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Different expression patterns of carbonic anhydrase IX in oral lichen planus and leukoplakia

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ABSTRACT
Tumor hypoxia is an important indicator of cancer prognosis. Among the different genes that are up-regulated by hypoxia is carbonic anhydrase IX, which combines carbon dioxide and water to form bicarbonate and hydrogen. Although expression of this enzyme is very low in normal tissues, carbonic anhydrase IX is overexpressed in several types of cancer. The aim of the present work was to analyze carbonic anhydrase IX expression in the two most frequent potentially malignant oral disorders: oral lichen planus and oral leukoplakia. Immunohistochemical analysis of oral lichen planus and oral leukoplakia biopsies was performed using anti-carbonic anhydrase IX antibody. Samples of normal mucosa served as controls. Statistical analysis was performed by Fischer's exact test. The enzyme was detected in the epithelium of both lesions. The staining was more intense in the basal layer and decreased towards the surface in oral lichen planus. Conversely, the most intense reaction was observed in the superficial layers in leukoplakia, and staining intensity decreased towards the basal membrane. No carbonic anhydrase IX expression was seen in normal mucosa samples. Carbon anhydrase IX expression in lichen and leukoplakia epithelia shows that hypoxia plays a role in the pathogenesis of both lesions. The different distribution patterns provides further evidence of the different biological behavior of these two entities, which under certain circumstances can have similar clinical and histological features.

Key words: *Carbonic Anhydrase IX; Oral Lichen Planus; Oral Leukoplakia.*

Diferentes patrones de expresión de la anhidrasa carbónica IX en liquen plano bucal y leucoplasia

RESUMEN
La hipoxia tumoral es un importante indicador de pronóstico en cáncer. Entre los distintos genes que son activados por hipoxia, uno de los principales es la anhidrasa carbónica IX (CAIX), que combina CO_2 con H_2O para sintetizar HCO_3^- y H^+. Aunque la expresión de esta enzima es muy baja en tejidos normales, se sobreexpresa en varios tipos de cáncer. La finalidad del presente trabajo fue analizar la expresión de CAIX en las dos lesiones orales potencialmente malignas más frecuentes: el liquen plano y la leucoplasia. Se utilizó una técnica inmunohistoquímica con un anticuerpo específico contra CAIX, en biopsias de liquen plano oral y leucoplasia oral. Se utilizaron mucosas normales como controles. Se realizaron análisis estadísticos utilizando test exacto de Fischer. La identificación de la enzima fue positiva en el epitelio de ambas lesiones. En los líquenes la reacción es más intensa en los estratos basales, disminuyendo hacia la superficie. Inversamente, las leucoplasias mostraron marcación más intensa en estratos superficiales, con disminución hacia la membrana basal. Las mucosas normales resultaron negativas. La expresión de CAIX en el epitelio de líquenes y leucoplasias indica que la hipoxia juega algún papel en la patogenia de ambas lesiones. El diferente patrón de distribución es una evidencia más del diferente comportamiento biológico de dos entidades las cuales en ciertas circunstancias pueden manifestar cuadros clínicos e histológicos semejantes.

Palabras clave: *Anhidrasa Carbónica IX; Liquen Plano Oral; Leucoplasia Oral.*

INTRODUCTION

Tumor hypoxia is an important indicator of cancer prognosis, since it is associated with aggressive growth, metastasis and poor response to treatment[1]. Genes that are up-regulated by microenvironmental hypoxia through activation of Hypoxia Inducible Factor-1 (HIF-1) include glucose transporters, glycolytic enzymes, and angiogenic growth factors[2]. Carbonic anhydrases (CAs), a family of metalloenzymes that require Zn^{2+} as a cofactor, are one of the most important groups. Sixteen isozymes that differ in their subcellular localization, catalytic activity

and susceptibility to different classes of inhibitors have been identified. Some of these isozymes are cytosolic (I, II, III, VII and XIII), others are membrane bound (IV, IX, XII and XIV), two are mitochondrial (VA and VB), and one is secreted in saliva (VI). There are also three acatalytic forms, called CA-related proteins (CARPs): CARP VIII, X and XI. In humans, CAs are present in various tissues, including the gastrointestinal tract, the nervous system, kidneys, lungs, skin and eyes, among others[3], but their expression in these tissues is very low. The most reactive site was found in the basolateral surfaces of the crypt enterocytes in duodenum, jejunum and ileal mucosa[4].

These enzymes catalyze the reversible hydration of carbon dioxide to bicarbonate

$$CO_2 + H_2O \leftrightarrow HCO_3^- + H^+$$

Membrane-bound CAs, including carbonic anhydrase IX (CAIX), have an extracellular active site and can provide H^+ or HCO_3^- ions formed during catalytic turnover for various physiological processes, such as extracellular acidification. Several isoforms of CA are thus involved in essential cell processes, like respiration and pH regulation, electrolyte balance, bone resorption, calcification and biosynthetic reactions requiring HCO_3^- as a substrate[5]. CAIX specifically, is important because together with its isoform XII, it is involved in malignant transformation processes[6].

CAIX is overexpressed in a variety of solid malignant tumors, including renal carcinoma and particularly clear cell adenocarcinoma[7-8], cervical carcinoma[9], ovarian carcinoma[10], oesophagal carcinoma[11], bladder carcinoma[12], non-small cell lung carcinoma[13] and mesothelioma[14]. In addition, overexpression of CAIX has been found to correlate with greater tumor aggressiveness[15].

CAIX has several functions in tumor cells. As it extrudes H^+ ions to the extracellular environment, it maintains the latter acidic while making intracellular pH slightly alkaline by incorporating HCO_3^- ions**.** This combination not only favors tumor cell growth but also facilitates events such as cell transformation, chromosomal rearrangements, extracellular matrix breakdown, migration and invasion, protease activation, and growth factor synthesis[16].

In addition, both *in vitro*[17] and *in vivo*[18] studies have shown CAIX to be a useful marker of hypoxia, exhibiting a pattern of expression around regions of necrosis. This distribution is similar to that of pimonidazol, the most validated exogenous marker of hypoxia. Few studies have addressed the expression pattern of CAIX in premalignant lesions. CAIX expression has been shown to increase in bronchial premalignant lesions[19], in breast[20], uterine cervix[21] and skin dysplasias[22], and in oral leukoplakia[23]. The aim of the present work was to study and compare immunohistochemical expression of CAIX in oral lichen planus (OLP) and oral leukoplakia, since both lesions have been classified as potentially malignant disorders, but differ greatly in their risk for malignant transformation.

MATERIALS AND METHODS

The study comprised 37 biopsies corresponding to cases with proven clinical and histopathological diagnosis of OLP (23 cases) and homogenous leukoplakia (14 cases), which were retrieved from the archives of the Oral Pathology Department of the School of Dentistry, University of Buenos Aires. The study was approved by the Ethics Committee of the School of Dentistry of the University of Buenos Aires, Argentina (Res CD 325/02).

All diagnoses were established following diagnostic criteria of the World Health Organization (WHO)[24] and modified WHO diagnostic criteria[25]. Nine of the 23 OLP cases were reticular lichen planus, and the remaining cases were the erosive or atrophic type of lichen. Two cases of leukoplakia showed moderate dysplasia. The specimens had been fixed in 10% formalin with PBS and embedded in paraffin. Seven specimens of normal oral mucosa obtained during surgery of deep-seated lesions were also studied. Endogenous peroxidase was blocked by immersion in 0.5% H_2O_2 in methanol for 30 min. Antigen retrieval was performed with Citra (Biogenex, Fremont, California, USA) in 3 cycles of 4 minutes each, at 400 W microwaves. The sections were then incubated overnight with the primary antibody, anti-human CAIX, raised in rabbit (Santa Cruz Biotechnology, Dallas, Texas, USA). Antibody binding sites were visualized using a streptavidin-peroxidase detection kit (Kit Multilink, Biogenex, Fremont, California, USA), and incubation in 3,3-diaminobenzidine substrate and 1% nickel chloride as intensifier. A section of a single block of a reactive oral squamous cell carcinoma was included in each staining batch as positive control. Sections in which the primary antibody was omitted were used as negative control.

The percentage of cases showing positive staining in the different localizations was analyzed using Fisher's exact test. Values of $p < 0.05$ were considered statistically significant.

RESULTS

CAIX expression was detected in 16 out of 23 cases of lichen and 12 out of 14 cases of leukoplakia, whereas all normal mucosa samples were negative. Difference in frequency was not satistically significant.

OLP and leukoplakia showed different expression patterns. In all positive lichen samples, CAIX was expressed in the basal layers of the epithelium A weaker reaction in superficial layers was also seen in 4 cases, whereas the staining was intense in the superficial and middle layers of 10 leukoplakia cases and only 4 cases were positive in the basal layers, In addition, CAIX was expressed mainly on the cell membrane (14 out to 16 cases on the membrane only) in lichen planus, but was observed on the membrane (5 cases), in the cytoplasm (5 cases), or both localizations (2 cases) in leukoplakia (Fig. 1).

Statistical analysis of results showed significant differences between leukoplakia and lichen when comparing localization of staining on the membrane or in the cytoplasm ($p < 0.05$), and in the layers expressing CAIX ($p < 0.02$).

The presence or absence of CAIX expression and the slight differences in staining intensity were not associated with type of OLP or with the presence or absence of dysplasia in leukoplakia.

DISCUSSION

The importance of studies on CAIX expression in malignant tumors lies mainly in its usefulness as a marker of hypoxia, which in turn influences tumor response to radio and chemotherapy[1]. Overexpression of CAIX has also been associated with more aggressive tumor behavior, irrespective of type of treatment[15]. Hence, it has been posited that determination of CAIX could be used as a marker of malignancy.

The availability of a tumor malignancy marker prompts investigating in which stage of malignant transformation induction of the gene encoding for expression of the marker occurs, with the aim to obtain a marker of early cancerization prior to neoplastic development. In this regard, increased CAIX activity has been demonstrated in premalignant lung lesions[19], breast ductal hyperplasia[20], and skin premalignant lesions[22]. Increased CAIX activity has also been detected in dysplastic epithelium adjacent to oral carcinomas[23] and dysplastic epithelium in oral leukoplakia[26].

Yang et al [27] demonstrated the presence of CAIX in plasma of oral cancer patients, and found increased expression of the enzyme and its mRNA not only in tumor stroma fibroblasts but also in fibroblasts of oral submucous fibrosis associated with areca nut chewing, a potentially malignant disorder.

However, a given molecular change cannot be considered in itself indicative of malignant transformation simply because that same change is more marked in malignant tumors, but rather a number of factors must come together for the risk of malignant transformation to increase. Oral leukoplakia carries an increased risk of cancer development. According to the literature, the annual malignant transformation rate of leukoplakia ranges from 2 to 3%[28], whereas the potentially malignant character of OLP is still under discussion, and most authors estimate the annual malignant transformation rate of OLP to be less than 0.7%[29]. The increased expression of CAIX observed in oral lichen and leukoplakia in the present work is therefore an indicator of an interesting biological behavior of these lesions, rather than a marker of increased risk of malignant transformation.

Although OLP and leukoplakia are recognized as two distinct pathological entities, some clinical and

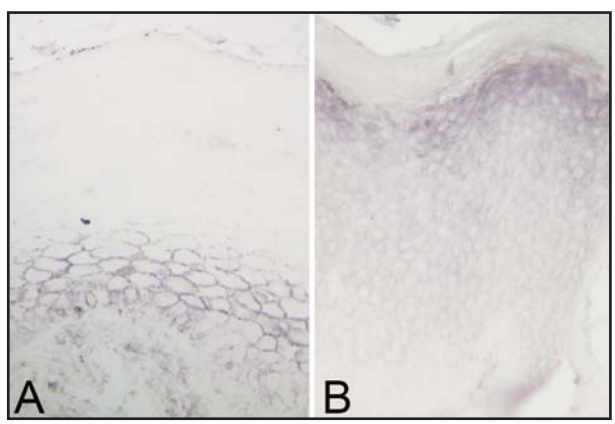

Fig. 1: CAIX immunohistochemical reaction. X 400.
A: Biopsy of oral lichen planus. Staining is more intense in cell membranes of the basal and suprabasal layers of the epithelium. B: Biopsy of leukoplakia. Staining is more intense in the superficial layers, and becomes fainter towards the suprabasal and basal layers of the epithelium.

histopathological features may pose differential diagnosis. In addition, the etiopathogenesis of both lesions was not yet fully clarified. Both entities undergo hypoxic stress. In fact, Pérez-Sayans et al[26] detected CAIX expression in oral leukoplakia; the authors associated their finding with the premalignant nature of the lesions, since they observed higher expression in lesions with dysplasia. Ding et al[30], found that expression of HIF1-α was higher in RNA isolated from lichen planus than in controls. HIF1-α is the main gene activated by hypoxia, and regulates transcription of a number of genes, including CAIX.

An interesting aspect is the markedly different expression pattern of CAIX in the entities studied here. CAIX expression was most prominent in the basal layers of the epithelium, and decreased towards the surface layers in lichen planus, whereas in leukoplakia, staining intensity increased from the basal cells towards the granular layer, where expression was strongest. It is known that OLP is an immune process mediated by T lymphocytes that are present in dense subepithelial infiltrates and cause alteration of the basal keratinocytes[31]. Hypoxia in the basal layers of the epithelium, as shown by higher intensity of CAIX expression, might mediate T lymphocyte recruitment and contribute to the onset of the disease. Leukoplakia is mainly triggered by external agents, such as smoking or alcohol consumption, which lead to an acanthotic response of the epithelium. This response, in turn, separates the epithelial cells from the blood supply, inducing higher CAIX expression in the superficial layers.

CONCLUSION

The different expression patterns of CAIX in oral lichen and leukoplakia provides further evidence of the differences in the biological behavior of both entities, and may be a contribution to the study of their pathogenic mechanisms.

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Parental perceptions of impact of oral disorders on Colombian schoolchildren's oral health-related quality of life

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ABSTRACT

There is no study assessing the impact of dental caries (DC), dental fluorosis (DF) and traumatic dental injuries (TDI) on oral health-related quality of life (OHRQoL) in school children from Colombia. The purpose of this study was to assess the impact of DC, DF and TDI on Colombian schoolchildren´s OHRQoL using their parents as proxies. The parents of 338 children aged 6 to 14 years from public and private schools of Cartagena, Colombia answered the Parental-Caregiver Perception Questionnaire (P-CPQ) on child's OHRQoL adapted to Colombian Spanish language and a socioeconomic questionnaire. Three calibrated examiners performed the clinical assessment for DC, DF and TDI. Poisson regression associated clinical and socioeconomic conditions to the outcome. Overall, 90.24% of parents reported children's oral impact (total P-CPQ score ≥ 1). The mean (standard deviation) P-CPQ scores were 12.49 (14.04). The multivariate adjusted model showed that children from public schools and who have dental caries experience (RR= 1.28; p=0.04 and RR= 1.37; p= 0.018, respectively) were more likely to experience negative impact on total P-CPQ scores.

DC was found to be associated to parental-caregiver perception of impact on their children's oral health-related quality of life, but DF and TDI were not.

Key words: *Dental caries, dental fluorosis, tooth injuries, quality of life, child.*

Percepción de los padres del impacto de desordenes orales de escolares colombianos sobre la calidad de vida relacionada con la salud oral

RESUMEN

No existen estudios que evalúen el impacto de la caries dental (CD), la fluorosis dental (FD) y el trauma dentoalveolar (TDA) sobre la calidad de vida relacionada con la salud bucal (CVRSB) de escolares de Colombia que pertenece a países de habla hispana. El propósito de este estudio fue evaluar el impacto de la caries dental (CD), la fluorosis dental (FD) y el trauma dentoalveolar (TDA) sobre la calidad de vida relacionada con la salud bucal de escolares colombianos usando sus padres como proxies. Los padres de 338 niños y niñas de 6 a 14 años de escuelas públicas y privadas de Cartagena, Colombia, contestaron el Parental-Caregivers Perception Questionnaire (P-CPQ) on child's OHRQoL adaptado al español colombiano y un cuestionario socioeconómico. Tres examinadores calibrados realizaron la evaluación clínica para CD, FD y TDA. La regresión de Poisson asoció las condiciones clínicas y socioeconómicas al puntaje total del P-CPQ y sus dominios. En general, el 90,24% de los padres reportaron el impacto oral de los niños sobre la calidad de vida (puntaje P-CPQ total ≥ 1). La media (DE) del P-CPQ fue de 12,49 (14,04). El modelo multivariado ajustado mostró que los niños de escuelas públicas que tenían experiencia de caries dental (RR = 1,28, p = 0,04 y RR = 1,37, p = 0,018, respectivamente) tuvieron mayor probabilidad de experimentar un impacto negativo en las puntuaciones totales del P-CPQ.

La CD mostró asociación con la percepción del impacto de los padres-cuidadores sobre la salud oral de sus hijos en relación con la calidad de vida. Sin embargo, FD y TDA no se encontraron asociados.

Palabras clave: *Caries dental, fluorosis dental, traumatismos de los dientes, calidad de vida, niñez*

INTRODUCTION

Oral health is currently considered an important pillar in overall human welfare, with strong influence on its development[1]. Everybody without exception should enjoy good oral health status guaranteeing certain activities and functions such as chewing, phonation and smiling as a sign of personal image, enabling social interaction, personal and emotional

development and having positive impact on quality of life[1, 2,] throughout their lifetime.

There is consensus in the literature regarding the negative impact of dental caries (DC) on school children's Oral Health-Related Quality of Life (OHRQoL) through proxy-reports from parents or caregivers[3-5]. Similarly, traumatic dental injuries (TDI) also have been shown to have negative impact on schoolchildren's OHRQoL[6,7]. In addition, a review of the literature showed that severe dental fluorosis (DF) was consistently reported to have negative effects on OHRQoL[8].In spite of this evidence, to the best of our knowledge, there is no study assessing the impact of these oral diseases and disorders, mainly TDI and DF, on schoolchildren, based on parents' proxy reports. Parents' perceptions concerning their children's oral health are important because they may enable clinicians to improve children's oral health, and thereby their OHRQoL[9]. Moreover, parents are usually the primary decision-makers on matters affecting their children's health and healthcare[10,11].

The parental-caregiver perceptions questionnaire (P-CPQ) is an instrument of the Child Oral Health Quality of Life Questionnaire (COHQLQ). It was developed in English in Canadá[12] and has been cross-culturally translated. Its validity has been demonstrated in English in the United Kindgom[13], Chinese in China[14], Portuguese in Brazil[15] and Spanish in Peru[16]. However, although the Spanish version of the P-CPQ[11] has been validated, it has not yet been tested for Spanish-speaking schoolchildren. The use of the P-CPQ in Spanish-speaking countries such as Colombia is essential, considering the high prevalence of DC and DF in schoolchildren, 51%[17] and 81% [18], respectively. Prevalence of TDI may have been neglected in Colombia, since there is no epidemiological survey of its extent. At present, the assessment of the impact of these oral conditions on Colombian schoolchildren's OHRQoL is unexplored. Therefore, the aim of this study was to assess the impact of DC, DF and TDI on the OHRQoL of 6- to 14-year-old Colombian schoolchildren in a population-based sample using parents' proxy-reports.

MATERIALS AND METHODS

This study followed Ethics Guidelines of the Declaration of Helsinki, Edinburgh amendment 2000, 1975 and Resolution 008430 of 1993 the former Ministry of Health of the Republic of Colombia. All parents received information regarding the aim of the study and signed informed consent forms.

Study population and data collection

A cross-sectional study was performed in 2015 on a population-based sample of children aged 6-14 years enrolled at public or private schools and living in Cartagena, Colombia. Cartagena city has an estimated population of 1,001,755 inhabitants, including 215,007 schoolchildren[19].

Sample size was calculated using a 94.5% prevalence of oral impact on schoolchildren's OHRQoL[15], with 95% confidence interval, a standard error of 3% and a design effect of 1.3. To cover non-response, the sample was increased by 15% to 338 children and their parents. Inclusion criteria were children of both genders, who had not received dental treatment in the past 3 months, with no systemic disease and/or neurological disease, with parents/caregivers who were fluent in Spanish language and who agreed to participate in the study.

A 2-stage random sampling procedure was adopted to select the sample. The first stage units were all public and private schools in the city. A total four schools, two public and two private, were randomly selected [WHO, 1997]. Since the schools were of different sizes, an equal probability selection method (probability proportional to the size) was used to ensure that all children would have the same chance of being selected [WHO, 1997]. The second stage units were the 6- to 14-year-old children enrolled at each selected school. At the school, the parents whose children were selected were summoned to be informed of the study objective, and one of the parents (preferably the one who spent most time with the child) was invited to answer two structured questionnaires in face-to-face interviews: one on socioeconomic conditions and another on the child's OHRQoL. Interviews were conducted by two dental assistants who were blind to the clinical oral examinations. They were trained in the reading and intonation of each question and the answer options in the OHRQoL instruments.

Socioeconomic conditions such as parental age, number of children and family income were collected as discrete quantitative variables, and parental level of education and family structure were collected as ordinal and nominal qualitative variables, respectively. All these socioeconomic data were then

categorized for statistical analysis as follows: child's age [children (6 – 9 years old), teenager (10 – 14 years old)], child's gender [female, male], type of school [public, private], age of parents [≤ 44 years old, > 44 years old], number of siblings [≤ 2 children, > 2 children], father's and mother´s education [< 10 years, ≥ 10 years], family income [measured in terms of the Colombia minimum wage-CMW, a standard for this type of assessment, which corresponds to approximately US$ 255.4 per month categorized into ≤ One CMW, ≥ Two CMW], housing ownership[yes, no], household crowding [≤ two members per room, > two members per room] and type of family [Nuclear family, Non-nuclear family].

OHRQoL instrument

The parental-caregiver perceptions of Child Oral Health-Related Quality of Life Questionnaire (P-CPQ) was used to assess children's OHRQoL. This instrument is applied to parents of children aged 6-14 years[15]. The P-CPQ contains 31 items grouped into four subscales: oral symptoms (6 items), functional limitations (8 items), emotional well-being (7 items), and social well-being (10 items). The questions are related to the frequency of events over the past three months. Answers are recorded on a Likert five-point scale used with the following response options: "Never" = 0, "once / twice" = 1, "sometimes" = 2, "often" = 3, "every day / almost every day" = 4. The P-CPQ scores are calculated as a simple sum of the response codes. Since there are 31 questions, the final score can range from 0 to 124, where a higher score indicates a higher degree of impact of oral conditions on children's oral health-related quality of life according to their parents.

In this study, the P-CPQ was adapted from the Peruvian Spanish version[16] to the Colombian context. This Peruvian Spanish version was pilot-tested on a convenience sample of 30 parents of children aged 6-14 years. These parents were not included in the final sample. Parents suggested that some words or expressions should be substituted by synonyms to facilitate the comprehension of the questionnaire. Modifications were made according the parents' comments. A Revision Panel consisting of three postgraduate professors in Pediatric Dentistry, Research and Family Health areas, all fluent in Spanish, who knew the objectives of the study and had experience in OHRQoL studies, reviewed the results and in consensus developed the Colombian Spanish version of the PCPQ.

Children's oral examination

Four previously calibrated examiners performed the children's oral examinations. The examiners were all graduate dentists with experience in previous epidemiological surveys. All examiners underwent two 6-hour sessions of training and calibration exercises with pictures of clinical cases for the clinical study conditions, with a 1-week interval between sessions. Intra- and inter-examiner reliability was established using all examiners' assessments of 20 children who received dental treatment at Dental School of Cartagena University. These children did not form part of the study sample. Kappa values were calculated for intra- and inter-examiner for all clinical conditions.

Dental caries was assessed according to the World Health Organization criteria and calculated in terms of decayed, missing, and filled teeth in permanent dentition (DMFT) and primary dentition (dmft)[11]. For most children with mixed dentition, the caries index was obtained by the sum of the dmft and DMFT scores. To calculate mean dmft/DMFT index, we added the individual average values and divided by the total number of children examined. The prevalence of children affected by the disease was described using the Knutson index [20]: children who have dental caries or have experienced dental caries (dmft/ DMFT > 0) and children who have never had experienced dental caries (dmf/ DMFT = 0).

Dental fluorosis (DF) was assessed using the criteria proposed by Thylstrup-Fejerskov,[21] which is more sensitive than Dean's index for individual classification of teeth into ten categories[22]. A score of zero indicates healthy enamel; scores of one to four indicate spots on the enamel surface, which increase as the score increases. Enamel destruction is observed in scores five to nine, where score five represents mottled enamel with holes smaller than 2mm in diameter, which are fused in score six to form bands less than 2mm deep. For the current study, DFwas categorized as mild (code 1,2,3), moderate (code 4-6) and severe fluorosis (code 7-9)[21].

Traumatic dental injury (TDI) was evaluated using the criteria proposed by Andreasen et al.[23]and evaluated as a possible confounding variable, based on the system adopted by the WHO. It includes

injuries to hard dental tissues and pulp; injuries to hard dental tissues, pulp and alveolar process; and injuries to periodontal tissues. TDI data were analyzed according to presence of at least one kind of trauma or absence of TDI (tooth present and sound).

Data analysis

Data were analyzed using STATA 9.0 (Stata Corp, College Station, TX, USA). Descriptive analyses assessed measures of central tendency (mean, standard deviation and observed range) of the total and individual domain scores of P-CPQ.

Poisson regression with robust variance was performed to associate domains and total P-CPQ scores to oral clinical conditions (DC, DF and TDI) and socioeconomic conditions. Univariate Poisson regression was performed to select variables with a p-value ≤0.20 to enter the final model. Then the selected variables were tested in the adjusted multivariate model and only remained in the final model if p-value ≤ 0.05. In these analyses, the outcome was employed as a count outcome, and rate ratios (RR) and 95% confidence intervals (95% CI) were calculated.

RESULTS

Internal consistency of the P-CPQ was analyzed using Cronbach's alpha coefficient, providing values of 0.85 for total P-CPQ scores in the pilot test phase and 0.89 for total P-CPQ scores in the final sample size of the study, showing the stability of the instrument.

As a result of the calibration process, the examiners obtained inter-examiner reliability values of Cohen's Kappa agreement of 0.87 for DC and 0.92 for DF and TDI. For intra-examiner agreement, the examiners obtained kappa values of 0.89 for DC, 0.85 for DF and 0.90 for TDI.

A total 370 parents were invited to participate in the study, of whom 10 were excluded because they did not conform to the study inclusion criteria. Of the 360 eligible participants, 338 provided signed parental informed consent (positive response rate = 93.8%).

Table 1 shows the socioeconomic and clinical conditions of the sample. Overall, most children had dental caries experience (62.4%), whereas DF and TDI were present in 64.5% and 8.9%, respectively. All questionnaires were fully completed without any missing data, and there were no 'don't know' responses. Most of the questionnaires were answered by mothers (79.3%). Overall, 90.24% of parents reported children's oral impacts (total P-CPQ score ≥ 1). Table 2 contains the mean, standard deviation, and the range observed for the total P-CPQ scores and individual domains.

Table 3 shows the univariate unadjusted analysis of clinical and socioeconomic variables associated

Table 1: Sample sociodemographic features (n = 338).

Variables	n (%)
Child age	
Teenager (10 – 14 years old)	152 (45)
Children (6 – 9 years old)	186 (55)
Child gender	
Male	180 (53.3)
Female	158 (46.7)
Type of School	
Private	170 (50.3)
Public	168 (49.7)
Age of parents	
≤ 44 years old	297 (87.9)
> 44 years old	41 (12.1)
Number siblings	
≤ 2 children	205 (60.6)
> 2 children	133 (39.4)
Father's education level	
≥ 10 years	91 (26.9)
< 10 years	247 (73.1)
Mother's education level	
≥ 10 years	84 (24.8)
< 10 years	254 (75.2)
Family Income	
≥ Two CSW	263 (77.8)
≤ One CSW	75 (22.2)
Household crowding	
≤ 2 members	239 (79.7)
> 2 members	99 (29.3)
Type of family	
Nuclear family	196 (57.9)
Non-Nuclear family	142 (42.1)
Clinical features	
Dental Caries	
Absence	121 (35.8)
Presence	217 (64.2)
Dental Fluorosis	
Absence	119 (35.4)
Presence	217 (64.6)
Traumatic Dental Injuries	
Absence	308 (91.1)
Presence	30 (8.9)

Table 2: Mean, standard deviation, possible range, and range observed for overall and for each P-CPQ11–14 domain scores (n = 338).

P-CPQ	Mean(SD)	Range Observed
Oral symptoms	3.88 (3.5)	0-20
Functional limitations	3.43 (4.17)	0-24
Emotional well-being	2.09 (3.90)	0-30
Social well-being	3.09 (6.15)	0-48
Total Score	12.49 (14.04)	0-90

with total and individual domains of the P-CPQ. There was significant association between some independent variables, total scores, and individual domains ($p < 0.05$): oral symptoms and mother's education and family income; emotional well-being domain and dental caries experience; social well-being domain and children's education, number of siblings; and total P-CPQ and members in family and dental caries.

The multivariate-adjusted model (Table 4) showed that children from public schools and children who have dental caries experience (RR= 1.28; p=0.04 and RR= 1.37; p= 0.018, respectively) were more likely to experience a negative impact on total P-CPQ scores. Children whose mothers have an educational level < 10 years and children who have dental caries experience showed positive and negative impact on the oral symptoms domain, respectively (RR= 0.75, p= 0.02 and RR= 1.22, p=0.04, respectively). Children who studied atpublic schools were more likely to experience negative impact on the emotional well-being and social well-being domains ($p<0.05$) (Table 4).

DISCUSSION

To the best of our knowledge, this is the first study to measure the impact of DC, TDI and DF on the OHRQoL of Colombian schoolchildren from parents' proxy-reports using a Spanish version of the P-CPQ[16]. The questionnaire showed semantic equivalence and good understanding by parents considering that Peru and Colombia share many similarities in their Spanish language and vocabulary. Cronbach's alpha coefficient was 0.89 for the total scale, indicating acceptable internal reliability, as values of 0.5 or above are considered acceptable[24]. Similar findings were reported for the Peruvian Spanish version[16]and in the original English and Brazilian versions[12,15], indicating its satisfactory use for assessing children's OHRQoL according to parents' perceptions in Colombia.

In this study, dental caries was the only oral disease that showed negative impact on total P-CPQ scores in the oral symptoms domain, though it did not affect other domains. A study in China also found negative impact of dental caries using the P-CPQ in 12-year-old children[25], but in this case, the impact was on the social and emotional well-being domains. There is no study using P-CPQ in Colombian and other Spanish speaking countries like Perú[16], but other instruments in Spanish that assess OHRQoL according to children and adolescent´s perceptions, such as the Child Perceptions Questionnaire 11-14 (CPQ11-14)[26] report impact of DC on the oral domain. This may indicate agreement between children and their parents/caregivers regarding the impact of DC on schoolchildren´s OHRQoL, and it will be important to explore the matter in future studies. Therefore, even when children and adolescents are able to provide self-reports, parents/caregivers' proxy reports should be obtained to provide additional supplementary information about the impact of different oral outcomes on childrens' OHRQoL[27]. Both views jointly may offer a more comprehensive basis for professional clinical decisions. This information may also be useful for health authorities in planning oral healthcare services[28].

In this study, we found no association between traumatic dental injuries and the perception of the impact of OHRQoL, but this maybe due to the low prevalence of TDI in the sample, in agreement with Abanto et al.[29]. Nevertheless, assessment of the impact of TDI on schoolchildren's OHRQoL using CPQ 11-14 shows a negative impact on OHRQoL. Crown discoloration also shows a negative impact using this scale[30]. This is another finding that shows different perceptions between children and their parents/caregivers regarding presence of TDI.

Dental fluorosis was not found to be associated with the perception of impact on OHRQoL, in agreement with a study performed in Pinheiro Preto, Brazil, but differing from a study in Colombia[31] which reported that children often avoided smiling because of the appearance of their teeth due to DF. It should be noted that said study was conducted in an endemic fluorosis zone. In contrast, our results show low prevalence of DF, and the fact that the

Table 3: Univariate analysis of socioeconomic variables and clinical conditions associated with the total score of P-CPQ and total score for domains.

	ORAL SYMPTOMS DOMAIN		FUNCTIONAL LIMITATIONS DOMAIN		EMOTIONAL WELL-BEING DOMAIN		SOCIAL WELL-BEING DOMAIN		TOTAL P-CPQ SCORE	
	Robust RR (95% CI)	P-value	Robust RR (95% CI)	P-value	Robust RR (95% CI)	P-value	Robust RR (95% CI)	P-value	Robust RR (95% CI)	P-value
Child age										
Teenager (10 - 14 years old)										
Children (6 - 9 years old)	0.94 (0.77 – 1.14)	0.54	1.23 (0.93 – 1.61)	0.13	0.76 (0.50 – 1.14)	0.19	0.84 (0.55 – 1.29)	0.44	0.95 (0.74 – 1.21)	0.68
Child gender										
Male										
Female	1.03(0.85 – 1.26)	0.70	1.12(0.86 – 1.46)	0.36	1.11(0.74 – 1.67)	0.59	1.13(0.74 – 1.73)	0.55	1.09(0.86 – 1.39)	0.44
Type of school										
Private										
Public	1.08 (0.89 – 1.31)	0.41	1.15 (0.89 – 1.49)	0.27	1.40 (0.91 – 2.15)	0.11	1.46 (0.94 – 2.28)	0.08	1.24 (0.97 – 1.57)	0.08
Number siblings										
≤ 2 children										
> 2 children	1.00 (0.81 – 1.23)	0.99	0.93 (0.71 – 1.22)	0.63	1.31 (0.87 – 1.98)	0.18	1.73 (1.14 – 2.62)	0.00*	0.18 (0.92 – 1.51)	0.18
Father's education level										
≥ 10 years										
< 10 years	0.87 (0.69 – 1.09)	0.22	1.09 (0.81 – 1.46)	0.56	0.99 (0.59 – 1.64)	0.98	1.18 (0.76 – 1.84)	0.44	1.02 (0.78 – 1.34	0.85
Mother's education level										
≥ 10 years										
< 10 years	0.77 (1.61 – 0.99)	0.04*	1.09 (0.81 – 1.46)	0.56	1.06 (0.65 – 1.72)	0.81	1.16 (0.74 – 1.82)	0.50	1.00 (0.75 – 1.32)	0.99
Family income										
≥ Two CSW										
≤ One CSW	0.78 (0.61 – 0.99)	0.04*	1.10 (0.79 – 1.52)	0.55	1.08 (0.68 – 1.73)	0.72	1.00 (0.65 – 1.56)	0.97	0.97 (0.74 – 1.27)	0.83
Household crowding										
≤ 2 members										
> 2 members	0.91 (0.72 – 1.16)	0.47	0.76(0.57– 1.03)	0.07	0.89 (0.58 – 1.37)	0.61	0.81(0.52-1.27)	0.37	0.84 (0.64 – 1.09)	0.21
Type of family										
Nuclear family										
Non-nuclear family	1.11 (0.92 – 1.35)	0.24	1.26 (0.97 – 1.64)	0.07	1.29 (0.86 – 1.94)	0.21	1.12 (0.73 – 1.71	0.58	1.18 (0.93 – 1.50)	0.15
Clinical features										
Dental Caries										
Absence										
Presence	1.19(0.97-1.45)	0.08	1.18(0.90-1.55)	0.22	1.61(1.03-2.5)	0.03	1.57(1.00-2.48)	0.05*	1.33(1.04-1.70)	0.02
Dental fluorosis										
Absence										
Presence	1.04 (0.85 – 1.28)	0.64	1.01 (0.77 – 1.31)	0.93	0.84 (0.54 – 1.29)	0.44	1.18 (0.75 – 1.84)	0.46	1.02 (0.80 – 1.31)	0.81
Traumatic dental injuries										
Absence										
Presence	1.16 (0.83 – 1.64)	0.37	1.04 (0.69 – 1.55)	0.84	1.33 (0.71 – 2.47)	0.36	1.44 (0.69 – 3.01)	0.39	1.21 (0.87 – 1.68)	0.25

Table 4: Multivariate analysis of sociodemographic variables and clinical conditions associated with the total score for domains and P-CPQ.

	ORAL SYMPTOMS DOMAIN		FUNCTIONAL LIMITATIONS DOMAIN		EMOTIONAL WELL-BEING DOMAIN		SOCIAL WELL-BEING DOMAIN		TOTAL P-CPQ SCORE	
Variables	**Robust RR (95% CI)**	**P-value**	**Robust RR (95% CI)**	**P-value**	**Robust RR (95% CI)**	**P-value**	**Robust RR (95% CI)**	**P-value**	**Robust RR (95% CI)**	**P-value**
Mother's education level										
≥10 years										
<10 years	0.75 (0.59-0.96)	0.02	†		†		†		†	
Type of school										
Private										
Public	†		†		1.75 (1.13-2.72)	0.012	1.96 (1.25-3.07)	0.003	1.37 (1.05-1.78)	0.018
Dental Caries										
Absence										
Presence	1.22(1.04-1.49)	0.04	†		†		†		1.28(1.02-1.65)	0.04

Oral symptoms: Xi2: 9.36, p=0.009. Functional limitations: no report, Emotional well-being: 10.82. p=0.004, Social well-being: Xi2 = 21.9, p = 0.0001, P-CPQ: Xi2 = 15.60, p = 0.001.
†Variables not associated with the respective domains in the final multivariate model after the adjustment.

DF variable was dichotomized may explain the different findings.

Our study found significant associations between P-CPQ scores, its domains and some socioeconomic conditions such as children who study at public schools with poor OHRQoL in emotional and social domains and mother's education with better OHRQoL in oral domains. This is similar to the findings in a systematic review assessing the impact of parental socioeconomic status and home environment on children's OHRQoL, which reports that children from an area with high deprivation had poorer OHRQoL than children from areas with low and medium categories of deprivation[32,33]. It also reports that higher educational level of the mother and father predicted better OHRQoL in children, but observed the mother's education – and not the father's education – was significantly related to OHRQoL scores.[34] Several studies also report that attending a poor school and maternal level education are meaningful aspects for children's oral health. In such conditions, some parents and their children do not have enough opportunities to access dental services or to purchase oral hygiene articles[29,30], which may explain the impact on social and emotional domains. Most families in Colombia live in areas with high deprivation and do not have oral health education or services to promote good OHRQoL. It is important to foster the role of schools in reducing health disparities among children[35]. With regard to mother's low level of education having positive impact on the oral symptoms domain of P-CPQ, one study reports how Latina mothers in the USA with higher educational status did not perceive cavities as more serious[36,37]. Previous qualitative studies describe how Latina mothers reported they did not perceive that dental decay was a condition that affected young children[38,39]. This may explain the positive impact in our study. Thus, it is important identify specific cultural beliefs that run counter to optimal oral health in young children.

This study is the first to test the P-CPQ in Spanish-speaking schoolchildren and indicates the need to design public policies in oral health providing comprehensive care to children and their parents and redirecting efforts towards providing proper treatment, reducing the prevalence of dental caries and improving quality of life. This study also contributes to public health by providing understanding of the psychosocial impact of DC, DF and TDI. In addition, the first use of the Spanish version P-CPQ has important implications for research and practice. In this regard, the first use of

the P-CPQ in a Spanish-speaking country enables comparisons with other cultural and ethnic groups around the world, and provides support for public oral health programs and dental care services for this age group. It also provides additional information to the results of a previous study on preschool children.[40]

CONCLUSIONS

According to parents' proxy-reports, the presence of dental caries and attending public schools have a negative impact on the OHRQoL of Colombian schoolchildren, whereas mothers with educational level < 10 year has a positive impact.

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Quality of life related to complete denture

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ABSTRACT

Full edentulism is characterized by the complete loss of permanent teeth, resulting in aesthetic, structural and functional changes which can negatively impact quality of life, and which are minimized through rehabilitation with complete dentures. The aim of this study was to compare oral health-related quality of life in patients with complete original dentures three months after installation of new dentures and two years after fabrication of new complete removable dentures. In this longitudinal comparative study, 15 volunteers of both genders, aged 50 to 82 years, who sought treatment at the Department of Dentistry of the Federal University of Rio Grande do Norte, participated in the preparation of new dentures. The Brazilian version of the Oral Health Impact Profile for edentulous patients (OHIP-EDENT) was used to evaluate quality of life. Data analysis was performed descriptively and with hypothesis testing using the Friedman and Wilcoxon tests with 5% significance level. In relation to the OHIP-EDENT domains, there was a difference for chewing discomfort and inability to chew between baseline and two years. However, there was no difference between the evaluated periods in the areas of pain and orofacial muscle discomfort, psychological inability and social disability. Improvement indicators in patient quality of life were observed in the area of discomfort and inability to chew between baseline and 2 years.

Key words: *Stomatognathic System; Mouth Edentulous, Mesh Pubmed; Complete Denture; Quality of life.*

Qualidade de vida relacionada à prótese total

RESUMO

O edentulismo completo caracteriza-se pela perda total dos dentes permanentes, resultando em alterações estéticas, estruturais e funcionais, podendo impactar negativamente na qualidade de vida, sendo minimizado através da reabilitação com a prótese dentária. O objetivo deste estudo foi comparar a qualidade de vida relacionada à saúde oral em pacientes com a prótese original completamaxilar e mandibular, três meses após instalação da nova prótese e dois anos depois da confecção da nova prótese total bimaxilar removível. Nesse estudo comparativo longitudinal, participaram 15 voluntários, com faixa etária entre 50 e 82 anos, de ambos os sexos, que buscaram tratamento no Departamento de Odontologia da Universidade Federal do Rio Grande do Norte, para a confecção de novas próteses. Utilizou-se a versão brasileira do Oral Health Impact Profile para pacientes edêntulos (OHIP-EDENT) para avaliar a qualidade de vida. A análise dos dados foi realizada de forma descritiva e analítica com os testes de Friedman e Wilcoxon, com nível de significância de 5%.Em relação aos domínios do OHIP-EDENT, verificou-se diferença para desconforto e incapacidade mastigatória entre a avaliação inicial e após dois anos. Nos domínios Dor e desconforto orofacial, Incapacidade Psicológica e Incapacidade Social, não ocorreram diferenças entre os períodos avaliados. Foram observados indicadores de melhora na qualidade de vida dos pacientes, no domínio desconforto e incapacidade mastigatória entre a avaliação e a 2 anos.

Palavras-chave: *Sistema estomatognático; Edêntulo; Prótese total; Qualidade de vida.*

INTRODUCTION

Complete edentulism is defined as a complete loss of permanent teeth[1], and is very common worldwide in the elderly population[2] aged 65[1] to 74 years[2,3]. It also characterizes a reality of the Brazilian population assessed in the latest epidemiological survey conducted in 2010 by the Ministry of Health. Survey results showed that 15.4% of the older adult

population was edentulous and needed complete dentures[4]. The 2013 National Health Survey found that 11% of individuals aged over 18 years were completely edentulous, with a higher proportion among women aged 60 years or older. Although complete loss of teeth is not necessarily a part of the natural aging process, age is one of the most prominent factors[5]. Other common factors are biological processes such as tooth decay, periodontal disease, trauma and oral cancer, and non-biological factors including dental procedures, the quest for healthcare, and socioeconomic and cultural factors[1]. Edentulism outcomes include changes to functional, neuromuscular and physiological[6] levels. Functional capacity includes chewing and speech, psychological status includes self-esteem and satisfaction with appearance, and social aspects involve pain and discomfort related to oral health[7-9]. Over time, the total loss of teeth leads to atrophy of support structures and loss of muscle tone, which have adverse effects on facial aesthetics[5] and also involve chewing, swallowing and speech functions[10]. Thus, proper oral function is not only associated with the ability to perform jaw movements and physiological parameters, but also with comfort and aesthetics, which can affect the quality of life[8].

The changes caused by total loss of teeth can be minimized through rehabilitation with dental prostheses, which is the most economical and common treatment[2,8,9,11]. Its purpose is to restore the harmony of the stomatognathic system and general health[9]. Acceptance of complete dentures requires psychosocial and functional adaptation, a process that can be influenced by patient expectations[12] and perceptions[7], which may involve quality of life. As defined by the World Health Organization, the term quality of life refers to "an individual's perception of their position in life in the cultural context and systems values in which they live and in relation to their goals, expectations, standards and concerns"[13].

Oral health-related quality of life thus plays a crucial role in the process of prosthetic rehabilitation, which includes functional, psychological and social aspects[7]. The aim of this study was to compare oral health-related quality of life with the previous dentures to oral health-related quality of life three months and two years after fabrication and fitting of the new complete removable dentures.

MATERIALS AND METHODS

A descriptive, comparative longitudinal study was approved by the Research Ethics Committee of the Onofre Lopes University Hospital under number 578.993. All participants were informed regarding study objectives and procedures, which were described in the Informed Consent Form, with patient consent and signature requested. Participants were selected among individuals who visited the Department of Dentistry of the Federal University of Rio Grande do Norte to have conventional complete dentures made. Inclusion criteria were individuals who had been fully edentulous for over a year and conventional complete upper and lower denture users needing new dentures. Participants who had motor difficulties, evident cognitive deficiencies in everyday actions or pathological changes of the alveolar edges were excluded.

The instrument used to assess the impact of quality of life was the Brazilian version of the *Oral Health Impact Profile* for edentulous patients (OHIP-EDENT), validated by Souza et al.[12]. Quality of life was evaluated in three stages: during the use of the old dentures, and after three months and two years of using the new dentures. The sample consisted of 15 individuals of both genders. It should be noted that much of the sample was lost over the course of the assessments, and the number of participants was reduced from 36 original participants to 22, and finally to 15 individuals. The OHIP-EDENT is an inventory consisting of 19 questions grouped into four subscales described by Souza et al.[14], emphasizing "pain and discomfort in orofacial muscles", "masticatory discomfort and inability", "psychological discomfort and inability" and "social inability". The options for the answers are: never, sometimes and almost always, which are assigned the scores of "0", "1" and "2", respectively. Higher scores represent worse oral health-related quality of life.

Statistical analysis was performed with SPSS version 20.0 for Windows. The Shapiro-Wilk test was used to evaluate the normality of data distribution, finding that the variables did not have normal distribution. The Friedman test was used to verify whether the sample showed any significant difference between the three periods evaluated by means of multiple comparisons. The *post hoc* analysis was performed using therection in order to identify in which periods there were differences. A 5% ($p \leq 0.05$) significance level was considered.

RESULTS

The final sample consisted of 13 females (86.67%) and 2 males (13.33%). Average age was 63.73 years, standard deviation 7.67, with minimum age 50 and maximum age 82 years. Average time of edentulism was 26.6 years with standard deviation 12.8. The usage time of the dentures was categorized into equal to or less than 5 years (4 - 26.67%), and more than 5 years (11 - 73.33%). The distribution of OHIP-EDENT areas between periods evaluated is shown in Table 1.

The area of masticatory discomfort and inability showed a difference between baseline and two years. There was no difference between the evaluated periods in the areas of orofacial muscle pain and discomfort and psychological inability and social inability (Table 2).

DISCUSSION

Average age above 60 years agrees with data reported in other studies[9, 15, 16]. Higher prevalence of females has also been reported in other studies[16,17], which may be explained by the fact that women are more concerned about caring for their oral health and seek treatment more often than men do[18]. For quality of life, we found no significant difference for discomfort and chewing inability between baseline and 2 years, highlighting the improvement in patient quality of life.

The analysis categorized the average time of denture usage, with up to 5 years being used to understand the main morphological and functional changes referred to edentulous individuals. Sixty percent of our sample used the dentures for over five years. The literature indicates that the quality

Table 1: Distribution of OHIP-EDENT areas between the evaluated periods. Natal 2017.

Areas	Median	Q25 - Q75	
	Evaluation	6.00	5.00 - 7.00
Orofacial muscle	3 months	5.00	3.00 - 6.00
Pain and Discomfort	2 Years	2.00	2.00 - 6.00
	Evaluation	5.00	1.00 - 7.00
Masticatory	3 months	2.00	2.00 - 4.00
Discomfort and inability	2 Years	1.00	0.00 - 3.00
	Evaluation	1.00	0.00 - 7.00
	3 months	0.00	0.00 - 3.00
Psychological inability	2 Years	0.00	0.00 - 1.00
	Evaluation	0.00	0.00 - 2.00
	3 months	0.00	0.00 - 0.00
Social inability	2 Years	0.00	0.00 - 0.00

Table 2: Comparison of OHIP-EDENT areas in three evaluated periods. Natal 2017.

Areas	n	Evaluation	3 months	2 Years
Orofacial muscle	15	6.00 ABC	5.00 BC	2.00 C
Pain and Discomfort				
Masticatory	15	5.00 EF	2.00 FG	1.00 G
Discomfort and inability				
Psychological inability	15	1.00 IJK	0.00 JK	0.00 K
Social inability	15	0.00 MON	0.00 NO	0.00 O

The groups of letters on the medians represent multiple comparisons of the Friedman test ($p<0.05$). The medians or pairs of median values with different letters indicate that there are significant differences between the corresponding medians after Bonferroni correction ($p<0.0167$).

of dentures may decrease over time, and individuals may present problems in mastication[15] after the fourth[15] and fifth[9] year of use, thereby making it necessary to replace the dentures, which is in accordance with our data.

The main reasons for the change in dentures observed in this study and in agreement with other studies relates to lack of stability and retention[11]. These factors can be explained by the period of bone loss which results in the first year after tooth extraction, when the bone may lose up to 25% of its width and approximately 4 mm in height due to the continuous process of bone resorption[11]. A decline in the alveolar ridge of approximately 1mm per year is expected over the years, being four times higher in the mandible than in the jaw[9]. Resorption can cause poor adaptation of the resin base for dental prostheses, making them slightly loose around the residual bone edge. This factor and the problems caused by the reduced vertical dimension cause great discomfort when eating, which can significantly reduce the efficiency of chewing, thereby influencing patient nutrition as a result of food not being crushed, thus reducing nutrient absorption[5].

Measuring oral health-related quality of life enables evaluation of patient's subjective perception of their condition and constitutes a key factor for clinical practice, which is complemented by physical indicators[19], with the aim of improving the understanding and therapeutic direction of the professionals involved[14]. It should be noted that the rehabilitation success of dentures is based on the opinion of the individual, emphasizing denture stability, comfort, speech, ease of removal for cleaning, chewing and aesthetics[20]. The literature suggests the OHIP-EDENT with psychometric properties is a specific instrument to assess any changes in clinical aspects and the quality of life of edentulous individuals after prosthetic treatment[14].

The results of this study showed differences in the OHIP-EDENT subscales for masticatory discomfort and inability between baseline and 2 years, representing an improvement in quality of life with the use of the new dentures. Masticatory discomfort and inability are improved by correct intermaxillary positioning, tooth anatomy, the shape and fit of the base of the dentures on the supporting tissues, generating comfort[5]. The difference in the masticatory inability index can be improved by the restoration of the vertical dimension of occlusion, correct centric occlusion[11], restoration of the cusps for crushing food, improved chewing efficiency and aesthetics. Moreover, new functional molding from the edge provides greater retention and stability, resulting in patient comfort with the new dentures[5].

In agreement with our findings, other studies have shown that a substitution for new dentures significantly improves quality of life[5,7,16,17,21]. Goiato et al.[5] evaluated the quality of life and patient perception before and after insertion of a new prosthesis in 60 patients with an interval of three months between evaluations, also using the OHIP-EDENT and resulting in significant impact on patient quality of life in all areas. However, in contrast to our study, they analyzed each issue in isolation. Komagamine et al.[16] determined factors related to self-assessment of dentures through the OHIP-EDENT and the masticatory performance before and after replacement of the dentures using a sample of 93 individuals, reporting that denture stability and retention provided better appearance and quality of life in edentulous patients, although they did not identify differences in mastication. In India[17], differences were observed after one month and six months of denture fitting when compared to pre-treatment. In relation to quality of life and gender, it was noted that women showed statistically significant differences in relation to men.

With regard to whether simplified and conventional manufacturing techniques of the dentures was related to oral health-related quality of life, one randomized study found no significant differences at three and six months[8]. Cardoso et al.[18] evaluated oral health-related quality of life and the efficiency of chewing in patients rehabilitated with mandibular overdentures of two implants and conventional dentures, finding that treatment with two mandibular implants on the dentures provides better efficiency in chewing and quality of life when compared with conventional prostheses.

It is interesting to note that while functional modifications provide a great deal of satisfaction to patients, facial appearance should also be taken into consideration. As stated by Nordenram et al.[22] regarding facial appearance, edentulous individuals live in a constant state of anxiety, and in some cases of self-recrimination for neglecting their oral health in the past and also worry about the perception of others. In addition, the appearance of premature

aging[22] is emphasized as a result of the loss of teeth causing a decrease in the lower third of the face, protrusion of the jaw, sharp nasolabial folds, arcuate cheeks, depressed labial commissures, and thin, drawn lips[23]. Changes in the orofacial muscles with the required prosthetic rehabilitation provide better self-esteem and confidence through rejuvenated appearance.

The use of dentures improves oral functions; however, denture adaptation deserves special attention because there are morphological and functional changes which may hamper denture fit and stability. The adaptation process seems to relate to the characteristics of the dentures, as well as the orofacial myofunctional situation due to the action of the muscular forces applied, which destabilize the dentures[10].

During the adjustment period, there may be functional difficulties such as lack of intelligibility in speech articulation, lack of saliva control, reduced mandibular and labial movements by virtue of the repositioning of teeth, refurbishment of the denture palate, and restoration of the vertical dimension of occlusion[24]. In addition to the aspects mentioned above, another important factor is the positioning and movement of the tongue, which operates in the functions of speech, mastication and deglutition. Furthermore, when people receive their new dentures, they may present tongue interposition in the production of dentilingual phonemes, so the aid of tongue contra movement for the retention and stability of jaw dentures through tactile sensation[25] should also be considered.

Concerning masticatory function, there is a decrease in perceptual and reduced or inaccurate sensorineural information which complicates the masticatory pattern organization because the texture of food is not accurately perceived as it is in subjects with teeth[10]. In addition to uncoordinated movements, a reduction in muscle strength for the incision and grinding of food occurs[23], as well as low masticatory efficiency[6,10,26]. A factor to be considered for the functional limitations described above refers to the time the evaluation was performed at approximately three months, and the adaptation process may occur in up to six months[10]. A limitation of this study is small sample size. It was noted that the adaptation period of conventional complete dentures can cause discomfort due to morphofunctional modifications, therefore myofunctional therapy is a possible treatment to assist in balanced performance of oral functions.

Regarding the sample and methodology employed, differences in discomfort and chewing inability between the initial evaluation and 2 years into wearing the dentures were confirmed, demonstrating an improvement in patient quality of life. This study emphasizes the importance of assessing relevant aspects of oral health-related quality of life of complete denture users. Further research should be conducted with larger samples and longer study periods.

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Omega-3 and Omega-6 salivary fatty acids as markers of dietary fat quality: A cross-sectional study in Argentina

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ABSTRACT

The use of saliva for analyzing biological compounds has recently been expanded. The aim of this study was to analyze the correlation between specific dietary sources of n-3 and n-6 fatty acids (FA) and their salivary levels to evaluate their role as intake markers.

Seventy-nine healthy volunteers were included. A validated food frequency questionnaire was used for data collection and Interfood v.1.3 software was employed to quantify food intake. Salivary samples were collected following international standards and FA profile was determined by gas liquid-chromatography. Multiple linear regression analyses were performed for dependent variables (salivary FA profile) to detect independent associations with n-3 and n-6 FA food source intake, adjusted by age, gender, body-mass index, total energy intake, regular exercise, alcohol intake and smoking. Salivary concentrations of alpha-linolenic acid (ALA) 18:3 n-3 were significantly associated with nuts intake (β=0.05, 95% CI 0.02-0.07, p=0.04). Salivary concentrations of linoleic acid (LA) 18:2 n-6 and arachidonic acid (AA) 20:4 n-6 were associated with the intake of n-6 vegetable oils and red meat, cold meat and viscera (β=-0.80, 95% CI 0.06-0.09 p=0.03; β=-0.40, 95% CI 0.30-0.50, p=0.02, respectively).

This study supports the hypothesis that salivary concentrations of n-3 and n-6 FA are related to food intake. Monitoring dietary FA though salivary markers is relevant for nutrition epidemiology and for prevention and management of several diseases related to fat intake.

Key words: *fatty acids, diet, saliva, biomarkers.*

Ácidos grasos salivales Omega-3 y Omega-6 como marcadores de la calidad lipídica de la dieta: un estudio de corte transversal en Argentina

RESUMEN

El uso de biomarcadores salivales está en continua expansión. El objetivo de este estudio fue analizar la asociación entre fuentes alimentarias de ácidos grasos (AG) n-3 y n-6 y sus concentraciones salivales como marcador de ingesta.

Participaron 79 voluntarios sanos. Se aplicó un cuestionario validado de frecuencia de consumo alimentario y el software Interfood v.1.3 para su procesamiento. Las muestras salivales se recogieron según estándares internacionales y se determinó el perfil de AG salivales por cromatografía gaseosa. Se desarrolló un modelo de regresión lineal múltiple ajustado por sexo, edad, índice de masa corporal, valor energético total, actividad física, consumo de tabaco y alcohol para analizar la asociación entre el perfil de AG salivales y la ingesta de alimentos fuente de AG n-3 y n-6. Las concentraciones salivales del AG alfa-linolénico (ALA) 18:3 n-3 se asociaron positivamente con la ingesta de nueces (β=0.05, IC 95% 0.02-0.07, p=0.04), mientras que las concentraciones salivales de ácido linoleico (AL) 18:2 n-6 y araquidónico (AA) 20:4 n-6 se asociaron con el consumo de aceites ricos en n-6 (β=0.80, 95% IC 0.06-0.09 p=0.03) y de carnes rojas, fiambres y embutidos y vísceras, (β=0.40, IC 95% 0.30-0.50, p=0.02). De acuerdo a estos resultados, las concentraciones salivales de AG n-3 y n-6 se relacionan a la ingesta de sus alimentos fuente. El monitoreo de la ingesta lipídica a través de biomarcadores salivales constituye un aporte a la epidemiología nutricional y a la prevención y tratamiento de patologías vinculadas a la ingesta de grasas.

Palabras clave: *ácidos grasos, saliva, dieta, biomarcadores.*

INTRODUCTION

The relationship between diet and the risk of developing chronic diseases has been documented in recent decades. Nutritional epidemiology, focusing on dietary intake, involves the development of several tools to estimate calories, proteins, carbohydrates, fats, vitamins, minerals, etc., such as diet records, dietary recalls and food frequency

questionnaires (FFQ). The FFQ is one of the most commonly used methods because it provides insight into the regular dietary intake of a population over time and is relatively cheap, quick and easy to use[1]. Although this progress in dietary recall tools is promising and cost-effective, the methods for assessing dietary intake are still not without intake error, a commonly cited research limitation. The combination of different methods (e.g. administration of different questionnaires and assessment of biomarker levels) could reduce respondent burden and reporting bias[2,3].

Blood is the most commonly used biological fluid for biomarker determination and is sensitive to dietary intake. Urine is a good indicator of hydrosoluble compound intake, since their concentration in urine depends on the nutrient saturation degree in tissues[4,5]. The use of saliva for analyzing biological compounds to evaluate nutritional status has recently been expanded[6-8]. Collecting saliva is non-invasive, more comfortable than venipuncture and requires no special equipment, and it is easily stored prior to analysis[9-11].

Fatty acid (FA) intake is reflected in a wide variety of biological tissue samples, such as serum and subcutaneous adipose tissue[12,13]. A previous study showed a significant increase of α-linolenic acid 18:3 n-3 (ALA) in saliva from vegetarians or semi-vegetarians compared to people who consumed a mixed diet rich in animal products, who had significantly higher levels of salivary arachidonic acid 20:4 n-6 (AA)[14]. This finding could be related to the significant intake of food rich in n-3 essential fatty acids (EFA), such as soy, dried fruits, sunflower and corn oils.

Competition between metabolic pathways may lead to changes in FA composition independent of dietary content. It is reasonable to expect that the best markers of dietary intake would be FA that cannot be endogenously synthetized such as *trans*, linoleic acid 18:2 n-6 (LA) and ALA. Other FAs whose synthesis depends on EFA intake include eicosapentaenoic acid 20:5 n-3 (EPA) and docosahexaenoic acid 22:6 n-3 (DHA), also present in fish and seafood[15]. EFA are important compounds which play a role in the complex biological process of inflammation. EFA and their metabolites are known to have pro- and anti-inflammatory actions and to regulate gene expression, enzyme activity, immune response and gluconeogenesis[16].

We hypothesized that FA salivary levels may be affected by dietary sources of FA. The main aim of this study was to analyze the correlation between specific dietary sources of n-3 and n-6 FA and salivary levels of EFA and derivatives, to evaluate their role as intake markers.

MATERIALS AND METHODS

Study participants

A cross-sectional study was carried out involving non-probability sampling of 79 male and female adults aged eighteen to eighty years who visited two hospitals in Córdoba (Privado and Córdoba), Argentina for a routine check-up. Subjects on special diets and those with digestive and/or metabolic dysfunction (such as celiac disease, lactose intolerance or diabetes) were excluded. All participants provided informed consent to participate in this study. The study complies with the Helsinki Declaration and was carried out according to the guidelines for the protection of the volunteers' rights. It was approved by the Institutional Ethics Committees (Córdoba, Argentina).

Dietary assessment

Participants were interviewed by two well-trained professional nutritionists. A validated qualitative and quantitative FFQ was used to collect data. It included questions related to 257 types of food and drinks consumed, with emphasis on food sources of fat[17]. This instrument includes questions on intake of whole and low-fat dairy products, cheese, eggs, beef, poultry, pork, fish, canned fish, seafood, viscera, sausages, vegetables, tomato derivatives, herbs, fruits, nuts, legumes, cereals, bakery products, animal fat, oils, seasonings, sugar, confectionery, pastry, beverages (alcoholic and non-alcoholic), snacks, ice cream and soy products. The frequency references used were the number of times each item was consumed per month, per week or per day and never. Portion size was described as small, medium or large using a photographic guide to help subjects understand these definitions[18].

Food data were processed using a software package called *Interfood v.1.3*, to produce information in grams/day of each food item consumed[19]. This software is an open-source program that has three basic components: dietary intake FFQ; a database of common foods and their composition considering 131 substances (macro- and micronutrients and

phytochemicals); and a relational database that links the FFQ data with the food database.

Collection of saliva samples

Saliva was collected from 8:00 to 10:00 a.m. after a minimum of six to eight hours fasting. With the subject leaning forward, unstimulated but spontaneously flowing whole saliva was collected in sterile plastic test tubes (5 mL or more). Prior to saliva collection, the study subjects did not consume any drinks and were at rest with no previous physical activity, tooth brushing and/or oral rinse[9]. Samples were frozen at -20ºC until they were processed by centrifuging at 80xg at 5ºC for 30 minutes. The supernatant was analyzed.

Fatty acids analysis

Total salivary lipids were extracted with chloroform-methanol 2:1 according to Folch's method and FA were methylated by sodium methoxide[20,21]. Salivary FA was separated, identified and quantified using a capillary column (BPX 70.30 m long, 0.25 mm ID, 0.25 um film, Phenomenex, Torrance, USA) in a gas chromatograph (Clarus 500 Perkin Elmer, Waltham, USA). Oven temperature began at 170ºC, rising 1.2ºC/min up to 240ºC, with a total chromatographic run time of 30 minutes[22]. The free FA were identified using commercial standards. Values, expressed as a percentage of detected FA, corresponded to the mean of two chromatographic runs on each sample.

Assessment of other variables

Certified personnel following standardized procedures took all additional measurements. Body weight and height were measured using a professional mechanical scale equipped with calibrated stadiometer. Participants were in underwear and without shoes. Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters (kg/m^2). Other variables such as physical activity and alcohol and tobacco use were also evaluated.

Statistical analysis

Baseline demographic characteristics were calculated as means and standard deviations for continuous variables or as numbers and percentages for categorical variables. Gender differences in variables of lifestyle status, food intake and salivary FA concentrations were compared by Wilcoxon's test for continuous variables and Fisher's test for categorical variables. Multiple linear regression analyses were performed for dependent variables (salivary FA profile) to detect independent associations with n-3 and n-6 FA food source intake in grams/day (fish and seafood, nuts, vegetables oils rich in n-6, and red meat, cold meat and viscera), adjusted by age, gender, BMI, total energy intake, regular exercise, alcohol intake and smoking. All tests were two-sided and significance was considered at $p<0.05$. All analyses were performed with Stata® statistical software package (v.11.0).

RESULTS

Table 1 shows the basic characteristics of the study subjects. Mean age was 57.7 years for men and 49.2 years for women. 84.7% of men and 72.2% of women were current drinkers and 57.9% of men and 41.1% of women were current smokers. Average body mass index (BMI) was similar in men and women (27.7 and 25.5 kg/m^2, respectively). Regular physical activity was low in both men and women (47.3% and 45.1%, respectively). Calorie intake was 2678 ± 105 kcal/day for men and 2468 ± 387 kcal/day for women.

Dietary fat source profile is shown in Figure 1. Overall, the foods most often consumed were dairy products, red meat, confectionary and stuffed pasta. Regarding specific dietary n-6 FA food source

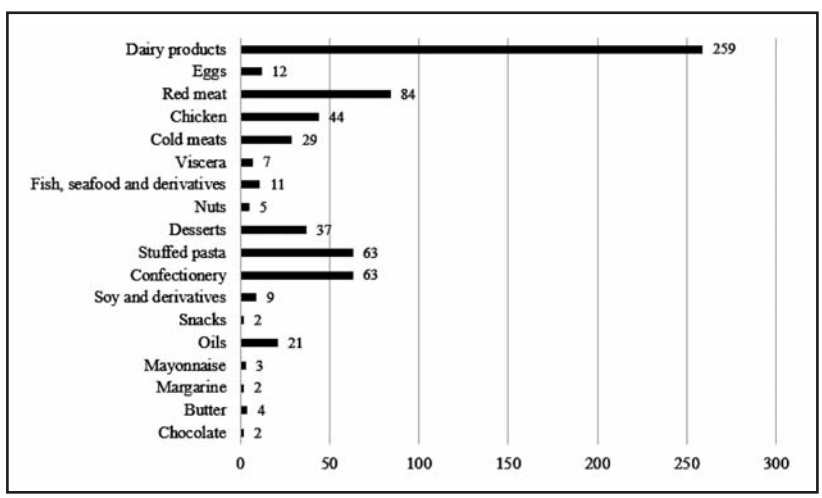

Fig. 1: Fat dietary source intake, grams per day.

intake, sunflower, soybean and corn oils were the oils most frequently consumed (oil average 21 ± 3 g/day) followed by red meat (84 ± 12 g/day). Total intake of red meat, viscera and cold meats was 120 ± 21 g/day). Regarding n-3 FA food source, fish, seafood and derivatives intake was 11 ± 5 g/day. Hake, mackerel, dorado and tuna were the most common fish species in the diet. Nuts intake (5± 2 g/day) included mainly walnuts and peanuts. Non-significant statistical differences were found for food intake, alcohol and tobacco use and physical activity by gender (p>0.05).

Table 1: Characteristics of study subjects.

	Men (n=37)	Women (n=42)	*p-value*
Age (years)	57.7 ± 12.4	49.2 ± 15.8	0.16
BMI	27.7 ± 3.2	25.5 ± 1.2	0.36
Energy intake (Kcal/day)	2678 ± 105	2468 ± 387	0.68
Current smoking (%)	57.9	41.1	0.27
Current alcohol drinking (%)	84.7	72.2	0.14
Regular physical activity (%)	47.3	45.1	0.88

p-values for sex differences are based on t tests for continuous variables and chi-square tests for categorical variables.
Values are mean ± standard deviation for continuous variables and number (percentage) for categorical variables.

Eighteen salivary FAs were analyzed by gas chromatography. Table 2 shows salivary FA composition. Non-significant statistical differences were found between men and women (p>0.05).

The results of multivariate analyses are shown in Table 3. Salivary concentrations of ALA 18:3 n-3 were significantly associated with the intake of nuts (β=0.05, 95% CI 0.02-0.07, p=0.04). On the other hand, salivary concentrations of LA 18:2 n-6 and AA 20:4 n-6 were associated with vegetable oils and with red meat, cold meat and viscera intakes, respectively (β=0.80, 95% CI 0.06-0.09 p=0.03; β=0.40, 95% CI 0.30-0.50, p=0.02, respectively).

DISCUSSION

This study showed a significant correlation between salivary ALA 18:3 n-3, LA 18:2 n-6 and AA 20:4 n-6, and dietary sources in a healthy adult

Table 2: Salivary fatty acid profile*.

FAs	Men	Women	Total	*p-value*	FAs	Men	Women	Total	*p-value*
4:0	5.8	3.1	4.4	0.69	**18:3 n-3**	3.9	4.4	4.1	0.20
	± 0.9	± 1.1	± 1.3			± 1.2	± 0.4	± 0.4	
6:0	4.3	2.8	3.5	0.20	**20:0**	1.8	0.5	1.1	0.37
	± 1.2	± 0.9	± 1.3			± 0.8	± 0.1	± 0.5	
12:0	3.1	4.2	3.6	0.42	**20:3 n-3**	1.5	2.3	1.8	0.43
	± 1.8	± 0.7	± 0.9			± 0.3	± 0.7	± 0.4	
14:0	2.3	4.1	3.6	0.22	**20:4 n-6**	5.2	6.3	5.6	0.41
	± 0.6	± 1.1	± 0.7			± 0.9	± 2.1	± 1.1	
16:0	22.2	23.6	22.5	0.40	**20:5 n-3**	1.3	0.9	1.0	0.46
	± 4.2	± 5.6	± 3.8			± 0.4	± 0.3	± 0.3	
16:1 n-7	2.2	3.1	2.7	0.36	**22:0**	1.7	0.9	1.4	0.19
	± 0.5	± 1.1	± 0.6			± 0.4	± 0.2	± 0.5	
18:0	13.5	15.4	14.7	0.45	**22:5 n-3**	2.6	1.8	2.2	0.80
	± 3.9	± 4.2	± 2.2			± 1.0	± 0.4	± 0.6	
18:1 n-9	11.2	8.3	9.2	0.44	**22:6 n-3**	2.2	1.8	2.1	0.22
	± 2.7	± 1.2	± 2.1			± 0.4	± 0.6	± 0.7	
18:2 n-6	13.4	15.5	14.1	0.66	**24:1 n-9**	1.8	1.1	1.5	0.52
	± 4.4	± 3.8	± 3.1			± 0.6	± 0.3	± 0.6	

*Values are expressed as **mean** percentage (**± standard deviation**) of total detected fatty acids.

population. Although the presence of FA in adipose tissue, serum or plasma is accepted as a reliable biomarker of their intake in food, there is still scanty literature referring to the correlation between dietary and salivary lipids[23,24].

Salivary LA 18:2 n-6 and ALA 18:3 n-3 would be expected to have the strongest association with dietary intake because they cannot be synthetized endogenously. In our study, nut intake, a source of n-3 FA, was associated with 18:3 n-3 salivary levels. Nuts are natural foods rich in unsaturated FA; most nuts contain substantial amounts of monounsaturated FA, while walnuts are especially rich in both n-6 and n-3 FA[25]. Although there is no evidence about nuts intake and salivary lipids, frequent consumption of nuts is related with lower concentrations of inflammation markers and is also recommended by the WHO Guideline on Sugar Intake for Adults and Children for dental health professionals[26-28]. The American Heart Association promotes the consumption of four servings (30-40 gram per portion) per week of nuts, seeds or legumes[29]. The study population shows inadequate intake of nuts according recommendations, but even though it was low, it was reflected in the salivary n-3 profile. On the other hand, we found no association between fish intake and salivary levels of long chain n-3 FAs due to the very low fish intake recorded, which was considerably lower than current guidelines (100 grams, twice a week). In this respect, other authors have shown a correlation between fish intake and plasma n-3 FA[30,31].

Our results showed significant association between oil intake –mainly sunflower oil, followed by soybean and corn oils– and salivary LA 18:2 n-6. Previous studies have reported high intake of sunflower oil in Argentina[32]. The aforementioned oils are the main sources of 18:2 n-6 FA[33,34]. The effects of 18:2 n-6 on health and disease are still controversial. An imbalance in the n-3:n-6 ratio, produced by an increased n-6 consumption or a reduced n-3 intake, may promote the endogenous synthesis of inflammatory molecules[35].

Significant association was observed between red meat intake, cold meat and viscera and AA 20:4 n-6 salivary concentrations. The presence of this FA in human saliva as related to diet and inflammatory conditions has been previously demonstrated [10,36]. Westernized diets characterized by high intake of red meat and processed foods have been related to inflammation biomarkers such as leukotrienes-2 and thromboxanes-4, which are associated with cardiovascular diseases and certain types of cancer[37]. EFA metabolites, derived by lipoxygenase and cyclooxygenase enzymatic pathways, have been quantified in human mixed saliva as well as in saliva fractions. The level of free AA and the quantitative hydroxyeicosatetraenoic acid (HETE) act as markers for the inflammatory processes occurring in the oral mucosa and in saliva in response to the development of squamous cell carcinoma[38]. In addition, pro-inflammatory lipid mediator precursors have been detected as potential markers for aggressive periodontitis[39]. In this sense, monitoring dietary FAs such as LA and ALA and their metabolites is a relevant strategy in the prevention and management of several fat intake-related diseases, not for only oral diseases, but also cardiovascular disorders, diabetes and tumors such as prostate and mammary, among others.

Secretion of saliva plays an important role in numerous significant biological processes. Although some salivary components are well characterized, data for characterizing salivary lipids and their functions is scarce and controversial. However, the

Table 3: Linear regression analysis (ß coefficient) by food intake.

Food intake	ß	95% Conf. Interval	*p-value*
Fish and seafood			
20:5 n-3	0.006	0.002-0.001	0.65
22:5 n-3	-0.0003	-0.0001-0.001	0.41
22:6 n-3	0.001	0.0004-0.002	0.59
Nuts			
18:2 n-6	0.02	0.008-0.07	0.36
18:3 n-3	0.05	0.02-0.07	**0.04**
Vegetable oils high in n-6 FA			
18:2 n-6	0.80	0.06-0.09	**0.03**
20:4 n-6	0.02	0.01-0.05	0.19
Red meat, cold meat and viscera			
20:4 n-6	0.40	0.30-0.50	**0.02**

ß: coefficient in multivariable model adjusted for age, sex, smoking status, body mass index, energy intake, alcohol consumption and physical activity.

qualitative and quantitative content of salivary lipids may change in pathological states (e.g. cystic fibrosis and diabetes), and according our results, due to nutritional exposure[40]. Thus, salivary lipids could be used for validating dietary measurement or as markers of food consumption.

Some limitations of the present study must be considered. It is cross-sectional design and therefore we cannot infer causality from these results. In addition, these findings cannot yet be generalized due to the sample size and the fact that a particular population was analyzed.

In conclusion, the present study supports the hypothesis that salivary concentrations of n-3 and n-6 FA are related to the intake of certain foods. Further studies are needed to confirm these results and to determine the causal direction of this relationship.

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Increased interpremolar development with self-ligating orthodontics. A prospective randomized clinical trial

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ABSTRACT

In compressed dental arches with mild to moderate crowding, space can be gained during the alignment phase to help improve tooth location.

The aim of this study was to compare transverse measurements before and after the alignment stage in two groups: Group A treated with passive self-ligating brackets, (Damon System) and Group B with conventional brackets (Roth philosophy). The change in transverse diameter between teeth was measured on dental casts taken before and after orthodontic alignment.

Twenty four patients of both sexes aged 13 to 36 years, with moderate tooth crowding (4 to 6 mm), were treated. They all received treatment at the Department of Orthodontics, School of Dentistry, University of Buenos Aires. Patients were distributed randomly into two groups of 12. No extraction was performed until the end of the alignment. Dental casts were measured before and after alignment.

The distance between upper first premolars increased more in Group B ($p = 0.008$), and the distance between canines was higher in Group B, with statistically significant difference ($p < 0.01$).

Both systems enable tooth alignment by increasing transverse diameter of the arches in patients with mild to moderate crowding. The greatest transversal development occurs in the inter-premolar area in both techniques and is significantly higher with self-ligating orthodontics.

Inter-canine distance increases significantly with conventional orthodontics compared to self-ligating orthodontics.

Key words: *dental arches - orthodontics; development.*

Mayor desarrollo interpremolar con ortodoncia de autoligado. Estudio clinico prospectivo randomizado

RESUMEN

En las arcadas dentarias comprimidas, con apiñamiento dentario de leve a moderado, es posible obtener ganancia de espacio que favorezca la ubicación de las piezas en la fase de alineación por remodelación alvéolo-dentaria de las arcadas.

El objetivo de este trabajo fue comparar las diferencias pre y post tratamiento en la etapa de alineación de los grupos A: brackets autoligables pasivos, (Sistema Damon) y grupo B: brackets convencionales (Filosofía de Roth). Se evaluaron las variaciones en el diámetro transversal, antes y después de alinear ortodóncicamente los dientes, utilizando modelos de estudio.

Participaron 24 pacientes de ambos sexos, entre 13 y 36 años con apiñamiento dentario moderado (entre 4 y 6 mm) tratados en la Cátedra de Ortodoncia de la Facultad de Odontología, Universidad de Buenos Aires. Los pacientes fueron distribuidos al azar en dos grupos de 12 pacientes cada uno. No se realizaron exodoncias hasta finalizar la alineación. Se midieron los modelos pre y post alineación.

La distancia entre primeros premolares superiores aumentó en el grupo A más que en el B ($p=0.008$) y entre caninos fue mayor en el grupo B, con diferencia estadísticamente significativa ($p< 0,01$).

En pacientes con apiñamiento leve a moderado, la expansión con ambos sistemas permite la alineación dentaria por aumento del diámetro transversal de las arcadas fundamentalmente en el área premolar. El mayor desarrollo transversal se produce en el área premolar para ambas técnicas y es significativamente mayor con ortodoncia autoligable.

En ortodoncia convencional, aumenta significativamente la distancia intercanina en comparación con la autoligable.

Palabras clave: *arcos dentales - ortodoncia; desarrollo.*

INTRODUCTION

Self-ligating brackets were introduced in 1946 by Stolzenberg[1] and the Damon system used in this study was introduced in 1996[2,3]. Different authors have highlighted advantages of the self-ligating system compared to conventional brackets: reduction in treatment time and greater comfort for the patient[4,5] shorter consultations, longer intervals between appointments[6-8]. Pandis et al. 2008 conducted a clinical trial comparing dental changes during the initial stages of alignment without extractions in lower jaw, finding no statistically significant difference[9].

Our team compared the gingival response in patients treated with conventional and self-ligating brackets and found that orthodontic treatment increased bacterial plaque and gingival inflammatory response in both groups without statistically significant differences between groups[10]. Moreover, adequate basic therapy can maintain gingival and periodontal health. These results are consistent with recent studies published by Kaklamanos et al.[11]

A review of the literature published by Miles[12] and Herradine[13] compared conventional with self-ligating treatment, finding that both treatments achieved alignment with a combination of dental arch expansion, with less incisive flaring when self-ligating treatment was used. Celar[14] published a systematic review in 2013 comparing initial pain, number of consultations and treatment time, concluding that further prospective studies are required to define whether there are any differences between the techniques. Reddy et al. reported in 2014 that self-ligating bracket treatments are more efficient regarding space closure than the conventional systems and that alignment occurs through lower incisor inclination[15].

Recently published studies quantify the increase and bone loss by vestibular and lingual / palatal with cone beam tomography in orthodontic patients. Transverse changes in dental arches have been analyzed in plaster study casts[16] and in digital models[17].

Our hypothesis is that the greatest gain in space with the self-ligating system occurs in the lateral areas of the arches, at the level of the premolars, with no alteration of inter-canine or inter-molar distance.

Our goal is to compare differences in the distance between canines, first and second premolars and first and second molars in both arches before and after alignment using both techniques.

MATERIAL AND METHODS

This project was approved by the Bioethics Committee of the school of Dentistry of the University of Buenos Aires, and follows the principles of the Declaration of Helsinki. Patients signed informed consent to participate in this prospective clinical trial.

Patients

Inclusion criteria: Patients 13 to 36 years old of both sexes, with permanent dentition, with little development in their dental arches, moderate dental crowding (-4 to -6 mm of dental discrepancy).

Exclusion criteria: patients with joint disorders, untreated caries or periodontal disease. Patients with mixed or temporary dentition. Patients with diastemas, teeth retained or absent except third molars.

Treatment plan: Patients were assigned randomly to treatment with one of the techniques. Treatment plans were made after diagnosis, which was based on cephalometric analysis, study of plaster casts and clinical analysis. Extractions were determined from the clinical analysis of facial aesthetics, sagittal problems, open bite, dental protrusion and dental discrepancy. When extractions were required, they were performed after the alignment stage.

Experimental design

Patients were treated by calibrated professionals from the Orthodontic department, with unified criteria. The sample was distributed into two groups of 12 patients each.

Group A: 12 patients of both sexes, 13 to 34 years old, were treated with the Damon System (low friction). Damon II brackets (A Company), nickel-titanium-copper archwires (Damon Cooper-Ni-Ti.014 and 0.016), Damon format.

Group B**:** 12 patients of both sexes, 13 to 36 years old, were treated with a straight wire technique (with friction). Roth brackets (A Company, Synthesis), nickel-titanium archwires (Ni-Ti .012, .014 and .016), True Arch format and elastomeric ligature (Fig.1).

The treatments followed the protocol of each technique. Routine diagnostic studies were performed (Panoramic Rx, Tele-Rx profile, cephalometric

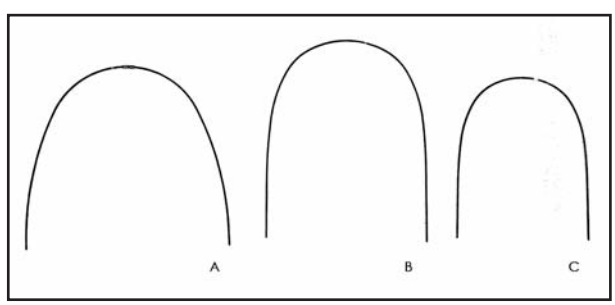

Fig. 1: The archwires used for the treatment with Damon System present Damon format, which is the same for both jaws (a). Treatments performed with Roth's Philosophy True Arch used archwires with different shapes for upper (b) and lower jaw (c).

analysis, articulator mounting, clinical examination and dental study casts). The dental arch was considered aligned when it was possible to place the rectangular archwire. During the initial stage, no extractions or interproximal wear were performed. Operational definitions of variables: Pre- and post-alignment dental casts were studied.

Dental cast study

The expansion of the dental arches in transverse direction was measured in upper and lower jaws. Measurements were performed taking as a guide the projection on the gingiva of the palatal sulcus of the first or second upper molars and the lingual groove of the lower molars, the projection on the gingiva of the tip of the palatine cusp of the first and second upper and lower premolars, and the projection on the gingiva of the cingulum of the upper and lower canines.

Measurements were taken with a digital caliper (Mitutoyo Digimatic NTD12-6" C; Mitutoyo Corp., Tokyo, Japan). Five measurements per jaw were taken on each patient for transverse analysis. The casts were measured by three calibrated operators who were blinded to the groups (pre- and post-treatment, identified with numbers)[18] (Fig. 2). Measurements were compared using descriptive statistics of mean, SD and 2-tailed Student's T-test for unpaired samples.

RESULTS

Analysis of dental casts: transversal variation

Table 1 shows the averages and Standard Deviations (SD) of the variation in the distance between contralateral teeth of 24 patients at the end of the alignment stage taking into account all transverse measurements.

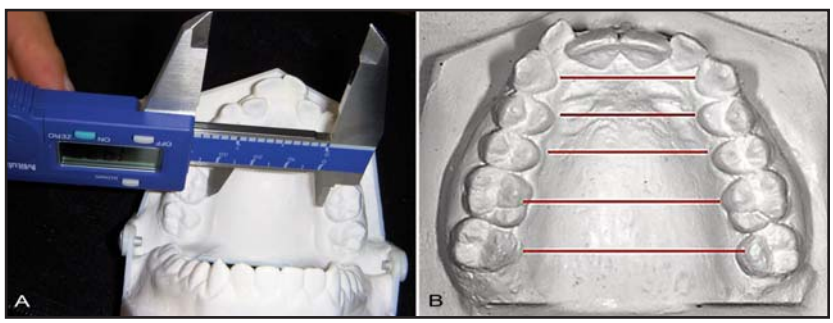

Fig. 2: A: Measurement with digital caliper of the transverse distance between homologous teeth in upper jaw casts. B: Transverse measurements performed pre- and post-alignment in upper jaw casts.

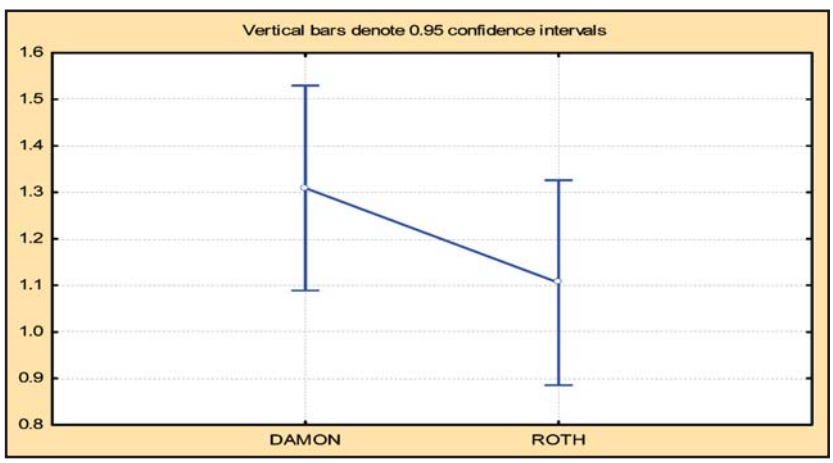

Fig. 3: Bar chart considering both jaws. Difference in the development of the arches with self-ligating (Damon System) (n= 12) and Roth Technique (n = 12) (p = 0.19).

Statistical analysis

The following descriptive statistics were performed: average, SD and Student T Test for two-tailed unpaired samples for comparison between samples. Comparative analyses were performed by jaws, by techniques and by teeth. Student's T-test showed statistically significant differences in the distance between first upper premolars, which was greater with self-ligating brackets (p=0.008). At the level of the canines, there was greater expansion with the conventional technique ($p \leq 0.01$). The rest of the comparisons showed no statistically significant difference. Considering both maxillaries, the sum of the distances between all contralateral teeth evaluated increased with the alignment by an average 1.3mm in group A (self-ligating) and 1.1mm in Group B (conventional); with no statistically significant difference (p = 0.19) (Fig. 3).

Table 1: Variation in the distance between contralateral homologous teeth in alignment treatment.

VARIATION	MAXILLARY	DAMON SYSTEM n=12		CONVENTIONAL ROTH n=12		TEST T UNPAIRED
BETWEEN		AVERAGE	DS	AVERAGE	DS	p
CANINES	UPPER	0,217	2,458	0,81	1,6476	***< a 0,001**
1PMs	UPPER	2,47	1,111	2,077	1,674	***0.008**
2PMs	UPPER	1,741	1,538	1,434	1,301	0,614
1Ms	UPPER	0,268	1,556	-0,227	0,957	0,357
2Ms	UPPER	-0,7	1,127	-0,296	2,33	0,594
CANINES	LOWER	0,416	1,637	1,903	0,923	***0,011**
1PMs	LOWER	1,744	2,394	1,879	1,177	0,862
2PMs	LOWER	1,66	1,847	1,111	2,016	0,493
1Ms	LOWER	-0,09	1,45	-1,07	1,645	0,135
2Ms	LOWER	0,055	1,465	-0,535	1,206	0,292

DISCUSSION

The Damon system uses self-ligating brackets and heat-activated copper-Ni-Ti archwires which are the same size for both jaws, whereas the Roth system uses True Arch Ni-Ti archwires, with the upper archwire being larger than the lower. Our hypothesis is that these two different orthodontic systems produce different responses in the tissues.

In the study subjects, who presented mild to moderate discrepancy, transversal development was slightly higher for the self-ligating system, although with no statistically significant difference. This would indicate that an archwire larger than the dental arch does not produce an expansion greater than needed when its composition is superelastic, as is the case of heat-activated copper-nickel-titanium, which exerts a very low force that can be controlled by the musculature

A paper published in 2014 by Atik et al[19] compared the two treatment systems taking into account the position of the incisors, the changes in transverse dimensions in the upper arch, inclination changes in upper teeth, the clinical periodontal parameters and the intensity of pain in patients with a Class I malocclusion by studying pre- and post-treatment casts and cephalometry in patients with dental crowding. The study concluded that there was no difference between the two systems relative to the position of the incisors, changes in the dental arch, transverse dimension, periodontal clinical parameters or intensity of pain. We believe that the same outcomes can be achieved with both systems because a Quad-Elix expander was used prior to the conventional treatment, enabling treatment without extractions. We understand that arches behave differently according to whether they are treated with passive self-ligating system or conventional system.

Analysis of transverse dimension between teeth

The increase in transverse diameter is mainly the result of variations in the shape of the arches. In compressed dental arches with moderate crowding, light arch wires can be used during the alignment phase to gain space and help improve tooth location. Franchi *et al.* (2006) compared the use of low-friction ligature instead of self-ligating brackets, reporting a similar increase in upper inter-molar width in comparison to that obtained by conventional ligation with elastomers. The study showed 4 degrees of vestibular molar inclination and concluded that this may imply that the molar expansion observed with self-ligating brackets is related to displacement or inclination of the molars rather than to bodily movement or basal maxillary expansion[20.]

In the present study, the evaluation of the distance between contralateral teeth used anatomical landmarks as reference: insertion of the teeth at the palatal or lingual level. This explains why the transverse increase is smaller in millimeters than when the increase is measured at the crown level.

Inter-canine distance

For inter-canine distance, the increase was slight: 0.2mm ± 2.4 with Damon system and 0.8 mm ± 1.6 with Roth. In the lower jaw, average variation was 0.4mm ± 1.6 with the self-ligating system and 1.9mm ± 0.9 with the Roth system. Differences were statistically significant in both cases.

Premolars and molars

For premolars and molars, the average increase in transverse diameter was 2.47 mm ± 1.11 for the self-ligating group and 2.07 mm ± 1.67 for the Roth group, with statistically significant difference ($p = 0.008$).

Vajaria et al included the measurement of the distance between premolars to compare the Damon system with straight-wire appliance, and found increased distances between canines and premolars for both groups, though with no significant difference in transverse level between groups[21].

Almeida et al studied the changes produced with both types of treatment only in the lower arch[16], finding no significant increase between premolars. However, in said study the measurements were taken at the cusps, which are anatomical landmarks different from those evaluated in the current study or in the study by Fleming et al[22].

Pandis et al. in 2009, carried out a comparative study between straight-wire appliance and self-ligating, finding that mandibular crowding was corrected through incisive flaring and expansion of the arches. However, they recorded measurements between canines and molars, without taking into account the inter-premolar distance, which in our study is the distance that varies the most and differs significantly between techniques[9].

In the individual evaluation of the response in the arches with the self-ligating system, it was found that the greatest modification in the transverse diameter of upper and lower arches occurs at the level of the inter-premolar distance, in agreement with papers recently published by Cattaneo et al[17]. They evaluated digital casts of patients treated with passive self-ligating and active orthodontics; finding a statistically significant increase at the level of premolars. Lineberger et al [23] used digital casts to study 25 patients treated with self-ligating brackets compared to untreated controls, and found a 2 - 2.2 mm increase in transverse diameter at the level of premolars and an increase in torque.

In the molar sector, the differences found were minimal. There was even reduction between upper and lower first and second molars with the conventional technique, and between second upper molar and first lower molar with the self-ligating technique, with no statistically significant difference between techniques.

Vajaria et al found a significant increase between molars in the treatment with Damon System, but the measurements were made using dental landmarks, so the results may be due to the vestibular inclination of the crowns[21].

In patients with mild to moderate crowding, transverse development with conventional and self-ligating treatments enable dental alignment, in both cases by increasing transverse diameter.

Most of the transverse development occurs in the premolar area with both techniques and is significantly greater with self-ligating orthodontics. Inter-canine distance increases significantly more for both jaws with conventional than with self-ligating orthodontics.

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Evaluation of apoptosis and osteopontin expression in osteocytes exposed to orthodontic forces of different magnitudes

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ABSTRACT

The in vivo response of osteocytes to different force magnitudes soon after they are applied remains to be elucidated. The aim of this study was to examine the early effects of applying a very light (LF: 0,16 N) and a very strong (SF: 2,26 N) orthodontic force during one hour on apoptosis and osteopontin (OPN) expression on alveolar bone osteocytes, in rats. Results: LF: compared to the control group, they showed a significant increase in OPN expression, and a significant decrease in the number of TUNEL-positive osteocytes. SF: compared to the control group, they showed a significant increase in OPN expression and a significant decrease in the number of TUNEL-positive osteocytes. Our results show that osteocytes respond very early to the application of tension and pressure forces of different magnitudes, and application of forces decreases the number of apoptotic osteocytes and increases OPN expression. These results allow concluding that osteocytes activate rapidly when subjected to locally applied forces, whether these forces be pressure or tension, light or strong forces.

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Key words: *osteocytes, mechanical stress, mechanotransduction, orthodontic tooth movement, apoptosis, osteopontin.*

Evaluación de la apoptosis y la expresión de osteopontina en los osteocitos luego de la aplicación de fuerzas ortodóncicas de diferentes magnitudes

RESUMEN

Hasta el momento no se ha dilucidado la respuesta temprana in vivo de los osteocitos a la aplicación de fuerzas de diferentes magnitudes sobre el hueso. El objetivo de este estudio fue examinar la respuesta temprana de la aplicación de una fuerza ortodóncica muy liviana (FL: 0,16 N) y muy fuerte (FF: 2,26 N) durante una hora sobre la expresión de apoptosis y osteopontina (OPN) en los osteocitos del hueso alveolar, en ratas. Resultados: FL: en comparación con el grupo control, mostraron un aumento significativo en la expresión de OPN y una disminución significativa en el número de osteocitos TUNEL-positivos. FF: en comparación con el grupo control, mostraron un aumento significativo en la expresión de OPN y una disminución significativa en el número de osteocitos TUNEL-positivos. Nuestros resultados muestran que los osteocitos responden muy temprano a la aplicación de fuerzas de tensión y presión de diferentes magnitudes, y la aplicación de fuerzas disminuye el número de osteocitos apoptóticos y aumenta la expresión de OPN. Estos resultados permiten concluir que los osteocitos se activan rápidamente cuando se los somete a fuerzas aplicadas localmente, ya sean estas fuerzas de presión o tensión, livianas o fuertes.

Palabras clave: *osteocito, fuerzas mecánicas, mecanotransducción, ortodoncia apoptosis, osteopontina.*

INTRODUCTION

Mechanosensation and transduction in osteocytes enables efficient exchange of physical and chemical signals among cells[1-3], mainly the signals that generate when bone is subjected to mechanical stress, as is the case of orthodontic forces applied to teeth[4].

Orthodontic tooth movement exerts different types of forces which have a different biomechanical effect: pressure and tension. It is assumed that the environmental changes in periodontal tissue during orthodontic tooth movement influence alveolar bone mechanically, acting on osteocyte activity and osteocyte network communication during the adaptive process[5-11]. Studies on alveolar osteocytes showed that application of tension forces during 48 hours was found to result in expression of connexin 43 protein[5], whereas application of pressure forces up to 24 hours resulted in a progressive increase in osteocytes undergoing apoptosis up to 1 day post-application, and a peak in the proportion of necrotic osteocytes and empty lacunae at 2 and 4 days respectively[6]. Results obtained using an experimental

model that combined both types of forces showed DMP-1 (dentin matrix protein-1) expression to increase after 6 hours and peak between days 3 and 7 post-application[7] and MEPE (matrix extracellular phosphoglycoprotein) expression to increase at 3 days[8]. According to reports in the literature, the percentage of osteopontin (OPN) positive osteocytes[9] and of connective tissue growth factor (CTGF) mRNA expressing osteocytes[10] also increased 12 hours after applying a force. Our previous studies showed that both the tensile and compressive forces exerted by orthodontia induced early changes in osteocytes and their lacunae, which manifested as an increase in lacunar volume and changes in lacunar shape and orientation, with an increase in canalicular width and increase in the length of cytoplasmic processes[11].

A study on *in vitro* response of osteoblastic cells to varying rates of fluid shear stress found that nitric oxide (NO) production was linearly dependent on the fluid shear stress rate, showing that strain rate (determined by the frequency and magnitude) is an important parameter for cell activation to stress[12]. However, *in vivo* osteocyte response to different magnitudes of orthodontic pressure and tension forces in terms of apoptosis and OPN expression has not been studied to date. The aim of this study was to examine the early effects of applying a very light (LF: 0,16 N) and a very strong (SF: 2,26 N) orthodontic force during one hour, on apoptosis and osteopontin (OPN) expression on alveolar bone osteocytes, in rats.

MATERIALS AND METHODS

Experimental Tooth Movement

Twenty four nine-week-old male Wistar rats, 220 g average body weight, were divided into groups of eight as follows: control group (C), a second group subjected to a 0,16N (16 gf) orthodontic force during 1 h (LF: light force) and a third group subjected to a 2,26 N (230 gf) orthodontic force during 1 h (SF: strong force). The appliance used to induce experimental tooth movement in the rats consisted of two stainless steel bands cemented to the upper first molars with a bracket welded to its palatal aspect through which a stainless steel wire spring was threaded[7,13]. Square section stainless steel wire (edgewise arch) was used to construct two springs designed to generate a 2,26 N (SF) and a 0,16 N (LF) respectively, towards the palatal aspect of the alveolus, exerting a compresssion force on the palatal site and a tensile force on the buccal site of the same alveolus (Fig. 1).

The animals were anesthetized by intraperitoneal injection of 50 mg/kg and 20 mg/kg body weight of 5% ketamine and 2% xylazine respectively prior to performing the procedures, and euthanized by ether overdose 1 h after applying the force.

All experiments were conducted in keeping with "The National Institutes of Health Guidelines for the Care and Use of Laboratory Animals (NIH Publication 85-23, Rev 1985)". The experiment was approved by the Ethics Committee of the School of Dentistry, University of Buenos Aires (Res CD 015/14).

Tissue Preparation

Maxillae were resected and fixed in 4% formaldehyde in 0.2 M sodium phosphate buffer (PBS) at 4 ºC during 48 hours. The tissues were decalcified in 10% ethylenediaminetetraacetate (EDTA) pH 7.4 at 4 ºC for 60 days, and processed for embedding in paraffin. Five micrometer-thick bucco-palatine oriented sections were obtained at the level of the mesial root of the first upper molar. The specimens were oriented in the microtome under a stereoscopic microscope; they were sectioned to visualize the most axial plane of the mesial root in which the root canal and the apex are completely open, in a longitudinal orientation; the study sections were obtained at this level.

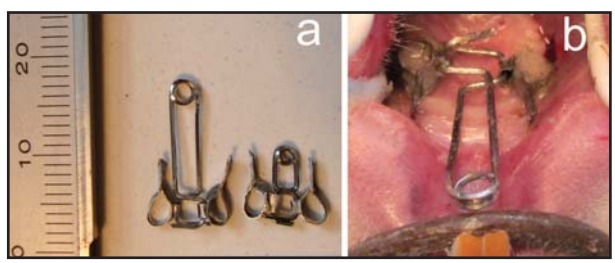

Fig. 1: Experimental orthodontic model. (a) Orthodontic springs: two different springs made of 0.016´´ X 0.016´´ stainless steel wire. Altering the diameter of the loop and the length of the spring allowed obtaining springs that exerted the force magnitudes used here, i.e. LF: 0,16N and SF: 2,26N. (b) Orthodontic appliance: two stainless steel bands were cemented to the upper first molars of experimental rats. Each band had a bracket welded to its palatal aspect through which a stainless steel wire spring was threaded. Because the springs exert force toward the palatal aspect of the alveolus, they exert a tension force on the periodontal aspect of the buccal wall, and a pressure force on the periodontal aspect of the palatal wall.

Detection of DNA fragmentation by TUNEL (transferase-mediated biotin-dUTP nick end-labeling)

The deparaffinized sections were treated with 20 µg/mL proteinase K (DakoCytomation, Carpinteria, CA, USA) in 10 mM Tris-HCL buffer, pH 7.4, at room temperature (RT) for 20 min, and then incubated with 0.30% H_2O_2 in methanol at RT for 30 min to block endogenous peroxidase activity. After rinsing with distilled water, the sections were incubated with TdT containing biotin-16-UTP buffer, pH7.2 (Chemicon, Temecula, CA, USA) at 37ºC for 90 min and then incubated with anti-digoxigenin antibody (Chemicon, Temecula, CA, USA), reacted with 3,3-diaminobenzidine (DAB) (Biogenex, San Ramon, CA, USA) and counterstained with methyl green. Sections of mammary gland after weaning were used as positive controls.

OPN Immunohistochemistry

The sections were incubated in 0.30% H_2O_2 in methanol for 30 min, followed by a wash in 10 mM phosphate-buffered saline (PBS) (pH7.2) during 10 min and incubated with 1% bovine serum albumin (BSA) (Sigma Chemical Co., St Louis, MO, USA) at RT for 30 min to block nonspecific binding of the antibody. The anti-OPN primary antibody (anti-rat OPN monoclonal antibody (Akm2A1:sc21742), Santa Cruz Biotechnology Inc., California, USA) was diluted 1:3000 in 10 mM PBS containing 0.10% BSA and 0.05% Tween 20 (Sigma-Aldrich, St. Louis, MO, USA). The sections were incubated with the primary antibody at 4 ºC for 18 h. For control experiments, sections were incubated with non-immune rabbit IgG in place of the primary antibody. The primary antibody was detected using the avidin-biotin-peroxidase complex (ABC kit) (Biogenex, San Ramón, CA, USA) following instructions on the data sheet and reacted with DAB (Biogenex, San Ramón, CA, USA). The sections were counterstained with hematoxylin. Human bone marrow sections were used as positive control.

The number of TUNEL-positive osteocytes, the number of OPN-expressing osteocytes, and the proportion of OPN-expressing bone matrix were determined in an area measuring 100 µm wide and covering the full length of the periodontal aspect of both the buccal and the palatal walls of the alveolus. The immunolabeled sections were photographed using a 40X objective and imported into image analysis software for quantification. The number of TUNEL or OPN-positive osteocytes, defined as osteocyte cell bodies exhibiting brown staining, and the number of TUNEL or OPN-negative osteocytes, defined as osteocyte cell bodies exhibiting (methyl) green or hematoxylin staining respectively, were counted on each section. The percentage of immuno-labeled-positive osteocytes was calculated as the number of positive cells divided by the total number of osteocytes (positive and negative).

Statistical analysis

Results are shown as the mean ± standard deviation (SD). Data were compared using one-way analysis of variance (ANOVA) and Dunnett post-hoc test. Values of $p < 0.05$ were considered statistically significant.

RESULTS

Histological observations

At the experimental time points studied herein, the width of the periodontal ligament (PDL) was narrower on the palatine wall of the alveolus (pressure strain side) and wider on the buccal wall of the alveolus (tension strain side) in experimental groups compared to the control. This compression and stretching of the PDL was even along the full length of the corresponding wall and no hyalinized tissue was evident in any of the cases (Figure 2).

TUNEL detection of apoptosis.

A significant decrease in the number of TUNEL-positive osteocytes was observed on both the tension and pressure strain sides in the light-force (82.22% and 64.3% decrease respectively) and the strong-force (74.5% and 53.8% respectively) groups, compared to controls. The number of TUNEL-positive osteocytes was lower on the tension side in both experimental groups (Fig. 3).

OPN Immunohistochemistry

The number of OPN-expressing osteocytes was found to increase significantly on both the tension and pressure strain sides in both experimental groups compared with controls (148% and 172.7% increase corresponding to the light force and 117.6% and 116% increase in the case of the strong force). In addition, the proportion of OPN-expressing bone matrix was found to increase significantly on both

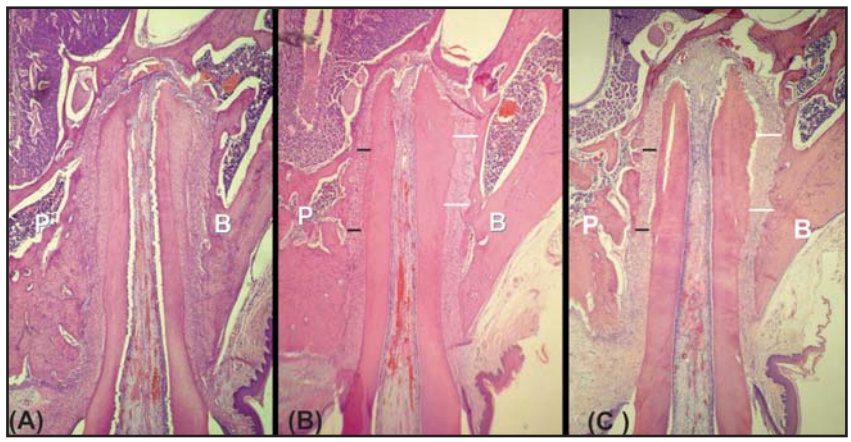

Fig. 2: Histological observations. Microphotographs of the mesial root of the first molar of a control rat (A) and experimental rats (B: LF; C: SF), showing the areas on the buccal side (tension strain side) and on the palatal side (pressure strain side) where we performed the immunohistochemical determinations. H-E,10X. White and black bars show stretching (B: buccal side) and compression (P: palatal side) of the PDL respectively occurred evenly along the full length of the corresponding wall. H-E, 10X.

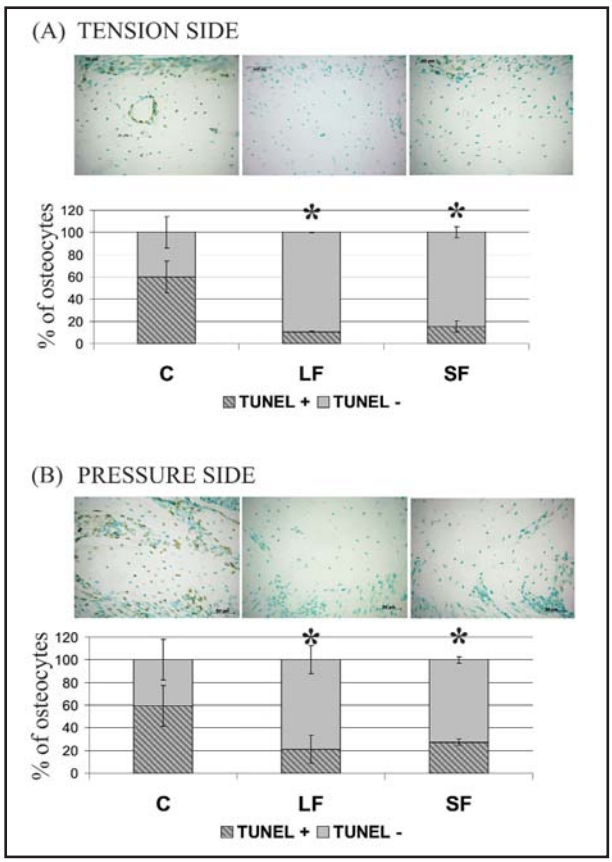

*Fig. 3: Immunohistochemical analysis of apoptosis using TUNEL. Tension (A) and pressure (B) strain sides. The figure shows that the percentage of TUNEL+ osteocytes decreased significantly on both the pressure and tension strain sides in animals under orthodontic forces compared to controls. Values are expressed as mean ± SD. *Statistically significant difference between control and experimental groups, $p<0.05$. SF: strong force, LF: light force, C: control. Representative microphotographs of paraffin-embedded sections immunostained for DNA fragmentation (brown) and counterstained with methyl green to show the number of osteocytes undergoing apoptosis.*

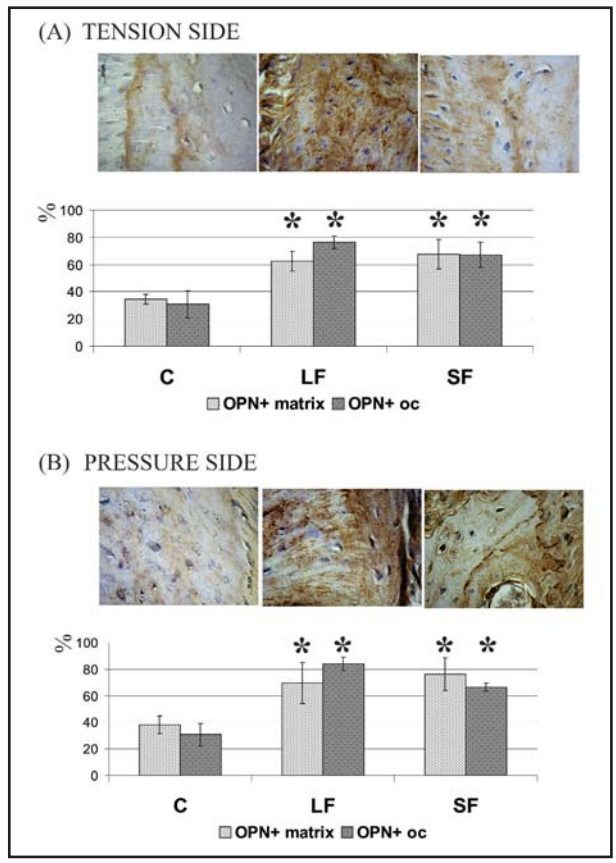

*Fig. 4: OPN immunostaining. Tension (A) and pressure (B) strain sides. The figure shows the percentage of OPN+ osteocytes and the percentage of OPN+ mineralized matrix. The percentage of OPN+ osteocytes increased significantly on both the pressure and tension sides in groups subjected to orthodontic forces compared to the control group; the increase was greater in those subjected to LF. The percentage of OPN+ mineralized matrix also increased significantly on both the pressure and tension sides in groups subjected to orthodontic forces compared to the control group. Values are expressed as mean ± SD. *Statistically significant differences between control and experimental groups, $p<0.05$. SF: strong force, LF: light force, C: control. Representative microphotographs of paraffin-embedded sections immunostained for OPN (brown) and counterstained with hematoxylin to show the overexpression of the protein both inside the cell and in the bone mineralized matrix.*

the tension and pressure strain sides in both experimental groups compared with controls (81.66% and 82.12% increase in the case of the light force and 96.85% and 100.16% increase in the strong force group) (Fig. 4).

DISCUSSION

This is the first study to evaluate the early *in vivo* response of alveolar osteocytes to different force magnitudes applied using an experimental model of orthodontic tooth movement. In this regard, the design of the spring used in this study allowed comparing the effect of a very light and a very strong force on both the tension and pressure strain sides. The orthodontic model used in the present study allows adapting the shape of the spring in order to vary the magnitude of the force and therefore analyze the biological effect of force magnitude on the cells involved in the adaptive remodeling process.

In the present study, osteocytes exhibited an early response to both tension and pressure forces of different magnitudes applied to bone. Specially, a significant decrease in the number of TUNEL-positive osteocytes was observed both in the light and strong force groups, with a marked diminution on tension strain sides. In other study[6], alveolar bone subjected to orthodontic forces exhibited a gradual increase in TUNEL positive osteocytes on the pressure side after 3h, peaking at 24 h. It is noteworthy however, that the authors encountered hyalinization in the periodontal ligament (PDL) on the pressure side where they performed the determinations, and they suggested that hyalinized PDL would impair transport systems resulting in ischemia or hypoxia, which would trigger osteocyte cell death. Conversely, though bearing in mind the differences between ours and their studies as regards the experimental conditions, our results showed neither an increase in the number of TUNEL-positive osteocytes nor hyalinization. It is therefore possible that since the mechanical stress in our experiment does not induce hyalinization of the PDL not impairing nutritional supply, osteocytes are prevented from entering apoptosis. In addition, it is known that forces exert an antiapoptotic effect. Shear stress reverses apoptosis of endothelial cells induced by different stimuli[14]. Moreover, shear stress upregulates the expression of integrins, and potential mechanotransducers located at the cell surface[15]. Various integrins prevent apoptosis[16,17] and it has been suggested that antiapoptotic integrin signaling involves the activation of the extracellular signal–regulated kinase (ERK) 1/2[18]. Several *in vitro* studies have shown that mechanical stimulation inhibits osteocyte apoptosis caused by serum-starvation[19], dexamethasone[20], and TNF-α[21]. Osteocytes detect fluid shear stress inside the lacuno-canalicular network via integrins[22-24], and NO production is initiated immediately[12]. NO prevents apoptosis of endothelial cells[25] and could be thought to have the same effect on osteocytes. NO production *in vitro* was found to be linearly dependent on fluid shear stress rate[12], and this may account for the differences, though not significant, in the percentage of the decrease in apoptosis observed between the force magnitudes used in the present study. Another response of osteocytes to mechanical loading is the immediate release of several growth factors[26]. By binding to cell-surface receptors, growth factors activate signaling pathways involving Bcl-2 family members that suppress apoptosis. In an unloaded model, apoptosis of osteocytes is associated with a transient decline in integrin and Bcl-2 survival protein levels[27]. Moreover, it has been demonstrated that mechanical stimuli preserve osteocyte viability via activation of ERKs and new gene transcription *in vitro*[20] and prevent glucocorticoid-induced apoptosis in MLO-Y4 osteocytic cells[28]. Even, a more recent study shows that mechanical stimulation for just 10 min is sufficient to trigger survival signaling in osteocytic cells[29]. In our work, mechanical reversion of osteocyte apoptosis was observed in osteocytes of groups subjected to orthodontic forces. It is therefore possible that NO production inside the lacuno-canalicular system, ERKs activation and autocrine effect of mechanical-induced growth factors secretion may be some of the mechanisms involved in protecting osteocytes from apoptosis, when subjected *in vivo* to mechanical stress exerted by orthodontic forces under the experimental conditions used herein. Based on the above, we conclude that forces within the physiological range seem to be an important survival factor for osteocytes at the experimental time points used in this study. Further *in vivo* studies must be conducted in order to confirm our observation that mechanical stimulation prevents, or at least delays osteocytes from entering apoptosis, triggering a

response that mediates activation of the remodeling process induced by the mechanical loads.

In our study, the potential association between force magnitude and OPN expression was evaluated on both the pressure and tension strain sides after applying orthodontic forces, showing a significant increase in OPN expression in all cases, and highest values in osteocytes subjected to the light force. OPN is considered to play an important role in bone remodeling since it is thought to promote or regulate the chemotaxis and attachment of osteoclasts to the bone surface during bone resorption[30]. Studies reported in the literature found that osteocytes located near resorption sites expressed OPN during physiological tooth movement[31], and almost all osteocytes expressed OPN 48 h after applying an orthodontic force, prior to osteoclast recruitment to the bone surface[9]. We found that OPN expression also increased significantly in the mineralized bone matrix of bone under stress, suggesting that this OPN was synthesized by pre-existing mature osteocytes and not by osteocytes recently included in the matrix, or by active osteoblasts located on the bone surface. This phenomenon suggests clear activation of osteocytes associated with communication via the lacuno-canalicular network inside the mineralized bone matrix. Interestingly, we also found that OPN expression also increased significantly in the tension side, where bone formation is stimulated. Accordingly with our results, Morinobu *et al* showed that OPN expression was enhanced during bone formation under tensile stress to calvarial sutures suggesting that the presence of OPN is one of the positive factors for osteoblastic bone formation in the suture under mechanical stress[32]. Our results demonstrate that tension and pressure forces of physiological magnitude applied *in vivo* seem to activate mechanotransduction and to promote the remodeling process via early expression of osteocyte OPN.

It is noteworthy that both pressure and tension forces were found to generate immediate osteocyte response. The marked decrease in apoptosis, and the significant increase in OPN expression observed soon after applying different forces (as regards type and magnitude) allow concluding that osteocytes activate very rapidly when subjected to locally applied forces *in vivo*, whether these forces be pressure or tension, light or strong forces, and lends support to the role of osteocytes as mediators of the activation of bone resorption and formation, which appear as the final response to the application of orthodontic forces.

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Self-medication in patients seeking care in a dental emergency service

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ABSTRACT

The aims of this work were: To determine what percentage of first-time patients to the Dental Emergency Department at the School of Dentistry of Buenos Aires University had taken medications to relieve or treat their condition. To determine what percentage of these had used self-medication, and which were the most frequently taken medicines. To determine whether there is an association between self-medication and educational level, and between self-medication and whether the patient has health coverage. This was an observational, cross-sectional study which reviewed 567 clinical histories of patients who visited the Dental Emergency Department from March 2015 to September 2016. The following parameters were assessed: sex, age, reason for consultation, medication, dose, interval, duration and indication. Patients' educational level and whether they had health coverage were ascertained. Confidence intervals of 95% were calculated for percentages using the Wilson score method. Inferential analyses were performed using the Chi-square test (χ^2). Significance level was set at 5%.

Eighty five percent (85%,.n=481) of the patients had taken at least one medication; 77% (n=372) had used self-medication. The most frequently used medicines were non-steroid anti-inflammatory drugs (61%), antibiotics (34%) and glucocorticoids (2%). No association was found between self-medication and patients' having health coverage (χ2=13; p=0.08). No significant association was found between educational level and self-medication (χ2=10; p=0.22). Nevertheless, the lowest percentages of self-medication were found in subjects with complete university studies (77%; CI95: 60% to 89%), while the highest percentages were found in subjects with incomplete primary education (89%; CI95: 69% to 97%), complete primary education (92%; CI95: 82% to 96%) and incomplete secondary educations (90%; CI95: 84% to 94%).High levels of self-medication were found in the study population. Although no association was found between educational level and self-medication behavior, the percentage of self-medication was higher among patients with lower educational levels. The high level of self-medication highlights the importance of conducting campaigns to raise awareness about the adequate use of medicines.

Key words: *self-medication, emergency service, hospital, dentistry.*

Automedicación en pacientes que concurren a un servicio de guardia odontológica

RESUMEN

Los objetivos del presente trabajo fueron: Determinar qué porcentaje de pacientes que concurrió por primera vez al Servicio de Urgencias de la Facultad de Odontología de la Universidad de Buenos Aires consumió medicamentos para aliviar o tratar su dolencia. Determinar qué porcentaje de pacientes fueron automedicados, y cuáles fueron los medicamentos más utilizados. Determinar si existe relación entre la automedicación y el nivel de estudio y entre la automedicación y la presencia de cobertura médica. Se realizó un estudio observacional y transversal. Se relevaron 567 historias clínicas de pacientes que concurrieron entre marzo 2015 y septiembre 2016 y se valoraron los siguientes parámetros: sexo, edad, origen de la consulta, medicación, dosis, intervalo, duración, e indicación. Se indagó el nivel educacional alcanzado y la existencia de cobertura médica. Se calcularon intervalos de confianza al 95% para porcentajes mediante el método score de Wilson. Se realizaron análisis inferenciales mediante la prueba Chi-cuadrado (χ2). Se fijó un nivel de significación del 5%.

El 85% (n=481) de los pacientes había consumido al menos un medicamento. El 77% (n=372) de los pacientes estaba automedicado. Los medicamentos más utilizados fueron anti-inflamatorios no esteoroideos (61%), antibióticos (34%) y glucocorticoides (2%). No se encontró asociación entre la

automedicación y la presencia de cobertura médica (χ2=13; p=0,08). No se encontró asociación significativa entre el nivel de estudios y la automedicación (χ2=10; p=0,22). Sin embargo, los sujetos con estudio universitario completo presentaron el menor porcentaje de automedicación (77%; IC95: 60% a 89%), mientras que los mayores porcentajes se encontraron en sujetos con primario incompleto (89%; IC95: 69% a 97%), primario completo (92%; IC95: 82% a 96%) y secundario incompleto (90%; IC95: 84% a 94%).

Se encontraron niveles elevados de automedicación en la población estudiada. Si bien no se observó asociación entre nivel educativo y la conducta de automedicación, fue mayor el porcentaje de automedicación en pacientes con menor nivel educativo. La alta presencia de automedicación refuerza la importancia de realizar campañas de concientización sobre el consumo adecuado de medicamentos.

Palabras clave: *Automedicación, servicio de urgencia, odontología.*

INTRODUCTION

According to the World Health Organization, self-medication is the selection and use of medicines by individuals to treat self-recognized diseases or symptoms[1,2].

Acute dental pain is, in many cases, invalidating and is often described as one the most severe a person can suffer in everyday life. In dentistry, patients often recur to self-medication to reduce dental pain, mainly by taking analgesics and sometimes including antibiotics[3]. This practice has numerous consequences, including an increased risk in untoward effects, drug-drug interactions, increased bacterial resistance to antibiotics, masking of underlying pathologies and/or conditions and reduction of the efficacy of treatment due to inadequate or insufficient use of medicines[4].

Self-medication today is still relevant to public health worldwide, with 8% to 13% prevalence in European and North American populations. This high frequency may be attributed to the availability of medicines to society and sometimes to poor coverage in healthcare services[5].

In Argentina, a survey conducted by the Argentine Pharmaceutical Confederation found that out of 1500 respondents in the cities of Buenos Aires and Córdoba, 82% take over-the-counter medicines and more than half of the individuals are unaware of the untoward effects they can cause. It is estimated that 11% of all cases of chronic kidney failure are a consequence of the use of analgesics and 40% of high digestive hemorrhage cases can be attributed to acetylsalicylic acid and other non-steroid anti-inflammatory drugs (NSAIDs)[6].

To date, no study has been published in Argentina reporting the extent of self-medication in situations of urgent dental conditions. We aim here to study the prevalence of self-medication in patients seeking dental care at the Dental Emergency Service of the School of Dentistry at the University of Buenos Aires. We also aimed to determine a possible association between self-medication and type of health coverage and/or patient's educational level.

MATERIALS AND METHODS

A total of 567 clinical histories of patients who spontaneously visited the Dental Emergency Department of the School of Dentistry at the University of Buenos Aires seeking dental care from March 2015 to September 2016 were analyzed. Exclusion criteria were patients younger than 16 years, patients consulting for esthetic reasons and patients failing to provide written informed consent to participate in the study. Before case resolution, clinical histories were prepared, type of urgency determined, and patients were asked to declare any prior care or medicine they have taken whether prescribed or self-indicated. Patients on medication were asked to indicate type of medicine, origin of treatment, dose, route of administration, interval and duration. Self-perceived improvement of patient condition, total duration and/or possible discontinuation of the treatment was also recorded. The sample consisted of 381 males (67.2%) and 186 females (32.8%) with median age 34 and a range between 16 to 89 years.

Confidence intervals of 95% were calculated for percentages using the Wilson score method. Inferential analyses were performed using the Chi-square test (χ^2). Significance level was set at 5%.

RESULTS

Of recorded cases, 85% (n=481; CI95: 82% to 88%) had taken at least one medicine, while 15% (n=109; CI95: 12% to 18%) declared no medication related to the dental episode prior to consultation (χ^2=275; p<0.05). Of total medicated patients, 77% (n = 372; CI95: 73% to 81%) recurred to self-indication, while 23% (n=109; CI95: 19% to 27%) had taken medicines under professional prescription (82% dentists, 18% physicians).

No significant differences (χ2=1.55; p=0.21) were observed when data was analyzed by gender, with 85 (325/381) and 84% (156/186) medication rates for males and females, respectively.

Among the 567 urgencies treated, the reason for consultation was primarily endodontic in 60% of included cases (n=340; CI95: 56% to 64%), infectious in 16% (n=92; CI95: 13% to 19%), post-surgical problems in 12% (n=67; CI95: 9% to 15%), periodontal in 5% (n=31; CI95 4% to 8%), temporomandibular joint disorders in 1% (n=5; CI95: 0% to 2%), and miscellaneous reasons in 6% (n=32; CI95: 4% to 8%). Fifty-six percent (n=269; CI95: 51% to 60%) of patients had taken one medicine, 36% (n=173; CI95: 32% to 40%) had taken 2, and 8% (n=39; CI95: 6% to 11%) had taken three or more.

Self-medicated patients used medicines from a variety of therapeutic classes (χ^2=667; p<0.05). The most frequently used category was NSAIDs (61%; CI95: 57% to 64%), followed by antibiotics (34%; CI95: 31% to 38%), glucocorticoids (2%; CI95: 1% to 3%), and others (3%; CI95: 2% to 5%).

Among NSAIDs, the most frequently used was ibuprofen (45%; CI95: 41% to 50%), followed by ketorolac (33%; CI95: 28% to 37%), diclofenac (11%; CI95: 8% to 14%), and others 11%; CI95: 9% to 15%) (Table 1).

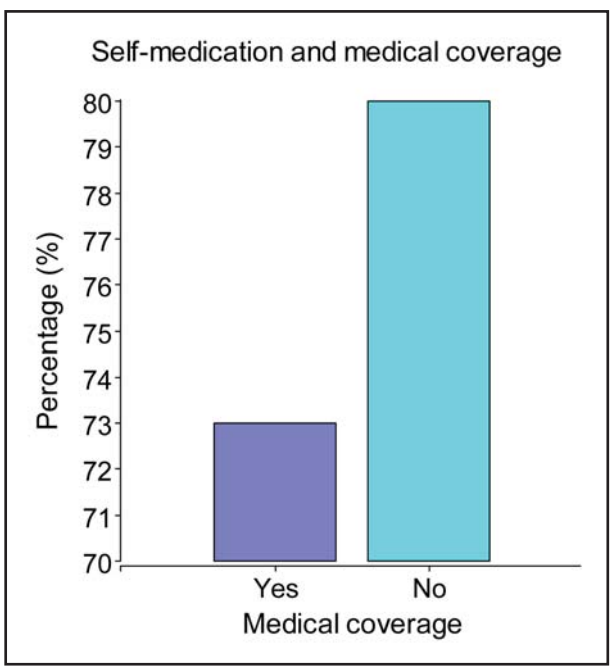

Fig. 1: Percentage of self-medicated patients and medical coverage.

A wide range of antibiotics was used. Among self-medicated patients, the most frequent antibiotic was amoxicillin (80%, CI95: 75% to 85%), followed by amoxicillin/clavulanate association (10%; CI95: 7% to 14%), azithromycin (6%; CI95: 4% to 10%), cefalexin (2%; CI95: 1% to 5%), and others (2%; CI95: 1% to 5%) (Table 2).

The rate of self-medication was also studied in terms of type of health coverage presented by patients. As shown in Fig.1, when analyzed against health coverage, a trend that did not reached statistical significance (χ^2=3.18; p=0.08) towards a higher use of medicines without prescription was detected in patients lacking health insurance.

No significant association was found either between educational level and self-medication (χ2=10; p=0.22). Nevertheless, the percentage of subjects with complete university studies who used self-medication was lower (77%; CI95: 60% to 89%), as compared to subjects with incomplete primary education (89%; CI95: 69% to 97%), complete primary education (92%; CI95: 82% to 96%) and incomplete secondary education (90%; CI95: 84% to 94%) (Fig. 2).

Table 1: Frequency of NSAID use in the self-medicated group.

Drug	%
Ibuprofen	45%
Ketorolac	33%
Diclofenac	11%
Acetaminophen	5%
Flurbiprofen	1%
Clonixin	4%
Piroxicam	1%
Total	100%

Table 2: Frequency of antibiotic use in the self-medicated group.

Drug	%
Amoxicillin	80%
Amoxicillin / Clavulanate	10%
Azithromycin	6%
Cephalexin	2%
Others	2%
Total	100%

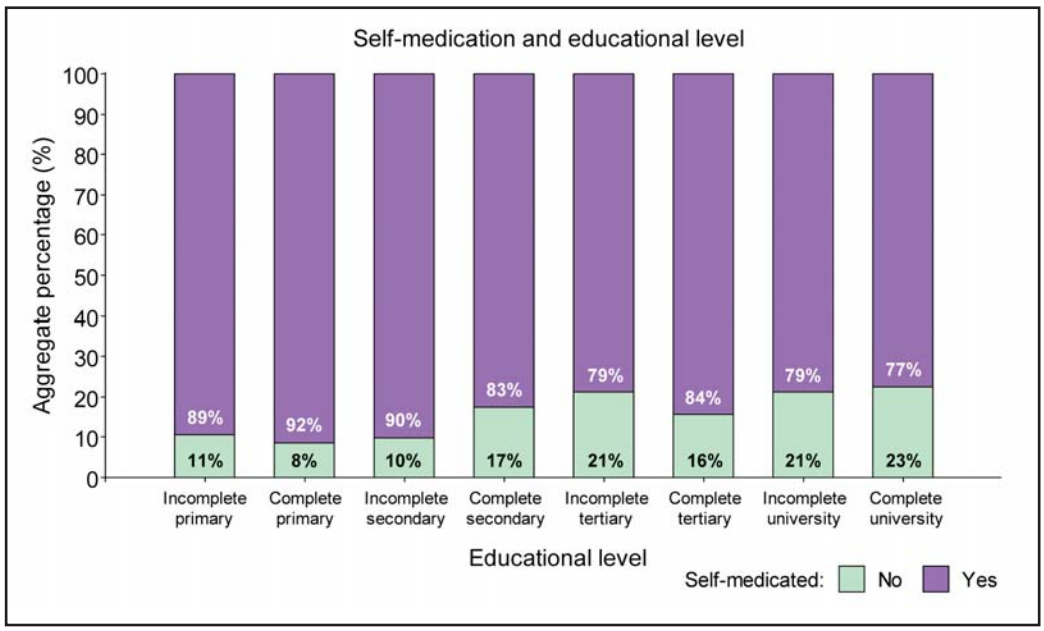

Fig. 2: Educational level for all patients in the study.

DISCUSSION

Self-medication is a very common habit in Latin America. We report here that 77% of the patients surveyed at the Dental Emergency Service of the School of Dentistry at the University of Buenos Aires incurred in self-medication. Our results agree with those presented by Ramay, et al that reported 79% self-medication with antibiotics in suburban areas and 77% in central areas of Guatemala City[9]. Other investigators also reported similar results in Bolivia[13], with 72% and Peru[14, 15] with 81 and 69% prevalence of self-medication, respectively. Even higher numbers for self-medication were reported among university students in Brazil[11] and medical students in Cuenca, Ecuador[12] while a 41% prevalence was informed in Mexico[10]. Differences in results may be accounted by the type and size of population surveyed and institution in which the study was performed, among other variables. Our study included patients who spontaneously sought attention by the Emergency ward at a Dental University Hospital in central Buenos Aires, Argentina. Our study surveyed also all types of medications related to the dental condition affecting the sampled individuals and is, thus, not limited to a single class of therapeutic agents.

Prescription legislation in Argentina is extensive and current laws were originally established in the 1960s. The fact that 70% of the medicines that enrolled patients declare to use in our study require prescription, shows that neither patients nor pharmacies comply with the enforced regulation. Analgesics were most frequently used by patients, as reported in other studies [4,7,10,12-15]. Although not statistically significant, a lower prevalence of self-medication was detected among patients with complete university studies, something that points out to a possible relationship between instruction and a more responsible use of medication.

Some of the causes of these high figures in Latin America might be deficient healthcare services, long waiting times at urgent care service providers, cost of medical care, media advertising and the role of pharmacists and healthcare professionals. Many patients are unaware of the consequences of self-medication, which include increased risk of untoward reactions to drugs, drug-drug interaction, increase in bacterial resistance, masking of underlying pathologies and/or conditions and reduction in the efficacy of treatment as a result of inadequate or insufficient use of medicines.

CONCLUSION

Strategies should be developed to ensure that physicians, dentists, nurses and pharmacists make rational use of medicines. Other effective strategies may be campaigns to raise awareness among the population regarding the consequences of self-medication and improved access to primary healthcare centers.

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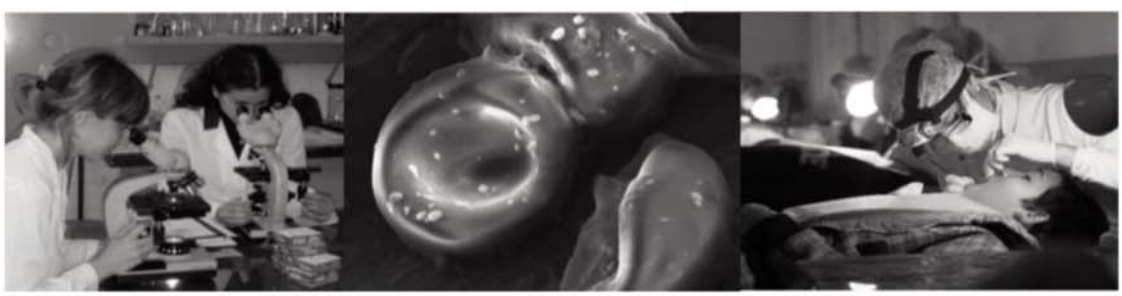

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