the mean temperature of cold sensation thresholds in each quadrant, the comparison between the values of each quadrant (uppercase letters) and the comparison between values for the same quadrant according to the evaluation period (lowercase letters).

In the periods BB and AB, there was no statistically significant difference between quadrants. However, in period AD, a significant difference (p =0.0000) was observed between the right and left quadrants.

Fig. 4: In-office bleaching procedure using 35% hydrogen peroxide.

Fig. 5: Desensitizing agent application.

Table 3: Composition of the products used in this study.

Product	Composition	LOT
Whiteness HP MAXX 35%*	30%–35% hydrogen peroxide, thickener, pigments, glycol, inorganic compound and de-ionized water	260111
Desensibilize KF 2%*	5% potassium nitrate, 2% sodium fluoride, de-ionized water, glycerin, neutralizer and thickener	281111
Saline	0.9% NaCl solution in distilled water	11099311

* FGM– Dental products.

Table 4: Mean temperatures and standard deviation of cold sensation threshold (°C) in right and left quadrants.

Periods	Right quadrant	Left quadrant
Before bleaching (BB)	13.898 (4.81) Ba	14.102 (3.22) Ba
After bleaching (AB)	19.241 (3.68)Aa	19.646 (4.82)Aa
After desensitizing (AD)	20.646 (3.72) Aa	13.835 (3.63) Bb

Means followed by different letters (uppercase in columns and lowercase in rows) represent a statistically significant difference according to Student's t-test, $p \leq 0.05$.

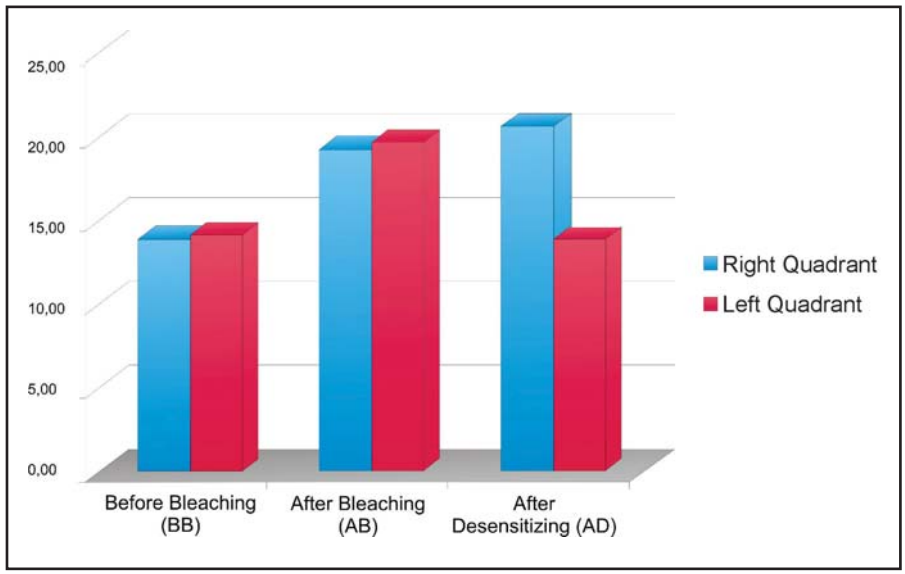


Fig. 6: Data of cold sensation threshold on the right and left quadrants during the periods of evaluation.

Dental bleaching increased the mean values of cold sensation thresholds, which means that the teeth became more sensitive. In the right quadrant, dental cold sensation after bleaching remained the same after use of saline solution. However, in the left quadrant, the values measured at AD were similar to the initial values obtained at BB ($p = 0.7645$). Overall, results revealed a reduction of dental sensitivity after desensitizing (Fig. 6).

DISCUSSION

Quantitative Sensory Testing (QST): Advantages and Limitations of the Method

Different results in dental sensitivity show that sensory function is not consistent and uniform, therefore, reliable and reproducible methods should be applied.[18] In this regard, the development of an accurate pain-testing methodology is helpful for the evaluation of dental sensitivity and for indicating proper treatments. The cold sensation threshold can be defined as the moment at which patients feel the cold temperature (in degrees Celsius) in contact with the dental surface. This detection can show their ability to feel discomfort during the bleaching treatment because it indicates whether or not the tooth was more sensitive. This is why we chose to determine the experimental and control group in the same person, who had similar initial mean values of cold sensation (baselines) in both quadrants.

The device provides a slow temperature decrease (from 36ºC to 0ºC) that allows the patient to detect the threshold accurately, and the data are parametric quantitative values. It is important to note that our findings were described considering a 5% level of significance to decide whether the difference is statistically significant.

Quantitative Sensory Testing (QST), widely used in skin and oral mucosa, was adapted here for testing cold sensation thresholds on tooth surfaces. Because QST requires patients' collaboration by stopping the thermal stimulus as soon as it is perceived, the test must be conducted in a silent environment with a constant temperature.[7,19] In addition, test reliability depends on the control group. In this study, the control consisted of use of saline as a desensitizer in the right quadrant. [8,20]

The TSA II should be carefully configured to avoid any influence of repetition on tests on the same tooth. An interval between tests was crucial to achieve 36ºC, a temperature comfortable for patients, and to avoid the unwanted addition of stimuli. Similarly, cooling speed was relevant in the transference of thermal stimuli to the dentine-pulp complex.

Bleaching and Desensitizing Treatments

High concentrations of hydrogen peroxide[21] and the pH of the bleaching products have been associated with histomorphological alterations in enamel, increase in enamel permeability, and penetration of byproducts generated by the breakdown of bleaching agents.[22,23] These phenomena are related to a high frequency of dental sensitivity, which is considered the most common side effect of dental bleaching.[24,25]

The physiology of dental sensitivity after bleaching remains unclear due to the functional and structural complexity of teeth, including nerves, odontoblastic features, and internal movement of fluids.[10,11,26-28] The hydrodynamic theory[29] is based on the movement of dentin fluid as the result of several stimuli that

excite the pulp nerve fibers and cause pain.[28,30] The excessive oxygen generated by bleaching products in the pulp also stimulates the release of inflammatory chemical mediators, such as substance P, which sensitize the pulp nociceptors and play a role in pain modulation,[31] as can be observed in the values of cold sensation thresholds near patients' comfortable temperature (36ºC).

The presence of surface alterations on teeth suggests that the topical action of some products may reverse those alterations or treat dental sensitivity. The positive response to desensitizing after dental bleaching is consistent with the results of studies by Tay et al., 2009[4] and Reis et al., 2011[31] which demonstrate the effectiveness of 5% potassium nitrate used with 2% sodium fluoride. Such evidence may result from the neurological action of potassium nitrate, which disturbs the depolarization of neurons and reduces the effectiveness of sensory nerve conduction.[4,31,32] Quadrants treated with this desensitizing agent exhibited lower temperatures of cold sensation thresholds than the quadrants treated with saline. In addition, the formation of calcium fluoride on flaws, diffusion channels, and gaps in the dentine-pulp complex represented a physical barrier with this desensitizer.[31] Thus, the null hypothesis was rejected because similar data were obtained in the periods BB and AD in the left maxillary quadrant treated with desensitizer.

An interesting complement to our study would be the inversion of the quadrant treatments, using saline solution on the left quadrant and the desensitizing agent on the right. However, the interpretation of the data would be impaired, since the mechanism of action of this topical agent takes place by means of neural blockade. Thus, in this second stage of the study there may already be inhibition of the neural response due to the previous treatment.

Considering the data found in this study and previous results related to desensitizing effectiveness,[4,31] the quantitative analysis of dental sensitivity using a neurosensory method is a promising approach for further clinical studies of this symptom caused by several dental treatments.

CONCLUSIONS

- Dental bleaching with highly concentrated peroxides increased dental cold sensation thresholds.
- Topical desensitizer containing sodium fluoride and potassium nitrate reversed the immediate cold sensation thresholds produced by the cold stimulus.

ACKNOWLEDGMENTS

We would like to thank FAPESP for financial support (2010/11627-6) and FGM Produtos Odontológicos Ltda. for providing dental products.

CORRESPONDENCE

Dr. André Luiz Fraga Briso
Departamento de Odontologia Restauradora,
Faculdade de Odontologia de Araçatuba (UNESP).
Rua José Bonifácio, 1193 - Bairro Vila Mendonça
Cep 16015-050 Araçatuba, São Paulo, Brasil
alfbriso@foa.unesp.br.

REFERENCES

1. Dos Santos Medeiros MC, de Lima KC. Effectiveness of nightguard vital bleaching with 10% carbamide peroxide – a clinical study. J Can Dent Assoc 2008; 74:163-163e.
2. Da Silva Marques DN, Silveira JM, Marques JR, Amaral JA, Guilherme NM, da Mata AD. Kinetic release of hydrogen peroxide from different whitening products. Eur J Esthet Dent 2012; 7:344-352.
3. Delfino CS, Chinelatti MA, Carrasco-Guerisoli LD, Batista AR, Fröner IC, Palma-Dibb RG. Effectiveness of home bleaching agents in discolored teeth and influence on enamel microhardness. J Appl Oral Sci 2009; 17: 284-288.
4. Tay LY, Kose C, Loquercio AD, Reis A. Assessing the effect of a desensitizing agent used before in-office tooth bleaching. J Am Dental Assoc 2009; 140:1245-1251.
5. Sacerdote P, Levrini L. Peripheral mechanisms of dental pain: the role of substance P. Mediators of Inflamm 2012; 2012;2012:951920
6. Browning WD, Blalock JS, Frazier KB, Downey MC, Myers ML. Duration and timing of sensitivity related to bleaching. J Esthet Restor Dent 2007; 19:256-264.
7. Shukla G, Bhatia M, Behari M. Quantitative thermal sensory testing - value of testing for both cold and warm sensation detection in evaluation of small fiber neuropathy. Clin Neurol Neurosurg 2005; 107:486-490.
8. Juhl GI, Svensson P, Norholt SE, Jensen TS. Long-lasting mechanical sensitization following third molar surgery. J Orofac Pain 2006; 20:59-73.
9. Kalantzis A, Robinson PP, Loescher AR. Effects of capsaicin and menthol on oral thermal sensory thresholds. Arch Oral Biol. 2007; 52:149-153.

10. Pavlakovic G, Klinke I, Pavlakovic H, Züchner K, Zapf A, Bachmann CG, Graf BM, Crozier TA. Effect of thermode application pressure on thermal threshold detection. Muscle Nerve 2008; 38:1498-1505.
11. Lang PM, Stoer J, Schober GM, Audette JF, Irnich D. Bilateral acupuncture analgesia observed by quantitative sensory testing in healthy volunteers. Anesth Analg 2010; 110:1448-1456.
12. Olaleye D, Perkins BA, Bril V. Evaluation of three screening tests and a risk assessment model for diagnosing peripheral neuropathy in the diabetes clinic. Diabetes Res Clin Pract 2001; 54:115-128.
13. Cruccu G, Sommer C, Anand P, Attal N, Baron R, Garcia-Larrea L, Haanpaa M, Jensen TS et al. EFNS guidelines on neuropathic pain assessment: revised 2009. Eur J Neurol 2010; 17:1010-1018.
14. Pfab F, Valet M, Sprenger T, Toelle TR, Athanasiadis GI, Behrendt H, Ring J, Darsow U. Short-term alternating temperature enhances histamine-induced itch: a biphasic stimulus model. J Invest Dermatol 2006; 126:2673-2678.
15. Baad-Hansen L, Arima T, Arendt-Nielsen B, Neumann-Jensen B, Svensson P. Quantitative sensory tests before and 1 (1⁄2) years after orthognathic surgery: a cross-sectional study. J Oral Rehabil 2010; 37:313-321.
16. Pandis N. Sample calculation for split-mouth designs. Am J Orthod Dentofacial Orthop 2012; 141:818-819.
17. Rosner B: Fundamentals of Biostatistics. Boston, Massachusetts, USA: Brooks/Cole, 2011.
18. Dyck PJ, Zimmerman I, Gillen DA, Johnson D, Karnes JL, O'Brien PC. Cool, warm, and heat-pain detection thresholds: testing methods and inferences about anatomic distribution of receptors. Neurology 1993; 43:1500-1508.
19. Chong PS, Cros DP. Technology literature review: quantitative sensory testing. Muscle Nerve 2004; 29: 734-747.
20. Juhl GI, Jensen TS, Norholt SE, Svensson P. Central sensitization phenomena after third molar surgery: a quantitative sensory testing study. Eur J Pain 2008; 12: 116-127.
21. Price RB, Sedarous M, Hiltz GS. The pH of tooth-whitening products. J Can Dent Assoc 2000; 66:421-426.
22. Cooper JS, Bokmeyer TJ, Bowles WH. Penetration of the pulp chamber by carbamide peroxide bleaching agents. J Endod 1992; 18:315-317.
23. Bistey T, Nagy IP, Simó A, Hegedus C. In vitro FT-IR study of the effects of hydrogen peroxide on superficial tooth enamel. J Dent 2007; 35:325-330.
24. Krause F, Jepsen S, Braun A. Subjective intensities of pain and contentment with treatment outcomes during tray bleaching of vital teeth employing different carbamide peroxide concentrations. Quintessence Int 2008; 39:203-209.
25. Dawson PF, Sharif MO, Smith AB, Brunton PA. A clinical study comparing the efficacy and sensitivity of home vs combined whitening. Oper Dent 2011; 36:460-466.
26. Nair PN, Luder HU, Schroeder HE. Number and size-spectra of myelinated nerve fibers of human premolars. Anat Embryol (Berl) 1992; 186:563-571.
27. List T, Leijon G, Svensson P. Somatosensory abnormalities in atypical odontalgia: a case-control study. Pain 2008; 139:333-341.
28. Magloire H, Maurin JC, Couble ML, Shibukawa Y, Tsumura M, Thivichon-Prince B, Bleicher F. Topical review. Dental pain and odontoblasts: facts and hypotheses. J Orofac Pain 2010; 24:335-349.
29. Brännström M, Aström A. The hydrodynamics of the dentine; its possible relationship to dentinal pain. Int Dent J 1972; 22:219-227.
30. Charakorn P, Cabanilla LL, Wagner WC, Foong WC, Shaheen J, Pregitzer R, Schneider D. The effect of preoperative ibuprofen on tooth sensitivity caused by in-office bleaching. Oper Dent 2009; 34:131-135.
31. Reis A, Dalanhol AP, Cunha TS, Kossatz S, Loquercio AD. Assessment of tooth sensitivity using a desensitizer before light-activated bleaching. Oper Dent 2011; 36:12-17.
32. Pinto SC, Pochapski MT, Wambier DS, Pilatti GL, Santos FA. In vitro and in vivo analyses of the effects of desensitizing agents on dentin permeability and dentinal tubule occlusion. J Oral Sci 2010; 52:23-32.

INDEXES TO VOLUME 28 (2015)

SUBJECT INDEX

1beta 167
access to health care 13
acid etching.....192
aging 149
alginates 156, 258
alveolar bone.....174
angle class II 222
angle class III 222
anti-bacterial agents 72
apexification 144
biofilm.....210
bisphosphonates 215
bite force 22
bone.....83
bone regeneration 231
bone remodeling 203
cell viability 114
cephalometry.....222
child 167, 185
children 237
clinical trial 263
composite resins 64
computed tomography 58
core needle biopsy 245
cryopreservation 114
dental caries 3, 13, 95, 185
dental caries susceptibility 48
dental- diagnosis 95
dental enamel 79, 192
dental etching 79
dental health services 13
dental implantation 42
dental impression materials 156
dental leakage 144
dental polishing.....64
dental prosthesis 149
dental pulp.....114
dentin 139
dentin-bonding agents.....72
dentine sensitivity 263
dentist-patient relationships 35
dentistry 35
dexamethasone 83
dimensional measurement accuracy 156, 258
disuse 174
drinking water.....203
drug delivery systems 122
drug users 48
edentulous 149
elastic modulus 64
endodontics 28, 144
enterococcus faecalis 210
experimental model 174
facial pain 237
family 251
fascia lata 231
giant cell granuloma 103
gingival hyperplasia 103
gingival recession 162
gingival thickness 162
glass ionomer 132
hexavalent chromium 203
histiocytosis 167
hydrogen peroxide 263
hypomineralization 192
immunoblotting.....3
immunoglobulin A 3
immunoglobulin G 3
interleukin 167
irrigating solutions 139
jaw diseases 245
langerhans cells.....167
leakage 132
malocclusion 222
mandible 83
masticatory muscles 22
mesenchymal stem cells 114
microbial sensitivity tests 180
mineral trioxide aggregate 144
minocycline.....122
mouth 149
mouth disease 167
mouth mucosa.....103
mouthwashes.....180
nutritional status 185
opg 215
oral 95
oral health 48, 251
orthodontic anchorage procedures 108
orthodontics 42, 108
osteoporosis 22, 215
pain 108

INDEXES TO VOLUME 28 (2015)

SUBJECT INDEX

periodontal disease 215
periodontitis 122
ph 139
phenotype 114
piezosurgery 231
pit and fissure sealants 132
polymerization 72
porphyromonas gingivalis 122
pyogenic granuloma 103
quality of life.....149, 251
radiography 95
rankl 215
rats.....231
regenerative medicine 114
resin cements 28
saliva 3, 167, 185
scanning electron microscopy.....210
school age populations 13
skull.....231
social perception 35
sodium hypochlorite 79
streptococcus.....180
streptococcus mutans 3
temporomandibular joint disorders 58, 237
titanium 42
tooth bleaching 263
tooth eruption 203
ultrasound 22

INDEXES TO VOLUME 28 (2015)

AUTHOR INDEX

Abanto, J 251
Adorno-Farías, D 103
Aguilar-Gálvez; D.....251
Aguilera, MA 35
Aitken-Saavedra, J 103
Albites, U 251
Alippi, RM 83
Amer, MAR 231
Andrade-Torres, A 79
Arciniegas, AJ.....149
Argentieri, AB.....185
Arriola-Guillén, LE.....222
Benchuya, C 167
Bento, PM.....95
Berger, SB.....156
Bermejo, D 215
Biondi, AM 192, 237
Bönecker, M 251
Borda, CX.....149
Borda, MG 149
Bordoni, N 13
Bozal, CB 174, 192
Bozzini, C 83
Bozzini, CE.....83
Briso, ALF 263
Bulacio, MA 210
Burmann, PFP.....42
Cabrini, RL 245
Caixeta, RV.....156
Cangemi, R 210
Cano-Gutiérrez, C.....149
Carrión-García, M.....35
Castillo, JL 251
Centenaro, CC.....72
Champin, G.....83
Chiappe, VB 122
Cintra, LTA 263
Collares, FM 72
Coqueiro, RS.....108
Cornejo, LS.....48
Cortese, SG 192, 237
da Rosa, HCV 28
de Almeida, J 144
de Andrade, D 180
de la Casa, ML 139
de Lucca, RC 203
de Rossi, M 22
de Sousa, LG.....22
de Souza, NC 28
de Verney, JCK 42
Domingos, V.....58
dos Santos, PH 263
Erimbaue, MI 210
Farah, CL 237
Farías-Vergara, M 103
Felippe, WT 144
Fenyo-Pereira, M 58
Fernandes, MI.....162
Fernández de Preliasco, V 167
Fernández, CN 13
Ferrari, BA 258
Filgueira, PTD 95
Fitzcarrald, FD 222
Flores-Mir, C 222
Franco-Romero, G 79
Freitas, CF.....58
Fresolone, M 122
Fridman, DE 237
Gaio, EJ.....162
Gallinari, MO 263
Galván, LR 210
Gamba, CA 185
García-Cano, E 79
Garrofé, AB.....258
Gaudioso, C 210
Gigena, PC 48
Gómez, MV 122
Gómez, SI 3
Gonzaga, AKG 95
Grenón, M.....215
Gruppi, A 215
Gualtieri, A 167
Guerreiro-Tanomaru, JM 180
Guglielmotti, MB 231
Guiraldo, RD.....156
Guitelman, I 237
Gutiérrez, N 114
Hernandez, A 58
Hernández-Ruíz, AK.....79
Iglesias, V 185
Jaramillo, LM 3
Jiménez, NT 114

INDEXES TO VOLUME 28 (2015)

AUTHOR INDEX

Kaplan, AE 64, 132, 192
Kaplan, AE 258
Keszler, A 245
Kramer, PF 42
Lafaurie, GI.....114
Leitune, VCB 72
Lescano- de- Ferrer, A 48
Lewicki, M 203
López, GL 139
López, ME 139
Manlla, AM.....139
Manto, MC 185
Marcondes, ML.....28
Maroso, FB 162
Martinelli, J.....156
Martínez Reinoso, J 185
Mateo, MT 185
Maturana-Ramírez, A 103
Melo, DP.....95
Mendoza-Pinto, C 79
Meneghel, LL 156
Missana, LR 132
Molgatini, SL 185
Montiel-Jarquín, A 79
Moreno, GC 3
Moreti, AFF 156
Moyaho-Bernal, A 79
Munévar, JC 114
Nascimento, AP 180
Nascimento, RC 108
Nieva, N 132
Ogliari, EA 72
Ortolani, A 192
Paiva, SM 251
Palinkas, M 22
Parolo, CF 72
Paván, V 167
Pecci, A 122
Perdigão, J.....263
Picca, M 258
Pimenta, AL 144
Pithon, MM.....108
Plascencia, AR 35
Pontual, AA.....95
Pozos-Radillo, E 35
Preciado-Serrano, L 35
Rahal, V 263
Raitz, R 58
Ramirez-Molina, R 64
Ramos-Perez, FMM 95
Razaboni, AM.....180
Regalo, SCH 22
Renou, SJ 231
Reyes-Rojas, M.....103
Ribeiro, MC 108
Rivera-Prado, H 79
Roa, NS.....3
Rodrigues, RS 108
Rodríguez, A 3
Rodríguez, C 122
Rodríguez, PA 231
Rodríguez, PN.....185
Romanelli, HJ 122
Rösing, CK 162
Rostirolla, FV 72
Ruellas, AC 108
Ruschel, HC 42
Sáez, MM 139
Salgado, PA.....185
Samuel, SMW.....72
Sánchez,LM 203
Santos, CM 22
Santos, MJ.....108
Scalize, PH 22
Semprini, M 22
Siéssere, S 22
Sinhoreti, MAC.....156
Sly, GE 132
Spohr, AM.....28
Squassi, A 13
Stolbizer, F 245
Tanomaru-Filho, M 180
Trojan-Cotumacci, AE 174
Ubios, AM.....174, 203
Vargas, IA 42
Vasconcelos, PB 22
Véra L.S.A. Barbosa 95
Verde, ME 215
Watanabe, E 180
Weber, JBB 28

On November 12, 13 and 14, 2015, the 48th Annual Meeting of the Argentine Society for Dental Research (SAIO), the Argentine Division of the IADR, was held in Tanti, Córdoba Province.
The meeting was honored by the presence of IADR President Dr. Marc Heft, LAR President Dr. Erik Dreyer, and SUIO President Dr. Marcelo Kreiner, attending as special guests.

The following conferences were given at the meeting:
1- "Aging and oral health: A global research perspective" by Dr. Marc Heft, President of the International Association for Dental Research (IADR) (2015-2016)

2- “Informed consent: opening, flexibilization or coercion?” by Dr. Susana Vidal

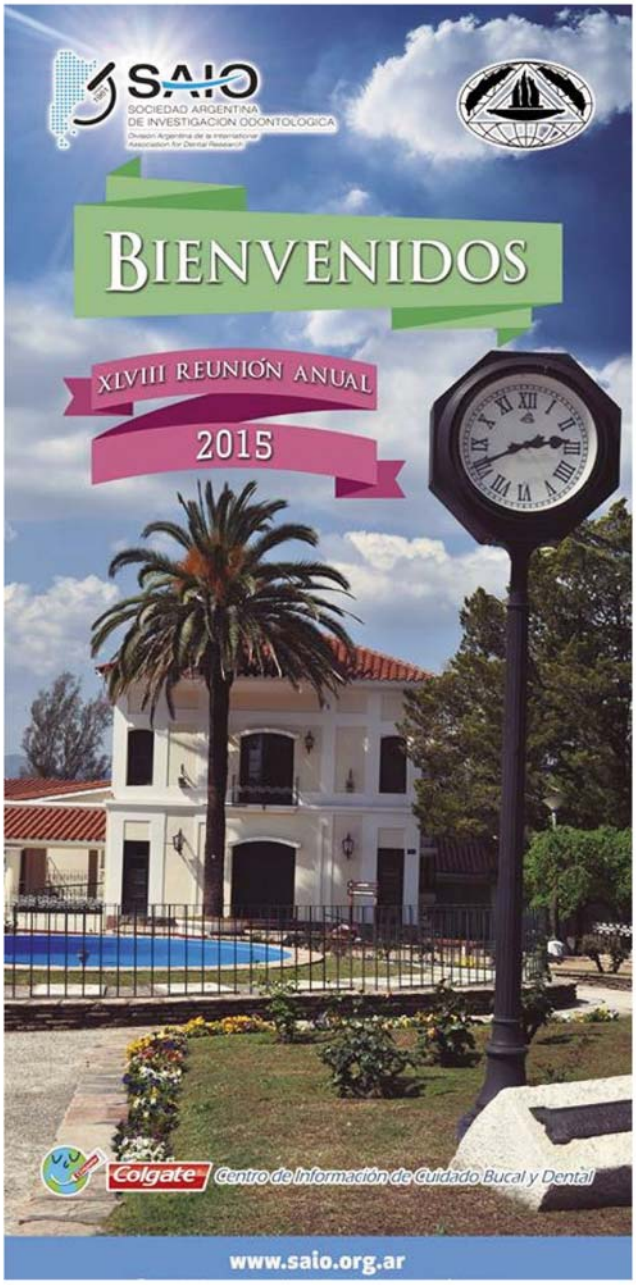
3-“Periodongal disease and its impact as an indicator of cardiovascular disease” by Dr. Liliana Nicolossi

A highlight of the meeting was the appointment of Dr. Rómulo Cabrini as Honorary Member, the late Dr. Marta Elena G. de Crosa as Honorary Member in Memoriam, and Dr. Beatriz Guglielmotti as Life Member.

The meeting was attended by 380 participants, and 274 research works were presented.

The following prizes and fellowships were awarded: Unilever Division Travel Award
Colgate - Palmolive Award for students
Colgate - Palmolive Award for Clinical Science Professionals
Colgate - Palmolive Award for Basic Science Professionals
“María Ines Egozcue” Award
Dental Education Award
Oral Health Award
Prof. Dr. H. Maddalena Award
Omar Tumilasci Award
“Rodolfo Erausquin” Award
“Rodolfo Erausquin” fellowship

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



Members of the SAIO Board and the Annual Meeting Organizing Committee, with Society President Dr. Daniel Olmedo and Organizing Committee President Dr. Alicia Malberti

From left to right: Drs. María Elena Itoiz, María Beatriz Guglielmotti, Daniel Olmedo, and Rómulo Cabrini.

Opening Ceremony of the 48th Annual Meeting of the Argentine Society for Dental Research (SAIO)

Colgate®

EL SECRETO PARA CUIDAR LA SALUD GINGIVAL DE SUS PACIENTES ES: SU CONOCIMIENTO, UNIDO A ESTOS PRODUCTOS QUE SON LOS ALIADOS MÁS EFICIENTES.

Sistema Completo
para el Cuidado Gingival.
Su mejor aliado.

www.colgateprofesional.com.ar / www.colgateprofesional.com.uy / www.colgateprofesional.cl

Presentamos **la Tecnología** que revoluciona el tratamiento preventivo de la caries dental.

Comprobado en estudios clínicos realizados
a lo largo de 8 años con más de 14.000 pacientes.

Tecnología NeutrAzúcar™ + Flúor

- Ayuda a neutralizar los ácidos del biofilm, la causa #1 de caries[1,2]
- 4X Veces Mayor Remineralización*[3]
- Revierte al menos 2X la caries temprana†[4]
- 20% mayor reducción de caries en 2 años‡[5]

Flúor

Colgate ayuda a tener un Futuro Libre de Caries

* Resultado de un estudio de remineralización en comparación con una crema dental regular con floruro.

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

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

QLF is a trademark owned by Inspektor Research Systems BV.

Estudios científicos citados: 1. Wolff M, Corby P, Klaczany G, et al. J Clin Dent. 2013;24(Spec Iss A):A45-A54. **2.** Data on file. Colgate-almolive Company. **3.** Cantore R, Petrou I, Lavender S, et al. J Clin Dent. 2013;24(Spec Iss A):A32-A44. **4.** Yin W, Hu DY, Fan X, et al. J Clin Dent. 2013;24(Spec Iss A):A15-A22. **5.** Data on file. Colgate-Palmolive Company.

www.colgateprofesional.com.ar

Colgate®

MARCA RECOMENDADA POR ODONTÓLOGOS