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

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# Association between history of orthodontic treatment and sociodemographic factors in adolescents

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***ABSTRACT***

*The aim of this study was to assess history of orthodontic treatment and its determinants in adolescents. This was a cross-sectional study conducted in the city of Passo Fundo, Brazil, on a representative sample of adolescents aged 15 to 19 years, regularly enrolled in 20 high schools. A structured questionnaire was applied to assess demographic, behavioral and health variables. The association between history of orthodontic treatment and the dependent variables was analyzed by the chi-square test or Fisher's exact test. Additionally, multivariate regression with robust variance was performed. A total 736 students were examined and interviewed, of whom 57.6% had undergone orthodontic treatment, while 42.4% had not. In the multivariable analysis, the following variables were significantly associated with history of orthodontic treatment: female (PR= 1.26; 95% CI: 1.11 – 1.43), white (PR= 1.32; 95% CI: 1.11 – 1.56), mothers with higher level of education (PR=1.49; 95% CI: 1.28 – 1.74), tooth loss (PR=1.21; 95% CI: 1.06 – 1.39), and concern about oral health (PR=0.69; 95% CI: 0.61 – 0.78). Attending a private school was not significantly associated with history of orthodontic treatment (p> 0.05). This study found a high rate for history of orthodontic treatment among adolescents, associated with gender, ethnicity, adolescent's mother with higher education, and tooth loss. Concern about oral health was a protective factor for orthodontic treatment.*

***Key words:*** *Adolescence; Esthetics, dental; Orthodontics.*

## Associação entre histórico de tratamento ortodôntico e fatores sociodemográficos em adolescentes

***RESUMO***

*Esse estudo objetivou verificar o histórico de tratamento ortodôntico e seus fatores associados. Esse estudo transversal foi conduzido na cidade de Passo Fundo, Brasil, com uma amostra representativa dos adolescentes regularmente matriculados no ensino médio, com idades entre 15 e 19 anos, de 20 escolas. Um questionário estrutura foi aplicado para acessar variáveis demográficas, comportamentais e de saúde. As associações entre histórico de tratamento ortodôntico e as variáveis independentes foram analisadas pelos testes de qui-quadrado ou exato de Fisher. Além disso, regressão multivariada com variância robusta foi realizada. 736 estudantes foram examinados e entrevistados. Tratamento ortodôntico foi realizado por 57,6% dos adolescentes, enquanto que 42,4% dos participantes não o mencionaram. Na análise multivariada, as seguintes variáveis estiveram significativamente associadas com histórico de tratamento ortodôntico: sexo feminino (PR= 1,26; 95%CI: 1,11 – 1,43), branco (PR= 1,32; 95%CI: 1,11 – 1,56), mães com alto nível educacional (PR=1,49; 95%CI: 1,28 – 1,74), perda dentária (PR=1,21; 95%CI: 1,06 – 1,39) e preocupação com a saúde bucal (PR=0,69; 95%CI: 0,61 – 0,78). Ir a uma escola privada não esteve significativamente associado com histórico de tratamento ortodôntico (p> 0,05). Esse estudo demonstrou que altos níveis de histórico de tratamento ortodôntico são encontrados em adolescentes e isso está associado com sexo, etnia, alta escolaridade da mãe do adolescente e perda dentária. Preocupação com a saúde bucal mostrou-se como um fator protetor para o tratamento ortodôntico.*

***Autores:*** *Adolescência-Estética, Dentária- Ortodontia.*

## INTRODUCTION

Malocclusion is the third largest oral health problem in terms of public health[1], and may influence appearance, with impact on self-esteem and quality of life[2,3]. Epidemiological studies on occlusion patterns in a given population are important because they reveal the prevalence of malocclusions and their impairment severity, indicating the normative need for orthodontic treatment[4]. Such data are used for planning treatment priorities in public health, based on the principle of equity in oral healthcare[5]. The Index of Orthodontic Treatment Need (IOTN) developed by Brook and Shaw[6], is one of the instruments that assess treatment need. It classifies

the need for orthodontic treatment considering the importance and severity of occlusal aspects for health and dental function[7]. According to the latest national survey in Brazil[8], prevalence of malocclusions among 15- to 19-year-olds was 35.6%, of which 20.3% were classified as definite malocclusion, 6.2% as severe malocclusion, and 9.1% as very severe malocclusion. Following these criteria based on the DAI, 64.4% of Brazilian adolescents would have little or no treatment need, 20.3% would have elective treatment need, 6.2% would have highly desirable treatment need, and 9.1% would have mandatory treatment need[9]. It is estimated that about one third of the population presents an obvious need for orthodontic treatment[10].

The national survey in Brazil did not report exposure to orthodontic treatment, either current or in the past. Generally, the country's public system does not provide such treatment, and these patients usually seek private clinicians. Moreover, their treatment needs are evaluated on an individual basis.

Prevalence of malocclusion varies widely depending on the method of evaluation. A study in India found that the prevalence of children with malocclusion who do not require immediate orthodontic treatment was 63% higher than those who do not need treatment[11]. Another study, conducted at the University of Leuven in Belgium, evaluated the orthodontic treatment needs of 11- to 16-year-olds[12], finding orthodontic treatment need for the esthetic component in 38.3% of the subjects. When the dental component was evaluated separately, the treatment need increased to 80.3%.

However, few studies have addressed the history of orthodontic treatment, either completed or in progress. There seems to be a difference between the prevalence of malocclusion and the normative need for treatment. The aim of this study was therefore to assess history of orthodontic treatment among high school adolescent students from public and private schools in a city in southern Brazil. The null hypothesis is that there is no statistically significant difference in the history of orthodontic treatment in adolescents regarding gender, ethnicity, mother's level of education, type of school, self-reported health problems, tooth loss, and concern about oral health in general.

## MATERIAL AND METHODS

### *Study design and location*

This cross-sectional study examined 15- to 19-year-olds enrolled in public and private high schools of the city of Passo Fundo, which is located in the north of the state of Rio Grande do Sul, Brazil, 300 km from the state capital, Porto Alegre. The population is about 190,000, according to the Brazilian Institute of Geography and Statistics[13]. More than 95% of the population lives in the urban area, with a poverty incidence of 27.91% and a Gini Index of 0.41. In 2012, the city recorded 7,558 students enrolled in regular high school in 23 schools, comprising 16 public schools and 7 private schools, all in the urban area. Of this total, 6,256 (82.78%) students attended public schools, and 1,302 (17.22%) attended private schools[14].

### *Ethical considerations*

The Institutional Review Board of the University of Passo Fundo approved the present study following the authorization from the 7th Regional Office of Education to carry out the study at public schools, and after formal approval by the principals of the private schools. All selected students provided an Informed Consent Form signed by their parents or legal guardians, and everyone present on the day of the survey was examined.

### *Sample*

The study coordinator visited all 23 high schools and invited them to participate. The sample consisted of thirty percent of the students from each accepting school, who were randomly chosen by draw from the lists of all students aged 15 to 19 years, regardless of their school schedule. Randomization was performed in two blocks, according to the distribution of male and female students.

The research team visited all classrooms that included selected students to present the aims of the study. After the explanation, the selected students received the Informed Consent Form to be signed by their parents or guardians. In case of absence, a second contact was made. The study included 736 students, who are representative of the adolescents regularly enrolled in the Passo Fundo high school system.

***Clinical examination and questionnaire completion***

A structured questionnaire, including demographic data, socioeconomic condition, general health behavior, health record, and oral health self-perception, was applied with the use of a set of questions from the PCATool-SB Brazil adult version, validated in Brazil[15]. Aspects regarding oral esthetics and concern about oral health were obtained through the application of a questionnaire validated for Brazilians[16]. The questionnaire explores the perception of the appearance of teeth and oral health concerns, which consider the last two months or the adolescent's current self-perception. We used four questions of the abovementioned questionnaire to assess the adolescents' concern regarding oral health, the appearance of their teeth, and tooth alignment and color[16].

All participants were asked the following question: "Have you ever been under any type of orthodontic treatment?" The answer to this question was used to classify the sample into "no orthodontic treatment", "history of orthodontic treatment", and "current orthodontic treatment". The research team asked the adolescents all the questions and completed the questionnaire.

Interviews followed by clinical examinations were conducted at the schools from April to July 2012. Clinical examinations were performed with the help of a wooden spatula, to verify ongoing orthodontic treatment and count all teeth except third molars.

The examinations and interviews were conducted by teams of five dental students who had been trained and validated by the study coordinator to ensure standard procedures. The training consisted of theoretical classes including a review of the literature on the subject, as well as reading and receiving an explanation of each question from the questionnaire. In order to assess reproducibility, after one week, the study coordinator re-examined and re-interviewed ten percent of the participating students randomly chosen by draw. The agreement rate between tests for tooth count was 98%. Teeth that could somehow be restored were counted, and teeth or roots indicated for extraction were not counted.

***Statistical analysis***

History of orthodontic treatment was considered the main outcome of this study. Adolescents under orthodontic treatment at the time of the examination and those who reported previous treatment were categorized as having a history of orthodontic treatment. All the independent variables analyzed are shown in Figure 1.

The independent variables were categorized as follows. Ethnicity was classified as white or non-white, with non-white including subjects who referred to themselves as being black, yellow, brown or indigenous. Socioeconomic condition was assessed by information on income and education. Mother's level of education was classified into three groups: complete or incomplete higher education, complete or incomplete high school, and up to elementary school. Type of school (public or private) was used as an income proxy, as students from public schools were considered to come from lower income families.

General health problem was dichotomized as either yes or no. Tooth loss was dichotomized as "yes" with at least one tooth loss, and as "no" for those who had 28 teeth. Concern about oral health was dichotomized as "yes" for those who were concerned about the health of their teeth, and "no" for those who were not concerned. Dental esthetics was assessed through three questions: whether the subjects were bothered by the appearance of their teeth, and whether they were concerned about tooth alignment and tooth color. These answers were dichotomized as yes or no.

Associations between the dependent variable and independent variables were assessed by either the chi-square test or Fisher's exact test. Uni- and multivariate analyses were performed using Poisson regression to assess the association between dependent and independent variables. The

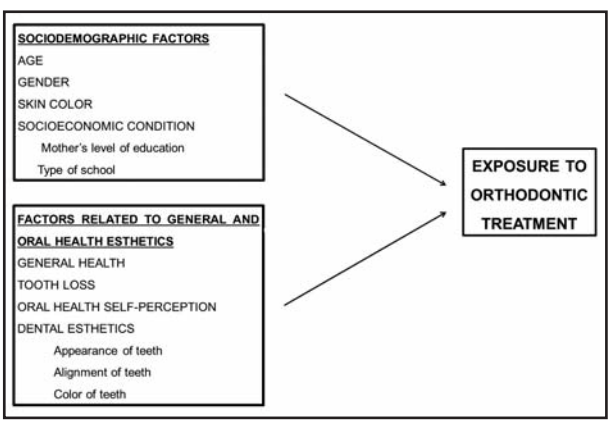


*Fig. 1: Explanatory variables for history of orthodontic treatment.*

covariates were chosen based on either association in univariate analysis (p<.25) or from conceptual basis. The significance level applied was 5%. Data analysis was performed using the statistical package SPSS 18 (SPSS Inc., Chicago, United States).

## RESULTS

Of the 23 schools invited, 20 accepted to take part in the study, including all 16 public and 4 private schools. Of the 6,122 students eligible for the study at the 20 schools selected, 1,836 students were chosen by draw and invited to participate. Seven hundred and thirty-six adolescents (736) accepted the invitation, all of whom were interviewed and received oral examination, generating a 40.08% rate of participation. The main reasons for exclusion are shown in Figure 2, and all adolescent were included in the final statistical analyses. Of these, 323 (43.9%) were males, and 413 (56.1%) were females. Of the total 736 participants, 620 (84.2%) were from public schools and 116 (15.8%) from private schools. These rates were similar to overall

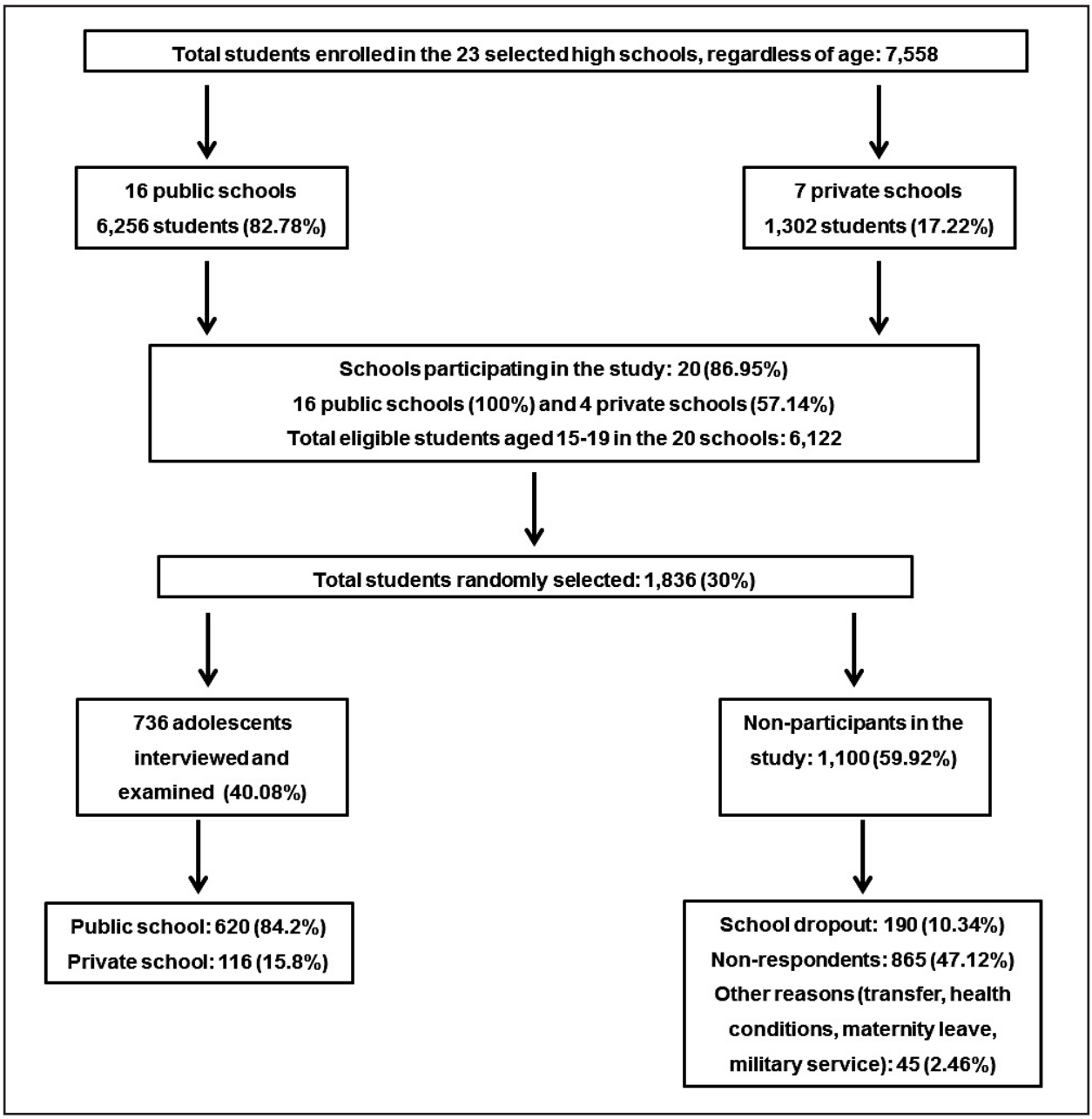


*Fig. 2:Flowchart of the participants through the study.*

city rates. History of orthodontic treatment was 57.6%. Of the total study population, 32.7% were under orthodontic treatment, 24.9% had completed orthodontic treatment, and only 42.4% had no experience of orthodontic treatment.

Sociodemographic aspects were associated with history of orthodontic treatment. Subjects who were female, white, enrolled at private schools, and with mothers with higher level of education were associated with higher history of orthodontic treatment (Table 1). Aspects related to oral health presented inverse association. Adolescents who reported no concern about oral health had 70.02% rate of history of orthodontic treatment, while 46.53% were concerned about oral health (p=0.0001). Tooth loss was not associated with history of orthodontic treatment in this analysis (p=0.13). Aspects related to appearance and esthetics were also associated with history of orthodontic treatment. Those who reported concern about appearance had 62.00% rate of history of treatment, compared to 51.46% for those not concerned about appearance (p=0.003). Concerns about alignment and color of the teeth were inversely and significantly associated with history of orthodontic treatment (p=0.0001 for both associations).

Table 2 shows the univariate analysis of this study. Female gender, white ethnicity, mother's level of education, attending to private schools, concern about oral health, appearance of the teeth bothering the adolescent, and concern about teeth alignment and color were significantly associated with history of orthodontic treatment

All those variables and tooth loss were included in the multivariable analyses. Only female gender

**Table 1: Frequency distribution of exposures regarding history of orthodontic treatment among adolescents aged 15 to 19 years, Passo Fundo, Brazil.**

| | | History of orthodontic treatment? | | |
|---|---|---|---|---|
| | | Yes | No | P-value* |
| | | n (%) | n (%) | |
| Age | 15 | 123 (52.11%) | 113 (47.89%) | 0.07 |
| | 16 | 161 (64.14%) | 90 (35.86%) | |
| | 17 | 99 (56.57%) | 76 (43.43%) | |
| | 18 | 34 (58.62%) | 24 (41.38%) | |
| | 19 | 7 (43.75%) | 9 (56.25%) | |
| Gender | Male | 164 (50.78%) | 159 (49.22%) | 0.0001 |
| | Female | 260 (62.95%) | 153 (37.05%) | |
| Ethnicity/skin colour | White | 330 (64.57%) | 181 (35.43%) | 0.0001 |
| | Non-white | 94 (41.78%) | 131 (58.22%) | |
| Mother's level of education | Complete or incomplete higher education | 123 (75.00%) | 41 (25.00%) | 0.0001 |
| | Complete or incomplete high school | 162 (61.13%) | 103 (38.87%) | |
| | Up to elementary school | 139 (45.27%) | 168 (54.73%) | |
| Type of school | Public | 333 (53.70%) | 287 (46.30%) | 0.0001 |
| | Private | 91 (78.44%) | 25 (21.56%) | |
| General health problem | Yes | 53 (58.24%) | 38 (41.76%) | 0.50 |
| | No | 366 (57.63%) | 269 (42.37%) | |
| Tooth loss | Yes | 96 (61.94%) | 59 (38.06%) | 0.13 |
| | No | 328 (56.45%) | 253 (43.55%) | |
| Concern about oral health | Yes | 181 (46.53%) | 208 (53.47%) | 0.0001 |
| | No | 243 (70.02%) | 104 (29.98%) | |
| Bothered by appearance | Yes | 266 (62.00%) | 163 (38.00%) | 0.003 |
| | No | 158 (51.46%) | 149 (48.54%) | |
| Concern about tooth alignment | Yes | 156 (48.29%) | 167 (51.71%) | 0.0001 |
| | No | 268 (64.90%) | 145 (35.10%) | |
| Concern about tooth color | Yes | 211 (51.84%) | 196 (48.16%) | 0.0001 |
| | No | 213 (64.74%) | 116 (35.26%) | |

*Chi-square test or Fisher's exact test

**Table 2: Univariate analysis model associating history of orthodontic treatment among adolescents 15 to 19 years old, Passo Fundo, 2012.**

| | | Prevalence Ratio (95%CI) | P-value |
|---|---|---|---|
| Gender | Female | 1.24 (1.09 – 1.41) | 0.001 |
| Age | | 1.01 (0.953 – 1.08) | 0.683 |
| Ethnicity | White | 1.55 (1.31 – 1.83) | <0.001 |
| Mother's level of education | Complete or incomplete higher education<br>Complete or incomplete high school | 1.66 (1.42 – 1.93)<br>1.35 (1.16 – 1.58) | <0.001<br><0.001 |
| Type of school | Private | 1.46 (1.30 – 1.65) | <0.001 |
| General health problems | Yes | 1.01 (0.84 – 1.22) | 0.913 |
| Tooth loss | Yes | 1.10 (0.95 – 1.27) | 0.203 |
| Concern about oral health | Yes | 0.66 (0.59 – 0.75) | <0.001 |
| Bothered by appearance | Yes | 1.21 (1.06 – 1.37) | 0.005 |
| Concern about tooth alignment | Yes | 0.74 (0.65 – 0.85) | <0.001 |
| Concern about tooth color | Yes | 0.80 (0.71 – 0.91) | <0.001 |

**Table 3: Multivariate analysis model associating history of orthodontic treatment among adolescents 15 to 19 years old, Passo Fundo, 2012.**

| | | Prevalence Ratio (95%CI) | P-value |
|---|---|---|---|
| Gender | Female | 1.26 (1.11 – 1.43) | <0.001 |
| Ethnicity | White | 1.32 (1.11 – 1.56) | 0.001 |
| Mother's level of education | Complete or incomplete higher education<br>Complete or incomplete high school | 1.49 (1.28 – 1.74)<br>1.26 (1.08 – 1.46) | <0.001<br>0.003 |
| Tooth loss | Yes | 1.21 (1.06 – 1.39) | 0.006 |
| Concern about oral health | Yes | 0.69 (0.61 – 0.78) | <0.001 |

(PR=1.26; 95% CI: 1.11 – 1.43), white ethnicity (PR=1.32; 95% CI: 1.11 – 1.56), higher mother's level of education (PR=1.49; 95% CI: 1.08 – 1.46), tooth loss (PR=1.21; 95% CI: 1.06 – 1.39) were significantly associated with history of orthodontic treatment in this analysis (Table 3). On the other hand, concern with oral health presented as a protective factor to history of orthodontic treatment (PR=0.69; 95% CI: 0.61 – 0.78).

## DISCUSSION

The aim of this study was to assess the history of orthodontic treatment among adolescents and to establish factors associated with such history, including sociodemographic factors, oral and general health factors, and oral esthetic factors.

The results showed that 57.6% of adolescents had at some time undergone orthodontic treatment, including both those who had already received treatment and those currently under treatment. These results show a high orthodontic treatment rate among Brazilian adolescents compared to studies that assess the true need for treatment. According to the latest national survey[9], the need for treatment obtained through the DAI was about 35%, of which 15% represented highly desirable and mandatory treatment. It is important to highlight that the DAI presents many disadvantages, such as its subjective nature, high variability in its classification, and the lack of assessment of all occlusal traits[17]. The higher history of orthodontic treatment in this sample is a matter of concern and should be contrasted with the true need of treatment.

The results of the present study show that adolescents who claimed to be white were more likely to receive this type of treatment. White adolescents in Brazil have a higher level of

education and income than adolescents who claim to be non-white [13]. Thus, they have more access to dental care, more knowledge, and more attitude regarding oral hygiene care and esthetic needs. Female adolescents were also associated with greater orthodontic experience, which may be explained by the fact that they seek more dental services, and are more concerned with oral health and aspects related to esthetics[18].

Adolescents with mothers with higher level of education were associated with higher orthodontic treatment experience. The association between mother's level of education and better oral health conditions of their children has been previously demonstrated in the literature[19]. This may include a higher concern from mothers about matters related to their children's appearance.

The multivariable analysis showed that tooth loss was significantly associated with history of orthodontic treatment, with tooth loss being 21% higher in adolescents who had undergone orthodontic treatment than in those who had not. Another study showed that 23.5% of tooth loss in children and adolescents was due to orthodontic reasons[20]. Although extractions may be needed during orthodontic treatment to gain space easily[21], it should be considered that they may not provide significant esthetic improvement.

Adolescents who reported concern about their oral health presented 31% less history of orthodontic treatment. The literature shows that the primary motivation for orthodontic treatment is improving dental appearance, and improvement in oral function is not necessarily involved in this process[22]. However, esthetic factors were not significant in the multivariate analysis. Furthermore, it may be hypothesized that adolescents who have already undergone orthodontic treatment and probably have regular access to oral health services could respond that they are not concerned about their general health. In contrast, adolescents who do not have access to these services may for that very reason express concern.

The literature reports that adults who express concern about their oral health tend to have more symptomatic visits to the dentist[23]. It may be speculated that the situation is similar in adolescents. Moreover, adolescents from private schools also presented more history of orthodontic treatment only in the univariate analysis. Another study found that the type of school is associated with oral health differences among adolescents[24]. Not only do these differences concern proper oral health behavior, but also show that students from private schools have greater access to sophisticated and expensive treatments.

Among adolescents who reported concern about appearance, 62% had orthodontic treatment history, while among those who were not concerned about appearance, only 51.46% had orthodontic treatment history (p=0.003). However, in the multivariable analysis, none of the factors related to oral esthetics differed significantly regarding history of orthodontic treatment. Another study showed that dissatisfaction with appearance is directly related to the desire for treatment, leading people to seek orthodontic treatment[25]. It should be noted that overtreatment is undesirable not only because of the expense involved, but also due to its potential association with adverse effects. Moreover, awareness needs to be raised regarding the fact that orthodontic therapy is not indicated strictly for esthetic problems.

Sample size was not calculated in this study. Analytical epidemiological studies need a minimum amount of 40-50 individuals for each variable tested. We estimated that inviting 30% of the adolescents at the schools would suffice for the different analyses to be performed. A census would not be possible, and other studies in this field have used smaller sample size than this study[26,27]. The literature also reports studies with similar sample sizes[28,29].

Among strengths of this study, we can highlight the number of participants, the reliability and use of validated methods, and the use of a random sample from all schools, achieving similar proportions to the actual distribution of enrolled students. The main limitation was the response rate, which was probably limited by the change of school and the need to provide informed consent from parents.

Studies providing associations are important in order to frame questions for further research. The evidence found in the current study contributes to understanding which factors predict whether or not adolescents have experience of orthodontic therapy. The findings of this study showed a high rate of history of orthodontic treatment among Brazilian adolescents. It was associated with sociodemographic factors, tooth loss and absence of concern about oral health. The history of orthodontic treatment in this sample does not match the data for malocclusion in Brazil, so overtreatment may be suspected.

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# Cytotoxicity assessment of 1% peracetic acid, 2.5% sodium hypochlorite and 17% EDTA on FG11 and FG15 human fibroblasts

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***ABSTRACT***

*The aim of this study was to evaluate the cytotoxic effects of 2.5% sodium hypochlorite (NaOCl), 17% ethylenediamine tetraacetic acid (EDTA), and 1% peracetic acid (PAA) on human fibroblasts. FG11 and FG15 cell lines were cultured in 24-well cell culture plates for cell proliferation assessment and 96-well cell culture plates for the methylthiazolyldiphenyl-tetrazolium bromide (MTT) assay; Dulbecco's modified Eagle's medium (DMEM) was used as control data. The experimental solutions were used at 0.01%, 0.05%, and 0.1% dilutions and assessed at 1-, 2-, and 4-hour intervals. Data were subjected to statistical analysis by two-way analysis of variance (ANOVA), followed by the Bonferroni test at a significance level of $p < 0.05$. The assessment of cell proliferation in this study showed cytotoxicity to the fibroblasts with 2.5% NaOCl for all three dilutions at all time intervals, 17% EDTA for the 0.05% and 0.1% dilutions at the 2- and 4-hour intervals, and 1% PAA for all three dilutions at the 4-hour interval. The cell viability assay (MTT assay) for fibroblasts showed 2.5% NaOCl to be cytotoxic at the 0.05% and 0.1% dilutions at all time intervals, 17% EDTA to be cytotoxic at the 0.1% dilution at the 2- and 4-hour intervals, and 1% PAA to be cytotoxic at the 0.1% dilution at the 2- and 4-hour intervals. In conclusion, 1% PAA was less cytotoxic than 2.5% NaOCl and 17% EDTA.*

***Key words:*** *EDTA, peracetic acid, sodium hypochlorite, toxicity.*

## Avaliação da citotoxicidade de ácido peracético a 1%, hipoclorito de sódio a 2,5% e EDTA a 17% em fibroblastos humanos FG11 e FG15

***RESUMEN***

*O objetivo do presente estudo foi o de avaliar os efeitos citotóxicos de hipoclorito de sódio (NaOCl) a 2,5%, ácido etilenodiaminotetracético (EDTA) a 17% e ácido peracético (PAA) a 1% em fibroblastos humanos. As linhagens celulares FG11 e FG15 foram colonizadas em 24-well cell plates para avaliação da proliferação celularr e em 96-well cell plates para o ensaio de MTT; O médio modificado Dulbecco's Eagle's (DMEM) foi usado como controle. As soluções experimentais foram usadas com diluições de 0,01%, 0.05%, e 0,1% e avaliadas com 1, 2 e 4 horas de intervalo. Os dados foram submetidos à análise estatística pelo two-way ANOVA, seguido do teste de Bonferroni com nível de significância de $p < 0.05$. A avaliação da proliferação celular neste estudo mostrou o NaOCL a 2,5% como citotóxico nas 3 diluições e 3 intervalos de tempo, o EDTA a 17% nas diluições de 0,05% e 0,1% nos intervalos de 2 e 4 horas, e o PAA a 1% em todas as diluições no intervalo de 4 horas. O teste de viabilidade cellular (MTT) mostrou o NaOCl a 2,5% citotóxico a 0,05% e 0,1% em todos os intervalos, o EDTA a 17% citotóxico nas diluições de 0,1% nos intervalos de 2 e 4 horas e o PAA a 1% citotóxico na diluição de 0,1% nos intervalos de 2 e 4 horas. Como conclusão o PAA a 1% mostrou-se menos citotóxico que o NaOCl a 2,5% e o EDTA a 17%.*

***Palavras Chave:*** *EDTA, ácido peracético, hipoclorito de sódio, toxicidade.*

## INTRODUCTION

Removal of bacteria is the key to successfully treating necrotic teeth. Root canal instrumentation alone cannot render a root canal completely disinfected, but needs to be complemented by the use of irrigants to achieve disinfection and prevent or heal apical periodontitis[1].

The ideal irrigant should meet the following requirements: effectiveness against a broad spectrum of bacteria, ability to dissolve vital and necrotic pulp tissue and remove the smear layer, and biological compatibility with periodontal tissues. Cytotoxicity is important because during irrigation procedures a solution may extrude and contact periodontal

tissues[2,3]. For many years, sodium hypochlorite (NaOCl) has been the most commonly used irrigant solution. Despite its antibacterial and tissue dissolution characteristics, NaOCl is not able to remove the smear layer, so chelant solutions have been used for this purpose. Ethylenediamine tetraacetic acid (EDTA) and Citric Acid (CA) have been recommended for smear layer removal[4, 5].

Several solutions that are effective against bacteria while removing the smear layer have recently been proposed. Chlorhexidine (CHX), tetraacetylethylenediamine with sodium perborate (TAE+P), maleic acid (MA), and iodine potassium Iodide (IPI) have been suggested as alternative irrigant solutions, although none of them possess all of the desired properties to be an ideal single irrigant solution[1,6]. CHX has been widely recommended; however, it cannot dissolve pulp tissue, and recent studies have shown it to be cytotoxic[7]. In addition, several new endodontic irrigants have failed to significantly remove or kill biofilm[6]. Citric acid has been used for root canal irrigation due to its ability to remove the smear layer and effectiveness against several bacterial species[8-10]. However, it is not an efficient antimicrobial solution, requiring more contact time to kill bacteria *in vitro*; therefore, its use as a single irrigant should be evaluated carefully[11].

Peracetic acid (PAA) has been used to disinfect medical devices[12] and has low levels of cytotoxicity[13]. Furthermore, 4% PAA has demonstrated efficacy in the reduction of live bacteria in biofilm[6], and 2% PAA has been shown to be effective against *Enterococcus faecalis*[14]. A low concentration of PAA has been shown to be useful in dissolving the smear layer[15], disinfecting gutta-percha cones[16], removing calcium hydroxide from the apical third[17], and providing antimicrobial activity against *Enterococcus faecalis* biofilm[18]. Additionally, recent studies have shown that 2.25% PAA increases dislodgment resistance of Biodentine filling material[19] and not does not affect the push-out strength of MTA Fillapex[20],

Some studies have shown the appropriate properties of a low concentration of PAA. In fact, a previous study of our research group demonstrated the efficacy of 1% PAA against *Entroccocus faecalis* biofilm; however, little is known about the cytotoxicity of 1% PAA on human fibroblasts. The aim of this study is therefore to assess and compare the cytotoxicity of 1% PAA, 2.5% NaOCl and 17% EDTA on human fibroblasts.

## MATERIALS AND METHODS

FG11 and FG15 fibroblast cells (110/mm$^2$) were maintained in Dulbecco's modified Eagle's medium (DMEM) (Invitrogen Srl, Milan, Italy) with 10% fetal bovine serum (Hyclone Laboratories Inc., Logan, UT) and 1% penicillin-streptomycin solution (Invitrogen). The cells were kept in a humidified atmosphere containing 5% $CO_2$ at 37°C until they reached confluence. Then they were enzymatically removed from the wells, and 10 uL of trypan blue (Sigma-ALdrich, St. Louis, MO) was added to 10 uL of the cells; 1 uL of this solution was counted by microscope in a Neubauer chamber. Cell proliferation, with the experimental solutions at 0.01%, 0.05%, and 0.1% dilutions, was evaluated by the trypan blue exclusion method in a hemocytometer (Neubauer Improved Bright-line, HBG, Western Germany) at 1-, 2-, and 4-hour intervals. Each dilution was assessed in sextuplicate per test.

Cytotoxicity was examined for the 0.01%, 0.05%, and 0.1% dilutions for each experimental solution after the 1-, 2-, and 4-hour intervals using the methylthiazolyldiphenyl-tetrazolium bromide (MTT) assay (Sigma Chemical Company, St Louis, MO). For the cytotoxicity assay, 110 cells/mm$^2$ in 96-well plates were cultured with the experimental solutions after 1, 2, and 4 hours. Then the cultured cells were added to 10 uL of the MTT assay (5 mg/mL) diluted in 90 uL of DMEM for 3 hours at 37°C. After this period, 100 uL of dimethyl sulfoxide (DMSO) was added; and after 15 minutes, the reading was performed. Cellular viability was determined using a microplate spectrophotometer reader (Epoch; BioTek Instruments Inc., Winooski, VT) at 590 nm, and the percentage of cell viability at each concentration was compared to the control. All the experimental procedures were conducted in sterile conditions under a laminar flow hood (Nuaire, Fernbrook Lane, Plymouth, MN).

Data were evaluated by two-way analysis of variance (ANOVA) followed by the Bonferroni test using Prism 5 software (GraphPad Software, San Diego, CA) at a significance level of $p < 0.05$.

## RESULTS

The trypan blue and MTT assays showed that the untreated cells remained vital at all time intervals. The trypan blue assay showed values for cell proliferation of the control group of ($0.53 \pm 0.03 \times 10^4$) at 1 hour, ($0.60 \pm 0.05 \times 10^4$) at 2 hours, and

(1.29 ± 0.16 x $10^4$) at 4 hours (Fig. 1). The MTT assay showed absorbance values for the control group of (0.34 ± 0.01) at the 1-hour interval, (0.34 ± 0.02) at the 2-hour interval, and (0.34 ± 0.04) at the 4-hour interval (Table 1).

Cell proliferation in the 2.5% NaOCl group was different from the control group with the 0.01% dilution at the 1-hour interval (0.30 ± 0.07 x $10^4$), the 2-hour interval (0.22 ± 0.05 x $10^4$), and 4-hour interval (0.92± 0.06 x $10^4$); the values for the 0.05% and 0.1% dilutions were the same (0 x $10^4$) at all time intervals and differed significantly from the control group ($p$ <0 .05). The MTT assay showed differences in cell viability for the 0.05% and 0.1% dilutions at all time intervals; no difference was found for the 0.01% dilution (p< 0.05).

Cell proliferation in the 17% EDTA group was different from the control group with the 0.05% dilution at the 2-hour interval (0.40 ± 0.06 x $10^4$) and the 4-hour interval (0.73 ± 0.06 x $10^4$) and with the 0.1% dilution at the 2-hour interval (0.37 ± 0.04 x $10^4$) and the 4-hour interval (0.22 ± 0.11 x $10^4$). No difference was found for the 0.01% dilution at any time interval. The MTT assay showed differences in cell viability for the 0.1% dilution at the 2-hour interval (0.24 ± 0.03) and the 4-hour interval (0.21 ±

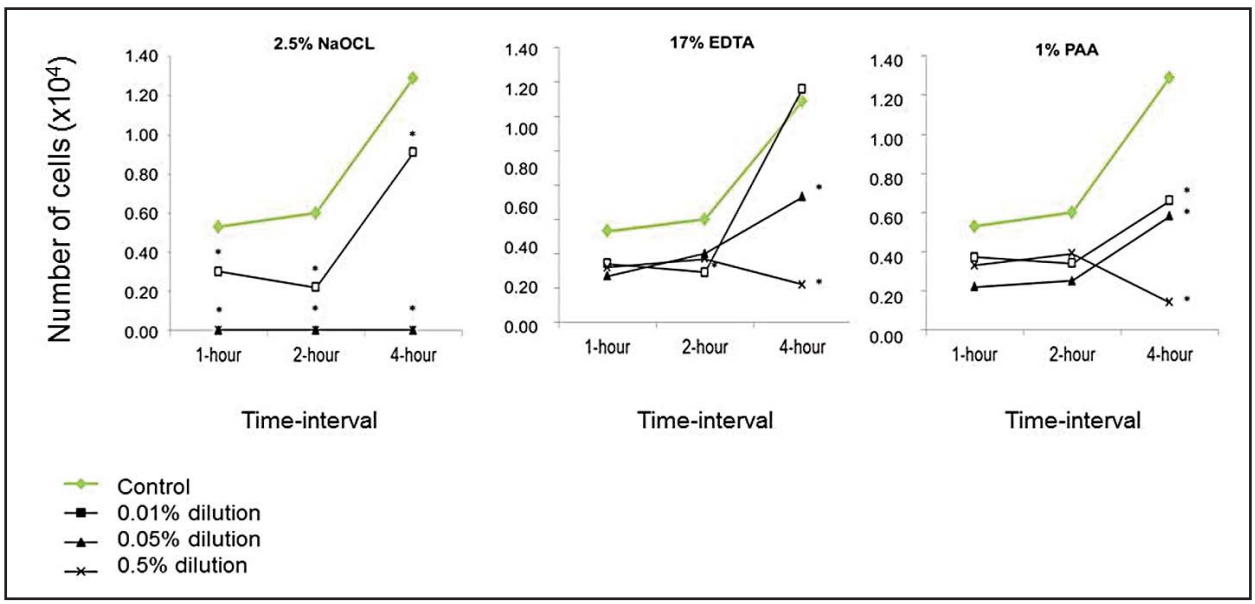


*Fig. 1: Number of cells (x 104) for each experimental solution at different time intervals and dilutions. *Values statistically different from control group at p < 0.05.*

**Table 1: Cell Viability for the Experimental Solutions at Different Time Intervals and Dilutions According to Optical Absorbance (590 nm).**

| Interval | Dilution | Control | 2.5% NaOCL | 17% EDTA | 1% PAA |
|---|---|---|---|---|---|
| 1-hour | 0.01% | 0.34 ± 0.03 | 0.40 ± 0.09 | 0.33 ± 0.03 | 0.33 ± 0.03 |
| | 0.05% | | 0.20 ± 0.02* | 0.31 ± 0.03 | 0.35 ± 0.03 |
| | 0.1% | | 0.10 ± 0.03* | 0.35 ± 0.06 | 0.32 ± 0.02 |
| 2-hour | 0.01% | 0.34 ± 0.02 | 0.32 ± 0.04 | 0.34 ± 0.02 | 0.34 ± 0.04 |
| | 0.05% | | 0.09 ± 0.004* | 0.35 ± 0.07 | 0.35 ± 0.03 |
| | 0.1% | | 0.08 ± 0.003* | 0.24 ± 0.03* | 0.25 ± 0.03* |
| 4-hour | 0.01% | 0.34 ± 0.04 | 0.30 ± 0.06 | 0.43 ± 0.06 | 0.38 ± 0.08 |
| | 0.05% | | 0.07 ± 0.004* | 0.40 ± 0.09 | 0.34 ± 0.03 |
| | 0.1% | | 0.08 ± 0.01* | 0.21 ± 0.05* | 0.13 ± 0.007* |

*Values statistically different from the control group at p < 0.05.

0.05). No difference was found for the 0.01% and 0.5% dilutions at any time interval ($p$ <0 .05).
Cell proliferation in the 1% PAA group at the 4-hour interval was different from the control group with the 0.01% ($0.66 \pm 0.11 \times 10^4$), 0.05% ($0.58 \pm 0.25 \times 10^4$), and 0.1% ($0.14 \pm 0.12 \times 10^4$) dilutions. No difference was found at the 1-hour and 2-hour intervals ($p < 0.05$). The MTT assay showed differences from the control group at the 2-hour ($0.25 \pm 0.03$) and 4-hour intervals ($0.13 \pm 0.007$) for the 0.1% dilution ($p < 0.05$).

## DISCUSSION

The aim of this study was to assess the cytotoxicity of different irrigant solutions used in endodontics. The trypan blue assay stains dead cells and is largely applied as a confirmatory test for measuring changes in viable cell number caused by a drug. The MTT assay measures the mitochondrial function and is commonly used to detect the loss of cell viability[21]. It is important to emphasize that the dilutions used in the present study (0.01%, 0.05%, and 0.1%) are based on the fact that *in vitro*, these cells do not present other shields such as phagocytic cells, lymphatic system or blood to decrease the cytotoxic effect of the experimental solutions[20]. Different cells have been used to evaluate cytotoxicity[12,21]; our study used FG11 and FG15 human periodontal fibroblasts for better simulation of cells that might be clinically affected by irrigant solutions.
The results of the present study confirm previous findings that NaOCl is cytotoxic to fibroblasts cells[7]. NaOCl was able to maintain cell viability only with the 0.01% dilution. These findings are in accordance with Simbula et al.[23], who have shown that higher concentrations are related to lower percentages of cell viability. It has also been suggested that low concentrations of NaOCl possess the antibacterial characteristics required to be an appropriate solution[24]. The present study is in agreement with a previous study that showed 17% EDTA to be less cytotoxic than 2.5% NaOCl[7].
PAA has been shown to be efficient against Ebola virus in culture plates and in dried blood[25]; and in its different dilutions has been suggested for use as a disinfectant solution for dental devices due to its rapid action against all microorganisms and absence of harmful products[12]. A previous study recommended PAA as a single irrigation solution in the treatment of teeth presenting necrotic pulp tissue[26]. Another study showed that 0.3% PAA was not cytotoxic when polyvinyl chloride (PVC), polyurethane, silicone tubes and polytetrafluoroethylene (PTFE) tubes were soaked in the solution and immersed in a culture of Henrietta Lacks cells (HeLa)[13]. To the best of our knowledge, no study has yet evaluated the cytotoxic effects of 1% PAA against human periodontal fibroblasts. Our results showed that 1% PAA presented lower cytotoxicity than 2.5% NaOCl and 17% EDTA. While our results suggest that 1% PAA appears to be an appropriate solution, another recent study showed 1% PAA to be more cytotoxic than 2.5% NaOCL, in disagreement with our findings[27].
PAA has been used in the past as a single irrigant solution for root canal therapy. Recently, PAA has attracted the attention of the endodontic community. Further clinical studies using current instrumentation, irrigation and filling protocols are necessary to evaluate whether 1% PAA can be used as a single irrigant solution. The present study evaluated only short-term cytotoxicity, but long-term evaluations are also recommended.
Within the limitations of this study, 1% PAA is less cytotoxic than 2.5% NaOCl and 17% EDTA.

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The authors deny any conflict of interest related to this study.

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# Comparison of instruments used to select and classify patients with temporomandibular disorder

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***ABSTRACT***

*The aim of the present study was to identify the relationship among instruments used to screen and diagnose temporomandibular disorders (TMD). A retrospective study was conducted using medical records of patients with temporomandibular disorder who had visited the institution for initial assessment between January and December 2015. Medical history and physical examination data were collected, particularly those focusing on the diagnosis of TMD and TMJ (temporomandibular joint) function. The following instruments were used to assess the severity of the TMD signs and symptoms: the Fonseca Anamnestic index (FAI), the Helkimo index (HI), the American Association of Orofacial Pain Questionnaire (AAOPQ) and the Jaw Symptom & Oral Habit Questionnaire (JSOHQ). Thirty-eight patient records were included, with prevalence of women (84.6%) and mean age 37.42 ± 14.32 years. The patients who were classified as having severe TMD by the FAI exhibited more positive responses on the AAOPQ (6.25 ±1.42; one-way ANOVA F=15.82), with a statistically significant difference when compared to patients with mild TMD (3.0 ±1.22; p<0.01). A positive correlation (r=0.78; p<0.01) was found between the number of positive responses on the AAOPQ and the sum of the JSOHQ scores. Patients who were classified with severe TMD on the FAI exhibited higher scores on the JSOHO (18.58 ±4.96/ one-way ANOVA F=14.43), with a statistically significant difference when compared to patients with moderate (12.08 ±5.64; p<0.01) and mild TMD (7.46 ±4.89; p<0.01). Conclusion: In the study sample, there was consistency among the instruments used to differentiate patients with severe and mild TMD. The selection of instruments should be rational, in order to improve the quality of the results.*

***Key words:*** *Temporomandibular joint disorders, signs and symptoms, surveys and questionnaires, facial pain.*

## Avaliação de instrumentos utilizados para selecionar pacientes com disfunção temporomandibular

***RESUMO***

*O objetivo deste estudo foi identificar a relação entre os instrumentos utilizados para selecionar e diagnosticar os pacientes com disfunção temporomandibular (DTM). Foi realizado um estudo retrospectivo utilizando prontuários odontológicos de pacientes atendidos devido a dor e disfunção na articulação temporomandibular, que haviam procurado a instituição para uma avaliação inicial entre janeiro e dezembro de 2015. Foram coletados dados da história médica e do exame físico, particularmente aqueles que se concentraram no diagnóstico de DTM. Os seguintes instrumentos foram utilizados para avaliar a gravidade dos sinais e sintomas da DTM: o índice anamnésico de Fonseca (FAI); O índice Helkimo (HI); o questionário da Associação Americana de Dor Orofacial (AAOPQ) eo Questionário de Sintomas e Hábitos Orais (JSOHQ). Foram incluídos trinta e oito prontuários de pacientes, com prevalência de mulheres (84,6%) e idade média de 37,42 ± 14,32 anos. Os pacientes que foram classificados com DTM severa pela FAI apresentaram maior número de respostas positivas no AAOPQ (6,25 ± 1,42; ANOVA F = 15,82), com diferença estatisticamente significativa em comparação com pacientes com DTM leve (3,0 ± 1,22; p < 0,01). Foi encontrada uma correlação positiva (r = 0,78; p <0,01) entre o número de respostas positivas no AAOPQ e a soma dos escores no JSOHQ. Os pacientes que foram classificados com DTM severa na FAI exibiram pontuações mais altas no JSOHO (18,58 ± 4,96 / ANOVA F = 14,43), com diferença estatisticamente significativa quando comparados a pacientes com DTM média (12,08 ± 5,64; p <0,01) e leve (7,46 ± 4,89; p <0,01). Na amostra estudada, houve congruência entre os instrumentos utilizados para diferenciar os pacientes com DTM grave e leve. A seleção de instrumentos deve ser racional, a fim de melhorar a qualidade dos resultados.*

***Palavras chave:*** *disfunção temporomandibular, sinais e sintomas, inquéritos e questionários, dor facial.*

## INTRODUCTION

Temporomandibular disorders (TMDs) is a collective term that defines a subgroup of painful orofacial disorders involving pain in the temporomandibular joint (TMJ), fatigue of the craniofacial and cervical muscles and limited mandibular movements.[1] Muscle-related conditions account for the largest subgroup.[2]

A number of assessment tools have been proposed for use in clinical practice and research on individuals with TMD, including the American Academy of Orofacial Pain questionnaire (AAOPQ), the Helkimo Index (HI), the Fonseca anamnestic index (FAI), and the Research Diagnostic Criteria for Temporomandibular Disorders (RDC/TMD), which can be used with clinical assessments, radiography, Magnetic Resonance Imaging, Computed Tomography and electromyography.[3,4]

An effective scale must identify patients correctly and discriminate normal subjects. Helkimo constructed an index by adding up the presence of symptoms and assigning a degree of severity when a certain level was exceeded. This index seems to provide a satisfactory indication of the severity of TMD. Helkimo also introduced a fixed set of symptoms, with well-defined assignments in the segments of the index and computation of the index-class, there by enabling the comparison of results. [5]

The severity of TMD is often analyzed. The Fonseca anamnestic index (FAI) has been widely employed for such purpose in clinical and epidemiological studies.[1,3,4,6] However, Chaves et al.[4] suggested that the FAI has not yet been completely validated and does not providea diagnostic classification of TMD. The data obtained using the FAI are therefore restricted to the classification of the severity of TMD signs and symptoms.

A number of authors have used two or more instruments to determine the level of agreement between them and with clinical findings.[7,8,4] It is essential to select a reliable instrument to assess TMD. Only scales that provide reliable reflections of the underlying problems can be used to differentiate between healthy and clinically affected individuals.[5] The aim of the present study was to assess the epidemiological profile of TMD patients treated at the dental clinic of Paulista University (Brazil). In addition, this study sought to identify the relationship among instruments used to screen and diagnose temporomandibular disorders.

**Materials and Methods**

A retrospective study was conducted using medical records of patients with TMD who had visited the institutionforan initial assessment between January and December 2015. It included male and female patients aged 18 to 60 years who exhibited at least mild TMD according to the FAI. The following exclusion criteria were applied: missing teeth without proper rehabilitation; deep bite; crossbite; use of misfit prostheses (partial or total dentures); history of trauma to the face or TMJ; systemic diseases (arthritis, arthrosis or dystonia).

The medical history and physical examination data, particularly those related to the diagnosis of TMD and TMJ function, were collected, including mouth opening (inter-incisor distance) and pain during muscle palpation (recorded on a scale of 0 to 10). The following instruments were used to assess the severity of the TMD signs and symptoms: Fonseca Anamnestic index (FAI), Helkimo index (HI), American Association of Orofacial Pain Questionnaire (AAOPQ), and Jaw Symptom & Oral Habit Questionnaire (JSOHQ).

The FAI was used to assess the severity of TMD based on signs and symptoms. It consists of ten items with three response options: yes (10 points), sometimes (5 points) and no (0 points). The score is determined by adding the scores of all items and provides the following classifications: absence of TMD signs and symptoms (0-15 points); mild TMD (20-45 points); moderate TMD (50-65 points) and severe TMD (70-100 points).[3]

Concerning the Helkimo index,[9] the present study used the clinical dysfunction index, which involves a functional assessment of the masticatory system. According to the presence and intensity of the symptom, a score of 0, 1 or 5 points was assigned to each patient. The following symptoms were analyzed: 1- Range of mandibular motion; 2- TMJ functional impairment; 3- Muscle pain during palpation; 4- TMJ pain during palpation; 5- pain during mandibular movement. The sum of the scores was used to classify the subjects as follows: 0 points - clinically free from symptoms; 1-4 points– mild dysfunction symptoms; 5-9 points – moderate dysfunction symptoms; 10-25 points – severe dysfunction symptoms.

The AAOP Questionnaire contains 10 self-explanatory questions (“yes” and “no” answers) on the most frequent signs and symptoms of oro facial pain and TMD. The Helkimo patient-history index (modified by Fonseca) contains 10 self-explanatory questions (“yes” and “no” answers) based on different symptoms of masticatory dysfunction.[7]

The Jaw Symptom & Oral Habit Questionnaire (JSOHQ) contains 13 questions, eight of which are related to jaw pain and five related to jaw function.

There are five possible answers to each question, ranging from no sign or symptom to extreme signs or symptoms. For analysis, the answers were converted into an ordinal ranking system (0 to 4).

All instruments were completed by dentistry undergraduate students and checked and corrected by a single researcher (PRP). The data were analyzed using descriptive and correlational statistics and SPSS v. 18.0 for Windows (SPSS Inc, Chicago, IL). The results were considered statistically significant for $p<0.05$. The present study was approved by the Research Ethics Committee of the Faculty of Dentistry of the UNIP.

## RESULTS

In the present study, the records of 57 patients who had received care for the first time during the study period were gathered. Of these, 38 fulfilled the inclusion criteria. There was prevalence of women (84.6%), white skin (76.9%), mean age 37.42 ± 14.32 years and mean body mass index 23.94 ± 3.98 kg/cm$^2$. Most patients reported some form of systemic disease (60.5%), with 18.4% mentioning depression. Ten women reported using oral contraceptives. Five main categories of pain were identified: facial pain (31.6%); difficulty while chewing (28.9%); headache (10.5%); bruxism and tooth clenching (7.9%) and clicking noises in the TMJ (5.3%). Twenty-two of the patient records mentioned difficulties while chewing and 21 patients reported parafunction. The maximum mouth opening values ranged from 31 to 60 mm (mean of 42.15 ± 9.34 mm).

Concerning the Helkimo index or clinical craniomandibular dysfunction, the most common form of disorder was severe (18 patients), distributed among the indices 3, 4 and 5 (n=11/5/2), followed by mild (11 patients) and moderate (9 patients). Concerning the FAI, there was a balanced distribution among the patients, who were classified as follows: mild TMD (n=14); severe TMD (n=13) and moderate TMD (n=11).

In the AAOPQ, there was a greater number of positive responses for question 7, referring to the presence of headaches, toothaches and neck pain (n=29), and question 5, referring to the presence of stiffness and fatigue in the jaw (n=26). The results related to the frequency of positive responses are shown in Fig. 1.

The mean score on the Mandibular Symptoms and Oral Habits Questionnaire was 12.34 ± 6.65. Higher scores were obtained for the questions related to difficulty while opening the mouth, discomfort while chewing and joint pain and noises. Fig. 2 shows the sum of the scores for each question.

Pain during muscle palpation was reported in 16 of the records. A greater intensity of pain in the palpated muscles was noted during the intraoral examination. During muscle palpation, the patients were asked to report zero for pain absence and ten

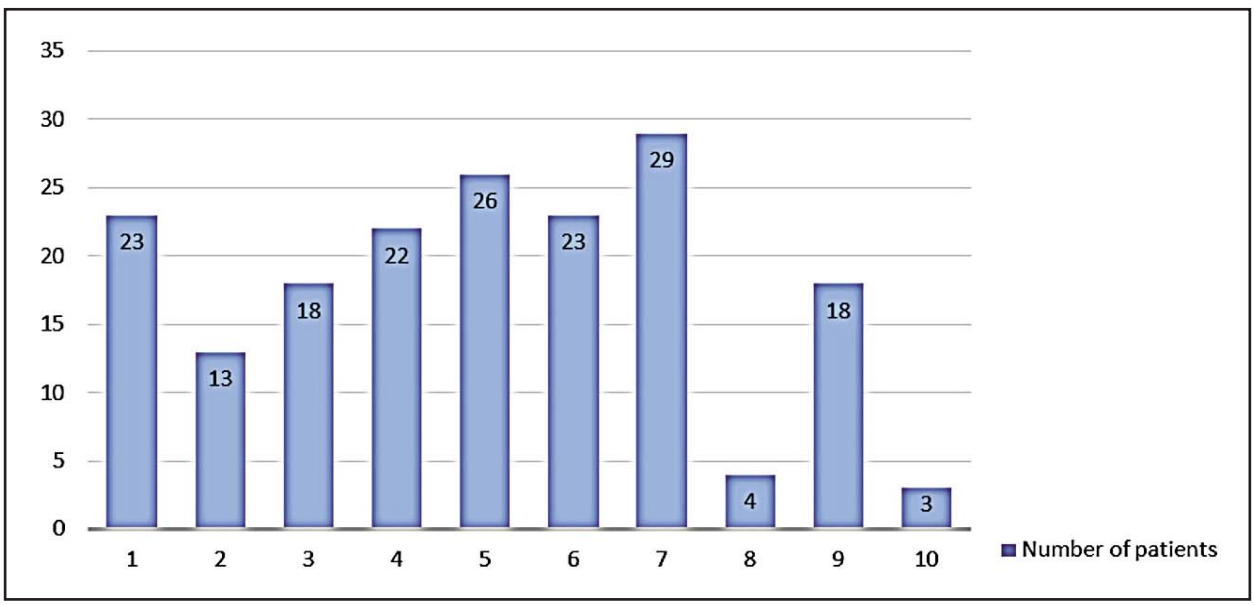
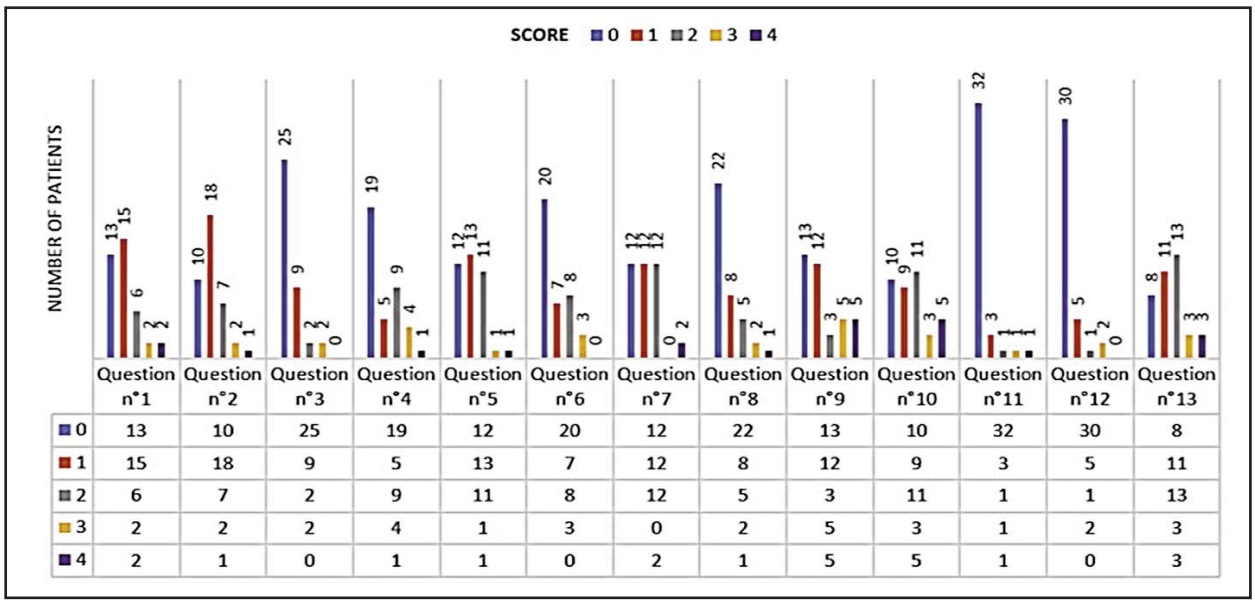



*Fig. 1: Frequency of affirmativeresponseson the American Academy of Orofacial Pain questionnaire.*




| | Question n°1 | Question n°2 | Question n°3 | Question n°4 | Question n°5 | Question n°6 | Question n°7 | Question n°8 | Question n°9 | Question n°10 | Question n°11 | Question n°12 | Question n°13 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0 | 13 | 10 | 25 | 19 | 12 | 20 | 12 | 22 | 13 | 10 | 32 | 30 | 8 |
| 1 | 15 | 18 | 9 | 5 | 13 | 7 | 12 | 8 | 12 | 9 | 3 | 5 | 11 |
| 2 | 6 | 7 | 2 | 9 | 11 | 8 | 12 | 5 | 3 | 11 | 1 | 1 | 13 |
| 3 | 2 | 2 | 2 | 4 | 1 | 3 | 0 | 2 | 5 | 3 | 1 | 2 | 3 |
| 4 | 2 | 1 | 0 | 1 | 1 | 0 | 2 | 1 | 5 | 5 | 1 | 0 | 3 |

*Fig. 2: Frequency of the scores for each question of the Mandibular Symptoms and Oral Habits Questionnaire.*

for worst pain experienced. The patients were divided according to average (above and below five). Table 1 shows the results. Table 2 shows the pain results for TMJ palpation.

**Table 1: Patient response in relation to pain during muscle palpation.**

| Muscle/ Side | Right (n) | | Left (n) | |
|---|---|---|---|---|
| **Score** | **Above 5** | **Below 5** | **Above 5** | **Below 5** |
| Anterior temporal | 05 | 11 | 06 | 10 |
| Medial temporal | 02 | 14 | 05 | 11 |
| Posterior temporal | 02 | 14 | 05 | 11 |
| Masseter | 08 | 08 | 09 | 07 |
| Sternocleidomastoid | 06 | 10 | 05 | 11 |
| Digastric | 02 | 14 | 04 | 12 |
| Platysma | 04 | 12 | 04 | 12 |
| Temporal (Intraoral) | 06 | 10 | 09 | 07 |
| Medial pterygoid | 05 | 11 | 08 | 08 |
| Lateral medial pterygoid | 06 | 10 | 08 | 08 |

**Table 2: Patient response in terms of pain during palpation of the TMJ.**

| | Right (n) | | Left (n) | |
|---|---|---|---|---|
| **Score** | **Above 5** | **Below 5** | **Above 5** | **Below 5** |
| Lateral pole | 05 | 11 | 03 | 13 |
| Posterior pole | 04 | 12 | 07 | 09 |

Higher scores were recorded on the Mandibular Symptoms and Oral Habits Questionnaire for patients with severe TMD, according to the Helkimo index (one-way ANOVA, 15.94±5.05, F=7.05; p<0.01), when compared with those with mild TMD (7.10 ±4.65; p=0.002). This difference was not found for patients with moderate TMD, when compared with those with severe TMD (12.27 ±7.87; p=0.258) or those with mild TMD (p=0.127). Table 3 shows the correlation between the Helkimo index and the FAI. A positive correlation (r=0.78; p<0.01) was found between the number of positive responses

**Table 3: Distribution and classification of patients according to the Helkimo Index (The Clinical dysfunction component) and the Fonseca Anamnestic Index.**

| | | Fonseca Anamnestic Index (n) | | | |
|---|---|---|---|---|---|
| | | **Mild TMD** | **Moderate TMD** | **Severe TMD** | |
| Helkimo Index (n) | Score | 20-40 | 45-65 | 70-100 | Total |
| Mild | 1-4 | 7 | 3 | 1 | 11 |
| Moderate | 5-9 | 4 | 3 | 2 | 9 |
| Severe | 10-13 | 2 | 3 | 6 | 11 |
| | 15-17 | 1 | 2 | 2 | 5 |
| | 20-25 | 0 | 0 | 2 | 2 |
| Total | | 14 | 11 | 13 | 38 |

n – number of patients

on the AAOPQ and the sum of the scores on the Mandibular Symptoms and Oral Habits questionnaire. Patients who were classified with severe TMD according to the FAI exhibited higher scores on the Mandibular Symptoms and Oral Habits questionnaire (18.58 ±4.96/ one way ANOVA F=14.43), with a statistically significant difference when compared to patients with moderate TMD (12.08 ±5.64; $p<0.01$) and mild TMD (7.46 ±4.89; $p<0.01$).

Patients who were classified with severe TMD by the FAI exhibited more positive responses on the AAOPQ (6.25 ±1.42; one way ANOVA F=15.82), with a statistically significant difference when compared to patients with mild TMD (3.0 ±1.22; $p<0.01$). No significant difference was found between patients with severe and moderate TMD (5.69 ±1.93; $p=0.648$).

## DISCUSSION

In general, all the indices used sought to assess the frequency and severity of the symptoms associated with TMD.[4,7] Patients with TMD may suffer from myalgia and joint disorders, which contribute to the diversity of the signs and symptoms reported. It was noted that the indices exhibited statistically significant differences when mild and severe disorders were compared using the Helkimo Index and the JSOHQ and when using the FAI and the AAOPQ, with no significant difference found for individuals classified with moderate TMD. This may be due to the fact that the moderate form of the disorder does not differ greatly from the other stages in terms of the frequency of symptoms.

Helkimo was a pioneer in developing indices to measure TMD severity. In an epidemiological study, Helkimo developed an index that was further divided into anamnesis, clinical and occlusal dysfunction. The index sought to identify the prevalence and severity of TMD in the general population.[5,9,10] However, the relationship between the anamnesis, occlusal and dysfunction components of the Helkimo index was not clear.[10] Thus, in the present study, only the dysfunction index was used, similarly to a previous study.[11] The Fonseca Anamnestic Questionnaire is a modified version of the Helkimo anamnestic index and is one of the few instruments available in Portuguese that assesses the severity of TMD symptoms[12] Despite the similarities in the results for TMD severity calculated by the FAI and the HI, they were not identical. These indices exhibit certain similarities among the symptoms studied, such as pain upon opening the mouth; pain in the TMJ and joint noises. Nevertheless, the HI is an objective clinical assessment, whereas the FAI is a questionnaire in which the patient indicates the presence or absence of the symptom studied. In addition, none of the indices provide a complete assessment and consequently, flaws are to be expected.

A positive correlation was found between positive responses on the AAOPQ and the sum of the scores on the JSOHQ. This can be explained by the fact that the questions deal with equivalent subjects, which contributed to the similarity of the results. The equivalent JSOHQ and AAOPQ questions are (question/question): 1/1 – pain or difficulty opening the mouth; 2/3 – pain during mandibular function/ while chewing; 9/4 – joint noises; 11-12/5 – locking of the jaws; 5/6 – pain in the TMJ.

Manfredi et al. [8] assessed the sensitivity and specificity of the questionnaire used for screening orofacial pain and TMD, as recommended by the American Academy of Orofacial Pain. A correlation was found between positive responses and the clinical findings of the specific anamnesis for TMD. Questions 3 and 5 deal with the characteristic pains of TMD such as difficulty and/or pain when chewing or talking, as well as a feeling of tiredness in the jaws. These are the most significant in the questionnaire, due to the link with occlusal conditions and the presence of habits such as grinding or clenching teeth. The authors noted that the questionnaire is sensitive and correlated with extracapsular pathologies or myogenic disorders in which the main complaint is diffuse facial pain. Franco-Micheloni et al. [13] showed that questions 8 and 10 of the AAOPQ demonstrated low and non-significant inter-item correlations with the clinical findings, corroborating their low contribution to the questionnaire. More than two positive answers for the eight item questionnaire could be used as a threshold for the detection of TMD. Campos et al.[6] conducted a study on 700 women to assess the validity and reliability of the FAI. They identified that questions 4, 8 and 10 hindered the internal consistency of the instrument. When these questions were excluded, the FAI exhibited satisfactory internal consistency. The FAI exhibited a high degree of diagnostic accuracy and can be used to identify myogenous TMD in women.[3]

Chaves et al.[4] suggested that the FAI has not yet been completely validated and does not offer a diagnostic classification of TMD. Thus, data obtained using this index are restricted to the classification of the severity of the signs and symptoms of TMD.

In the present study, the classification of severity (according to the FAI) enabled us to establish a statistically significant difference in relation to the JSOHQ scores for mild, moderate and severe stages of the disorder. This was possible due to the similarity of the questions concerning the presence of signs and symptoms, such as (FAI question/ JSOHQ question): 1/1- pain upon opening the mouth; 2/2- pain while chewing/moving the mandible; 3/3- muscle fatigue; 6/5- pain in the TMJ region; and 7/9 – joint noises.

The AAOPQ and the FAI seem to be ideal tools for initial patient screening because they are quick to apply and cost-effective, and are thus also appropriate for large epidemiological studies. The severity of TMD in the FAI and the number of positive responses in the AAOPQ can help clinicians decide whether a more comprehensive assessment is required to obtain a definitive TMD diagnosis.[13] The FAI and the AAOPQ are somewhat similar in terms of the signs and symptoms assessed (FAI question/AAOPQ question): 1/1: difficulty in opening the mouth; 2/3 – difficulty in moving the mandible; 3/5 – fatigue in the jaws; 4/7 - headaches; 6/6 – pain in the TMJ region (pre-auricular); 9/9 – abnormal occlusion/bite.

Several studies have sought to analyze the relevance of certain questions within the instruments used in the present study.[6,8] Several authors have proposed the removal of questions that do not seem to contribute to the diagnosis or classify the severity of the disorder. In fact, none of these instruments are flawless. However, considering that these indices include very similar questions and provide consistent results, is it necessary to use all of them? Which instrument should be selected for diagnosis and which should be used for classification? How should the results of a certain treatment protocol be monitored?

There is consensus in the literature concerning diagnostic instrument: the RDC/TMD has been accepted as a universal diagnostic instrument for TMD. It was proposed in 1992 by Dworkin and Leresche[14] and has been accepted and used in several clinical and epidemiological studies.[15] Moreover, it is continuously being improved.[16] Concerning classification, the present study considered two instruments (Helkimo and FAI), of which the latter seemed to be more adequate since it reducesthe number of categories and has been used recently in the literature. The authors of the present study monitored the results.

No diagnostic or assessment instrument should be used in place of a physical examination. Unfortunately, clinical data were not found for all of the patients, which prevented comparisons with the indices. The present study has the limitations that are inherent to retrospective studies: limited sample size; it did not use RDC/DTM in the diagnosis; and the inclusion of patients. Further studies could rationalize the selection of the assessment instrument in accordance with the objective, either to identify whether or not patients have the disorder or to classify, diagnose or monitor/compare the results of different treatment protocols. This study should be viewed as a preliminary study seeking to highlight an issue of paramount importance: how to select instruments correctly when assessing TMD.

In the study sample, there was consistency among the instruments used to distinguish between patients with severe TMD symptoms and patients with mild TMD symptoms, since the same topics (signs and symptoms) are covered by most of the instruments. The use of two or three of these instruments does not guarantee a more accurate diagnosis because most of them were created as a selection tool or to classify the severity of disease. Further studies could associate instruments for diagnosis such as RDC/TMD with instruments to classify the severity of disease such as Fonseca Anamnestic index and Helkimo index.

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# Parental perceptions of impact of oral disorders on Colombian preschoolers' oral health-related quality of life

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***ABSTRACT***

*There is no study assessing the impact of dental caries (DC), traumatic dental injuries (TDI) and dental malocclusions (DM) on the oral health-related quality of life (OHRQoL) of preschool children from Spanish-speaking countries in population-based samples. The purpose of this study was to assess the impact of DC, TDI and DM, on Colombian preschool children's OHRQoL through a cross-sectional study. The clinical setting included private and public preschools in Cartagena,Colombia. The sample included 643 preschool children aged 1-5 years and their parents, who answered the Colombian version of the Early Childhood Oral Health Impact Scale (C-ECOHIS) and socioeconomic questionnaire. Three calibrated examiners performed clinical assessment of severity of DC according to decayed, missing and filled primary teeth index, TDI and DM. Poisson regression associated clinical and socio economic conditions to the outcome. Overall, 48.2% of parents reported children's oral impacts (total C-ECOHIS score ≥1). The mean (standard deviation) C-ECOHIS scores were 2.20 (0.15). The multivariate adjusted model showed that children from non-nuclear families (RR=1.51; p=0.003),with low and high DC severity (RR=1.51, p=0.003; RR=1.53, p=0.009) and TDI (RR=1.56, p=0.003), were more likely to experience negative impact on total C-ECOHIS scores.*

*DC and TDI have negative impact on Colombian preschool children's OHRQoL. Children from non-nuclear families have worse OHRQoL at this age, independently of the presence of oral conditions.*

***Key words:*** *Dental caries; tooth injuries; quality of life; malocclusion; child, preschool.*

## Percepción de padres del impacto de desórdenes orales de preescolares Colombianos sobre calidad de vida relacionada con la salud oral

***RESUMEN***

*No existen estudios que evalúen el impacto de la caries dental (CD), el trauma dentoalveolar (TDA) y las maloclusiones dentales (MD) sobre la calidad de vida relacionada con la salud bucal (CVRSB) en niños preescolares en muestras de poblaciones de países hispanohablantes. El propósito de este estudio fue evaluar el impacto de CD, TDA y MD sobre la CVRSB en niños colombianos en edad preescolar a través de un estudio transversal. Las evaluaciones clínicas se realizaron en colegios privados y públicos de Cartagena, Colombia, en una muestra de 643 niños en edad de 1-5 años y sus padres quienes respondieron la versión colombiana de la Escala Early Childhood Oral Health Impact Scale (C-ECOHIS) y un cuestionario socioeconómico. Tres examinadores calibrados realizaron la evaluación clínica de la severidad de CD acorde con el Indice ceod para dentición decidua, TDA y MD. La regresión de Poisson asoció las condiciones clínicas y socioeconómicas al puntaje total del C-ECOHIS y sus dominios. En general, el 48,2% de los padres reportaron impactos orales de los niños (puntuación C-ECOHIS total ≥1). La media (DE) del C-ECOHIS fué de 2,20 (0,15). El modelo multivariado ajustado mostró que los niños de familias no nucleares (RR = 1,51; p = 0,003), que tienen baja y alta severidad de CD (RR = 1,51, p = 0,003; RR = 1,53, p = 0,009) y TDA(RR = 1,56, p = 0,003) tuvieron mayor probabilidad de experimentar un impacto negativo en las puntuaciones totales de C-ECOHIS.*

*La CD y la TDA tienen un impacto negativo sobre la CVRSB en niños preescolares colombianos. Los niños de familias no nucleares tienen peor CVRSB a esta edad, independientemente de la presencia de las condiciones orales.*

***Palabras clave:*** *Caries dental, traumatismos de los dientes, calidad de vida, maloclusión, preescolar.*

## INTRODUCTION

Oral health-related quality of life (OHRQoL) is important for measuring the impact of oral health disparities and access to care in different populations[1]. Children are the main priority of Public Health Dentistry, so urgent efforts should be made to apply the OHRQoL concept to children around the world, considering that their OHRQoL can be influenced by social, cultural and economic factors[2].

In Colombia, the latest national epidemiological survey for Oral Health indicated that the prevalence of dental caries (DC), traumatic dental injuries (TDI) and dental malocclusions (DM) in children aged 1 to 5 years is 38.3%, 34.5% and 15.8%, respectively[3]. At present, there is concrete evidence of the negative impact of DC on preschool children's OHRQoL[4-8], while the evidence for TDI and DM at this age remains controversial[9-14]. However, as far as we know, all the studies available in the literature are on non-Hispanic children.

The ECOHIS is the most frequently used instrument in the literature to assess OHRQoL in preschool children. Recently, the Latin American Spanish version of the ECOHIS has been cross-culturally adapted and validated to evaluate parents' perceptions of their preschool children's OHRQoL[15]. Nevertheless, to the best of our knowledge, it has not been tested among preschoolers in Spanish-speaking countries to assess the impact of oral diseases and disorders on preschool children's OHRQoL. The first use of the Latin American Spanish version of the ECOHIS would enable OHRQoL comparisons with other cultural and ethnic groups for which the ECOHIS has been adapted and validated, as well as being an outcome measure for evaluating service initiatives and oral health promotion programs in different countries.

The aim of this study was to evaluate the impact of DC, TDI and DM on the OHRQoL of Colombian preschool children and their parents in a population-based sample.

## MATERIALS AND METHODS

This study was approved by the Research Ethics Committee of the Public Health School of the University of Cartagena in compliance with Colombian National Health Council Resolution 8430/1993, and abided by the Helsinki Convention. Parents signed informed consent forms allowing their children to participate in the study.

### Sampling

A preschool-based cross-sectional study was performed in 2015 on children aged 1 to 5 years enrolled at private and public preschools in Cartagena,Colombia. The city of Cartagena has an estimated population of 1,001,755 inhabitants, including 116,293 preschool children.[16]

A 2-stage random sampling method was adopted to select the sample. The first stage units were all the public and private preschools in the city. A total of six schools, three public and three private, were randomly selected with a chance proportional to the number of enrolled children and considering their strategic distribution among the city districts. Since the preschools were of different sizes, an equal probability selection method (probability proportional to size) was used to ensure that all children would have the same probability of being selected[17]. The second stage units were the 1-to 5-year-old children enrolled in each selected preschool.

Sample size was calculated based on a 5% margin of error, 95% confidence interval and 1.4 correction factor for design effect. As there was no data on the prevalence of oral impacts on the OHRQoL of Colombian preschool children, a prevalence of 50% was used to obtain the largest possible sample and increase the power of the study. The minimum sample was determined to be 538 under five-year-old preschool children, to which 20% was added to compensate for possible dropouts, totaling 641 children. Children of both sexes and with parents/caregivers who were fluent in Spanish language and who agreed to participate in the study were included. Children undergoing dental treatment in the past 3 months or with systemic and/or neurological diseases were excluded.

### Data collection

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 at schools: one on socioeconomic conditions and another on the child's OHRQoL. Interviews were conducted by two dental assistants who were blinded to the children's oral examinations. They were trained in the reading and intonation of each question and answer options to the OHRQoL instrument, and in the use of modalizers.

Socioeconomic conditions such as parental age, number of siblings, and family income were collected

as discrete quantitative variables, whereas parental level of education and family structure were collected as ordinal and nominal qualitative variables, respectively. All these variables were then categorized for statistical analyses as follows: child's age [1-2 years old and 3-5 years old]; child's sex [female, male]; type of school [public, private]; age of parents [≤ 44 years old,> 44 years old]; number of siblings [≤ 2 children,> 2 children]; parental education [< 10 years, ≥ 10 years]; family income [as 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 and was divided into ≤ 01 CMW, ≥ 02 CMW]; household crowding [≤ 02 members per room, >02 members per room] and family structure [Nuclear family, Non-nuclear family (living with single parents or others)].

**OHRQoL instrument**

The Latin American Spanish version of the Early Childhood Oral Health Impact Scale (ECOHIS) assesses preschool children's OHRQoL according to parents' proxyreports[15]. It contains 13 items corresponding to four domains for items included in the child impact section: symptoms - 01 item; function – 04 items; psychological – 02 items; self-image / social interaction – 02 items; and two domains for the family impact section: parent distress – 02 items and family function – 02 items. The responses to each item are coded as follows: 0 = never; 1 = almost never; 2 = sometimes; 3 = often; 4 = very often; 5 = don't know. Total scores are calculated as the sum of the response codes. The answers "do not know" are counted, but excluded from the total ECOHIS score for each patient. A high score means a high degree of negative oral impacts on the child's OHRQoL.

In this study, the Latin American Spanish version of the ECOHIS[15] was adapted to the Colombian context. The Latin American Spanish version of the ECOHIS was pilot-tested on a convenience sample of 30 parents of children aged 1-5 years. These parents did not take part in the final sample. Parents suggested the substitution of some words or expressions by synonyms to facilitate de comprehension of the questionnaire; modifications were made according the parents' comments. Only one expression related to question 7, "has your child been annoyed or bad tempered?" was adapted to "has your child expressed frustration of been sad?". A Revision Panel consisting of three postgraduate professors in Pediatric Dentistry and Family Health Care, 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 version of the ECOHIS (C-ECOHIS), which was the instrument used in this study.

**Children's oral examination**

Four previously calibrated dental examiners independently carried out the children's oral examination. The examiners were all graduate dentists with experience in previous epidemiological surveys. All examiners underwent two 6-h sessions of training exercises with pictures of clinical cases for the study clinical conditions. The calibration process was established with an interval of 1 week between oral clinical sessions to obtain intra- and inter-examiner reliability Kappa values 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. Intra-and inter-examiner Kappa values were calculated for all clinical conditions.

DC was assessed according to the World Health Organization criteria[17] and calculated in terms of decayed, indicated for extraction owing to caries and filled primary teeth (dmft). The dmft was categorized according to the severity of DC, based on the previously proposed scores[4,18]: dmft 0 = caries free; dmft 1–5 = low severity; and dmft> 6 = high severity. The children were then classified by dental caries experience according to the Knutson index[19].

TDI was performed using the criteria proposed by Andreasen et al.[20], which is 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. The TDI data were then analyzed according to the presence of at least one kind of trauma or the absence (tooth present and sound) of TDI in the upper and lower arches.

DM were diagnosed according to published clinical criteria:anterior open bite – lack of a vertical overlap of the incisors in the occlusal position[4,11,21,22]; anterior cross bite – when the lower incisors were observed in front of the upper ones[4,23,24] posterior cross bite – when the upper primary molars

occluded in lingual relationship to the lower primary molars in centric occlusion[4,10,21,23], and increased overjet – horizontal distance between the incisal edges of upper and lower central incisors >3 mm[4,10,24]. The presence of at least one of these malocclusions classified the child as having malocclusions.

**Table 1: Sociodemographic features of the sample (n = 643).**

| Variables | n (%) |
|---|---|
| ***Child's age*** | |
| Infant (1 – 2 years old) | 157 (24.2) |
| Preschool (3 – 5 years old) | 486 (75.6) |
| ***Child's sex*** | |
| Female | 310 (48.2) |
| Male | 333 (51.8) |
| ***Type of School*** | |
| Public | 326 (50.7) |
| Private | 317 (49.3) |
| ***Age of parents*** | |
| ≤ 44 years old | 632 (98.3) |
| > 44 years old | 11 (1.7) |
| ***Number of siblings*** | |
| ≤ 2 children | 509 (79.3) |
| > 2 children | 134 (20.8) |
| ***Parental education level*** | |
| < 10 years | 121 (18.8) |
| ≥ 10 years | 522 (81.2) |
| ***Mother's education level*** | |
| < 10 years | 93 (14.5) |
| ≥ 10 years | 550 (85.5) |
| ***Family income*** | |
| ≤ 01 CMW | 277 (43.1) |
| ≥ 02 CMW | 366 (56.9) |
| ***Household crowding*** | |
| ≤ 2 members per room | 476 (74.0) |
| > 2 members per room | 167 (26.0) |
| ***Family structure*** | |
| Nuclear family | 476 (64.8) |
| Non-Nuclear family | 167 (26.0) |
| ***Dental caries experience*** | |
| Absence (dmft = 0) | 446 (69.4) |
| Presence (dmft ≥1) | 197 (30.6) |
| ***Dental caries severity*** | |
| Caries free: dmft = 0 | 446(69.4) |
| Low severity: dmft 1-5 | 160(24.9) |
| High severity; dmf<6 | 37(5.7) |
| ***Traumatic dental injuries*** | |
| Absence | 573 (86.0) |
| Presence | 90 (14.0) |
| ***Dental malocclusion*** | |
| Absence | 492 (76.5) |
| Presence | 151 (23.5) |

***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 domains of the C-ECOHIS scores. Poisson regression with robust variance was performed to associate domains and total scores of the C-ECOHIS to oral clinical conditions (DC, TDI and DM) and socioeconomic conditions. Univariate Poisson regression analysis was performed to select variables with a p-value ≤0.20 to enter the model. Then the explanatory variables selected were tested in the multivariate adjusted model and retained only when $p \leq 0.05$. 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 C-ECOHIS was analyzed using Cronbach's alpha coefficient, and was 0.87 for total C-ECOHIS scores in the pilot test phase and 0.90 for total C-ECOHIS scores in the final sample size of the study, showing the stability of the instrument.

As a result of the calibration process, inter-examiner reliability values for Cohen's Kappa agreement were 0.86 for DC, 0.90 for TDI and 0.91 for DM. Intra-examiner agreement kappa values were 0.87, 0.86 and 0.90 for DC, TDI and DM, respectively.

Altogether, 664 parents were invited to participate in the study, of whom 9 were excluded because they did not meet the study criteria. Of the 655 eligible participants, 643 provided signed parental informed consent to participate in the study (positive response rate = 98.2%).

Table 1 shows the socioeconomic and clinical conditions of the sample. It was observed that 197 children had dental caries experience (30.6%), whereas TDI and DM were present in 14% and 23.5% of the sample, respectively. All questionnaires were fully completed without omitting any answers. Most surveys were answered by children's mothers (84.1%).

Overall, 48.2% of parents reported children's oral impacts (total C-ECOHIS scores ≥ 1). The highest impact scores reported were 25 on the child impact section and 16 on the family impact section.

Table 2 shows the mean, standard deviation, and the range observed for the total C-ECOHIS scores and individual domains. The most strongly affected domains were the function domain and the parent distress domain in the child section and family section, respectively.

Table 3 shows the univariate unadjusted analysis of socioeconomic and clinical variables associated with total and individual domains of the C-ECOHIS. There was significant association between some independent variables, total scores, and individual domains ($P < 0.05$). In general, total C-ECOHIS scores was associated with child's age, type of school, mother's education, family structure, DC experience and its severity, and TDI.

The multivariate adjusted model (Table 4) showed that children from non-nuclear families, those with low and high DC severity and with presence of TDI were more likely to experience a negative impact on total C-ECOHIS scores (RR = 1.51, p = 0.003; RR = 1.53, p = 0.009; RR = 3.38, p =<0.0001; RR = 1.56; p = 0.003, respectively). Children from non-nuclear families, with low and high DC severity and TDI showed a negative impact on the oral symptoms domain (RR = 1.40, p = 0.034; RR = 1.46, p = 0.04; RR = 3.26, p = <0.0001 and RR = 1.60, p = 0.012, respectively). For the functional limitations domain, 3- and 5-year-old children, with mother's education ≥ 10 years, from non-nuclear families and those with high DC severity and TDI showed a negative impact (RR = 1.92, p = 0.002; RR = 1.72, p = 0.002; RR = 1.38, p = 0.03; RR = 1.69, p = 0.001; RR = 2.89, p< 0.0001; RR = 1.95, p <0.0001, respectively). Children from non-nuclear families, with low and high DC severity were associated with negative impact on the psychological domain (RR = 1.76, p = 0.036; RR = 1.48, p = 0.021; RR = 3.22, p = 0.003, respectively). The negative impact on the self-image social interaction domain was associated with children from non-nuclear families (RR = 2.54, p = 0.01), low and high DC severity (p<0.05). The low and high DC severity, and presence of TDI showed negative impact on the parent distress domain (RR = 1.75, p = 0.002; RR = 2.85, p<0.0001; RR = 1.57, p = 0.017, respectively). For the family function domain, 3- and 5-year-old children and with DC experience showed negative impact on the family function domain (RR = 2.53, p = 0.038;RR = 2.09, p = 0.009, respectively).

**Table 2: Mean,standard deviation, possible range and range observed overall and for each C-ECOHIS domain score (n = 643).**

| C-ECOHIS (variances) | Means(SD) | Range Observed |
|---|---|---|
| Oral symptoms (0-4) | 0.37 (0.03) | 0-4 |
| Functional limitations (0-16) | 0.65 (0.05) | 0-9 |
| Psychological (0-8) | 0.21 (0.02) | 0-7 |
| Self-image and social interaction(0-8) | 0.12 (0.2) | 0-8 |
| Parent distress (0-8) | 0.63 (0.05) | 0-8 |
| Family function(0-8) | 0.19 (0.02) | 0-8 |
| Total Score | 2.2(0.15) | 0-33 |

## DISCUSSION

This is the first study to asses parents' perceptions of the impact of DC, TDI and DM on the OHRQoL of Colombian preschool children using the C-ECOHIS in a population-based sample of 1-to 5-year-old children. The C-ECOHIS showed semantic equivalence and excellent internal consistency for this study.

Our adjusted results show that in general, low and high DC severities were associated with negative impact on total C-ECOHIS scores and most of the C-ECOHIS domains, while DC prevalence was only associated with the family function domain. This may be explained by the similarity of the two variables, which confound the associations in the analysis. Moreover, DC severity appears to be a more sensitive measure because the effect of the disease on children's OHRQoL can be observed in detail. Our results are also in accordance with previous studies assessing the impact of DC on preschool children's OHRQoL using other ECOHIS versions[4,5,7-9,25]. However, although there is conclusive evidence of the impact of DC on preschool children's OHRQoL, never before had this association been confirmed in Spanish-speaking preschoolers. This suggests that oral health perceptions concerning DC are not influenced by ethnic and cultural issues.

TDI showed negative impact on preschool children's OHRQoL, but only on the oral symptoms domain, functional limitations domain, parent distress domain and total C-ECOHIS scores. Most studies in the literature[11-13] found no association between the presence of TDI and preschool children's OHRQoL in total ECOHIS scores. Conversely, and

**Table 3: Univariate analysis of sociodemographic variables and clinical conditions associated with total ECOHIS score and total scores for domains.**

| | Oral Symptoms Domain | | Functional Limitations Domain | | Psychological Domain | | Self-Image And Social Interaction Domain | | Parent Distress Domain | | Family Function Domain | | Total Ecohis Score | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | RR (95% IC) | P | RR (95% IC) | P | RR (95% IC) | P | RR (95% IC) | P | RR (95% IC) | P | RR (95% IC) | P | RR (95% IC) | P |
| **Child age** | | | | | | | | | | | | | | |
| Infant (1 - 2 years old) | | | | | | | | | | | | | | |
| Preschool (3 - 5 years old) | 1.29 (0.84–1.97) | 0.235 | 1.07 (1.19–2.93) | 0.006 | 0.96 (0.53-1.74) | 0.917 | 1.77 (0.67-4.69) | 0.247 | 1.11 (0.74-1.65) | 0.597 | 3.12 (1.26-7.70) | 0.013 | 1.42 (1.00-2.04) | 0.049 |
| **Type of school** | | | | | | | | | | | | | | |
| Private | | | | | | | | | | | | | | |
| Public | 1.33 (0.97–1.83) | 0.071 | 1.58 (1.15- 2.18) | 0.005 | 2.05 (1.22-3.44) | 0.006 | 1.94 (0.90-4.20) | 0.091 | 0.93 (0.67-1.28) | 0.661 | 1.42 (0.78-2.55) | 0.242 | 1.34 (1.025.77) | 0.032 |
| **Number of siblings** | | | | | | | | | | | | | | |
| ≤ 2 children | | | | | | | | | | | | | | |
| > 2 children | 1.29 (0.88–1.88) | 0.178 | 1.39 (0.96–2.00) | 0.074 | 1.62 (0.88-2.98) | 0.012 | 1.13 (0.51-2.53) | 0.749* | 1.02 (0.70-1.49) | 0.893 | 0.74 (0.33-1.63) | 0.464 | 1.20 (0.85-1.70) | 0.279 |
| **Father's education** | | | | | | | | | | | | | | |
| < 10 years | 0.99 (0.64 – 1.53) | 0.984 | 1.43 (0.99–2.07) | 0.055 | 1.27 (0.63-2.58) | 0.495 | 1.48 (0.69-3.18) | 0.307 | 1.04 (0.70–1.56) | 0.816 | 1.56 (0.79-3.04) | 0.193 | 1.23 (0.86-1.76) | 0.237 |
| ≥ 10 years | | | | | | | | | | | | | | |
| **Mother's education** | | | | | | | | | | | | | | |
| < 10 years | 1.21 (0.77 – 1.90) | 0.387 | 1.86 (1.29-2.69) | 0.001 | 2.12(1.10-4.10) | 0.025 | 1.90 (0.86-4.17) | 0.108 | 1.01 (0.63–1.62) | 0.945 | 1.89 (0.94-3.76) | 0.070 | 1.51 (1.03-2.22) | 0.033 |
| ≥ 10 years | | | | | | | | | | | | | | |
| **Family income** | | | | | | | | | | | | | | |
| ≤ One CSW | 0.96 (0.69 – 1.32) | 0.804 | 1.24 (0.90-1.70) | 0.183 | 1.43 (0.85-2.42) | 0.170 | 0.96 (0.46-2.00) | 0.932 | 0.84 (0.61-1.16) | 0.286 | 1.09 (0.61-1.93) | 0.755 | 1.05 (0.79-1.38) | 0.715 |
| ≥ Two CSW | | | | | | | | | | | | | | |
| **Household crowding** | | | | | | | | | | | | | | |
| ≤ 2 members | | | | | | | | | | | | | | |
| > 2 members | 1.32 (0.93–1.87) | 0.119 | 1.56 (1.11-2.18) | 0.009 | 1.84 (1.07-3.17) | 0.027 | 1.50 (0.70-3.24) | 0.292 | 1.18 (0.83–1.67) | 0.342 | 0.87 (0.44-1.70) | 0.691 | 1.35 (0.99-1.84) | 0.051 |
| **Family structure** | | | | | | | | | | | | | | |
| Nuclear family | | | | | | | | | | | | | | |
| Non-nuclear family | 1.43 (1.04–1.97) | 0.027 | 1.78 (1.30-2.44) | <0.001 | 1.84 (1.09-3.09) | 0.021 | 2.79 (1.36-5.74) | 0.005 | 1.26 (0.90–1.75) | 0.169 | 1.34 (0.76-2.38) | 0.308 | 1.56 (1.18-2.06) | 0.002 |
| **Dental caries experience** | | | | | | | | | | | | | | |
| Absence | | | | | | | | | | | | | | |
| Presence | 1.81 (1.32–2.50) | <0.0001 | 1.62 (1.16-2.26) | 0.004 | 1.94 (1.14-3.30) | 0.014 | 3.80 (1.82-7.95) | <0.001 | 1.94 (1.40–2.68) | <0.001 | 2.69 (1.53-4.72) | 0.001 | 1.03 (1.00-1.06) | 0.011 |
| **Dental caries severity** | | | | | | | | | | | | | | |
| Caries free: dmft 0 | | | | | | | | | | | | | | |
| Low severity: dmft 1-5 | 1.52 (1.05-2.18) | 0.024 | 1.19 (0.81-1.73) | 0.356 | 1.57 (0.86-2.87) | 0.137 | 2.78 (1.30-5.94) | 0.008 | 1.76 (1.23-2.53) | 0.002 | 2.41 (1.28-4.52) | 0.006 | 1.61 (1.17-2.21) | 0.003 |
| High severity; dmf<6 | 3.28 (2.13-5.06) | <0.001 | 3.67 (2.35-5.71) | <0.001 | 3.33 (1.50-7.39) | 0.003 | 7.23 (2.37-22.0) | <0.001 | 2.80 (1.76-4.45) | <0.001 | 3.21 (1.42-7.25) | 0.005 | 3.42 (2.28-5.14) | <0.001 |
| **Traumatic dental injuries** | | | | | | | | | | | | | | |
| Absence | | | | | | | | | | | | | | |
| Presence | 1.53 (1.03– 2.28) | 0.035 | 1.67 (1.12-2.49) | 0.012 | 0.90 (0.39-2.09) | 0.818 | 2.11 (0.79-5.64) | 0.133 | 1.56 (1.06–2.30) | 0.024 | 1.00 (0.50-1.98) | 0.987 | 1.49 (1.05-2.10) | 0.022 |
| **Dental malocclusion** | | | | | | | | | | | | | | |
| Absence | | | | | | | | | | | | | | |
| Presence | 0.92 (0.65– 1.31) | 0.656 | 1.08 (0.74-1.56) | 0.672 | 0.96 (0.56-1.66) | 0.899 | 0.59 (0.26-1.31) | 0.198 | 0.21 (0.86-1.71) | 0.269 | 1.59 (0.84-3.00) | 0.152 | 1.09 (0.81-1.45) | 0.555 |

in line with our results, only one study[9] found a negative impact of the presence of TDI on total scores. Moreover, few studies[12,13] have assessed the impact of the presence of TDI on the ECOHIS domains using regression analysis, but no association was found. Notwithstanding, one of these studies also used a classification for severity of TDI[13], showing that complicated TDI has negative impact on the oral symptoms and self-image/social interaction domains. Our results corroborate the findings for the symptoms domain, however, we also found associations for other domains of the C-ECOHIS. Discrepancies between our results and previous studies can be explained by the use of different clinical criteria and analysis for TDI. Nevertheless, although some types of TDI can be expected to have negative effects on symptoms and to cause functional limitations and parental

**Table 4: Multivariate analysis of sociodemographic variables and clinical conditions associated with total scores for domains and ECOHIS.**

| | Oral Symptoms Domain | | Functional Limitations Domain | | Psychological Domain | | Self-Image And Social Interaction Domain | | Parent Distress Domain | | Family Function Domain | | Total Ecohis Score | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | RR (95% IC) | P | RR (95% IC) | P | RR (95% IC) | P | RR (95% IC) | P | RR (95% IC) | P | RR (95% IC) | P | RR (95% IC) | P |
| **Child age** | | | | | | | | | | | | | | |
| Infant (1 - 2 years old) | | | | | | | | | | | | | | |
| Preschool (3 - 5 years old) | † | | 1.92(1.26-2.93) | 0.002 | † | | † | | † | | 2.53 (1.05-6.17) | 0.038 | † | |
| **Mother's education** | | | | | | | | | | | | | | |
| < 10 years | † | | 1.72 (1.21-2.44) | 0.002 | † | | † | | † | | † | | † | |
| ≥ 10 years | | | | | | | | | | | | | | |
| **Household crowding** | | | | | | | | | | | | | | |
| ≤ 2 members | | | | | | | | | | | | | | |
| > 2 members | † | | 1.38(1.03-1.86) | 0.03 | † | | † | | † | | † | | † | |
| **Family structure** | | | | | | | | | | | | | | |
| Nuclear family | | | | | | | | | | | | | | |
| Non-nuclear family | 1.40 (1.02-1.91) | 0.034 | 1.69 (1.25-2.29) | 0.001 | 1.76 (1.03-3.01) | 0.036 | 2.54 (1.23-5.26) | 0.001 | † | | † | | 1.51(1.15-1.99) | 0.003 |
| **Dental caries experience** | | | | | | | | | | | | | | |
| Absence | | | | | | | | | | | | | | |
| Presence | † | | † | | † | | † | | † | | 2.09(1.20-3.6) | 0.009 | † | |
| **Dental caries severity** | | | | | | | | | | | | | | |
| Caries free: dmft 0 | | | | | | | | | | | | | | |
| Low severity: dmft 1-5 | 1.46(1.01-2.10) | 0.040 | 0.94(0.66-1.34) | 0.75 | 1.48(0.79-2.76) | 0.21 | 2.52 (1.15-5.51) | 0.021 | 1.75 (1.23-2.51) | 0.002 | 1.09(0.62-1.91) | 0.74 | 1.53(1.11-2.13) | 0.009 |
| High severity; dmf<6 | 3.26(2.16-4.92) | <0.0001 | 2.89 (1.93-4.34) | <0.0001 | 3.22(1.47-7.02) | 0.003 | 6.84 (2.36-19.81) | <0.0001 | 2.85 (1.80-4.51) | <0.0001 | 1.34(0.54-3.34) | 0.52 | 3.38(2.35-4.86) | <0.0001 |
| **Traumatic dental injuries** | | | | | | | | | | | | | | |
| Absence | | | | | | | | | | | | | | |
| Presence | 1.60 (1.10-2.31) | 0.012 | 1.95 (1.40-2.71) | <0.0001 | † | | † | | 1.57 (1.08-2.30) | 0.017 | † | | 1.56(1.16-2.11) | 0.003 |

†Variables not associated with the respective domains in the final multivariate model after the adjustment.

distress, our study did not assess whether this was true. Overall, the association between TDI and preschoolers' OHRQoL is still controversial. The negative impact of TDI on preschoolers seems to be more frequently reported in Spanish-speaking preschoolers, since presence of TDI affected not only total C-ECOHIS scores, but also most of its domains. DM did not show a negative impact on the OHRQoL of preschool children and their parents. Our results are in line with a recent systematic review[14] that did not find any type of association between malocclusions and impact on preschool children's OHRQoL according to the ECOHIS. At this age, children probably do not perceive this impact, which is often related to non-nutritive sucking habits. In addition, parents may attribute greater importance to oral symptoms than to any occlusal and aesthetic changes caused by the bad position of primary teeth[13] which will be replaced by permanent teeth in the future. Thus, the effect of malocclusions on OHRQoL is modified by the age of the children and their cultural environment[14]. Our study indicates that perceptions concerning the impact of DM on preschool children's OHRQoL is similar around the world when assessed by proxy reports. However, as this is the first study using the C-ECOHIS on Spanish-speaking preschoolers, further studies in other Spanish populations would be required to confirm our results.

A systematic review[2] that assessed the influence of parental socioeconomic status and home environment on children's OHRQoL suggested that children from families with high income, parental education and family economy had better OHRQoL. In addition, family structure, among other factors, was a significant predictor of children's OHRQoL[2]. In accordance with the systematic review, we have also found significant associations between C-ECOHIS scores, its domains and some socioeconomic and home environment conditions such as family structure, child's age and mother's education. Our study found that the variable most strongly associated with worse

preschool children's OHRQoL was family structure. Children from non-nuclear families were more likely to experience negative impact not only on total C-ECOHIS scores, but also on all the domains related to the child impact section. In this regard, living in a non-nuclear family predisposes preschool children to higher prevalence of DC[26], reflecting a situation of unstable economic circumstances and less ability to access health services and preventive tools, which could lead to higher oral impacts. Thus, mothers and children in non-nuclear families require substantially more attention and support than those from nuclear families to eliminate their disadvantage in developing oral diseases.[26]

This study is the first to test the C-ECOHIS in Spanish-speaking preschoolers and indicates the need for public policies providing comprehensive oral care to children at this age and redirecting efforts for prevention for DC and TDI in order to improve OHRQoL. In addition, the first use of the C-ECOHIS has important implications for research and practice. In this regard, the first use of the C-ECOHIS in a Spanish-speaking country enables comparisons with other cultural and ethnic groups around the world, as well as providing support for public oral health programs and dental care services for this age group.

## CONCLUSIONS

Dental caries and traumatic dental injuries have negative impact on Colombian preschool children's OHRQoL according to proxy reports, whereas malocclusions do not. In general, children from non-nuclear families have worse OHRQoL at this age, independently of the presence of oral conditions.

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# The effect of gingival aging in diabetic and non-diabetic status. An experimental study

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***ABSTRACT***

*Major gingival-periodontal changes according to age have been observed in both diabetic and non-diabetic rats. Male Wistar rats weighing 200-220 g were divided into two groups: 1) Non-diabetic (ND) and 2) Diabetic (D) by receiving an intraperitoneal (ip) dose of streptozotocin (STZ) (50 mg /kg). Animals from both groups (ND and D) were euthanized at 4, 8, 12, 17 y 25 weeks after treatment with saline solution or STZ. Glycemia values in ND rats were 5 to 6 mmol/L, while in D, glycemia increased progressively between weeks 4 and 25, with values ranging from 18.3±2.1 to 39.3±2.7 mmol/L. Oxidative stress differed significantly in gums of ND and D rats. ND: lipid peroxidation: Malondialdehyde (MDA): 8.52±1.2 to 15.5±2(nmol/mgP); superoxide dismutase (SOD): 37.1±4.2 to 21.2±1.3 (U/100mgP); D: MDA 13.1±1.6 to 22.9±2.7 (nmol/L); superoxide dismutase (SOD): 17.7±0.8 to 9.±0.2 (U/100mgP). Vascular permeability (VP) and gingival edema (E) showed significant changes between ND and D rats from 4 to 25 weeks. ND: PV: 10±0.2 to 16.1±1.3 (EB ug/g dry t); E: 0.9±0.1 to 4.1±1.3 ml; D: PV: 12±1.2 to 24.4±1.6 (EB ug/g dry t); E: 2.2±0.2 to 8.4±1.3 ml. Aging produced progressive natural changes in oxidative stress, VP and gingival E. In diabetic animals, changes in oxidative stress, VP and gingival E were observed early and were progressively more significant than for ND. According to these results, non-diabetic gingival modifications develop naturally with age, while in aging associated to diabetic disease, hyperglycemia increases progressively and early.*

***Key words:*** *Diabetes Mellitus, gingiva, periodontitis, oxidative stress, capillary permeability, edema.*

## El efecto del envejecimiento gingival en el estado diabetico y no diabetico. Estudio experimental

***RESUMEN***

*Se han observados importantes cambios gingivo-periodontales en función de la edad tanto en ratas no diabéticas como en ratas diabéticas. Ratas machos Wistar de 200-220 g de peso corporal fueron separadas en dos grupos: 1) No diabéticas(ND) ; 2) Diabéticas (D), por haber recibido una dosis intraperitoneal (ip) de estreptozotocina (STZ) (50 mg /kg). Ambos grupos de ratas (ND y D) fueron sacrificados a las 4, 8, 12, 17 y 25 semanas de edad después del tratamiento con solución salina o con STZ. En ratas ND las los valores de glucemia fueron de 5 a 6 mmol/L, en tanto que en las D las glucemias se observaron progresivamente aumentadas entre las 4 y las 25 semanas con valores entre 18.3±2.1 a 39.3±2.7 mmol/L. El estrés oxidativo mostró diferencias significativas entre las encías de animales ND respecto a los D; ND: peroxidacion lipidica: Malondihaldeido (MDA): 8.52±1.2 a 15.5±2(nmol/mgP);superoxido dismutasa (SOD):37.1±4.2 a 21.2±1.3 (U/100mgP); D : MDA 13.1±1.6 a 22.9±2.7 (nmol/L); Superoxidodismutasa :SOD 17.7±0.8 a 9.±0.2 (U/100mgP). La permeabilidad vascular(PV) y el edema(E) gingival mostraron cambios significativos entre las 4 y las 25 semanas de edad entre los animales ND respecto a los D : ND : PV : 10±0.2 a 16.1±1.3 (EB ug/g t seco); E :0.9±0.1 a 4.1±1.3 ml; D: PV :12±1.2 a 24.4±1.6 (EB ug/g t seco); E 2.2_/- 0.2 a 8.4± 1.3 ml. El envejecimiento produjo cambios progresivos naturales en el estrés oxidativo, PV y E gingival. En tanto que en el estado diabético los cambios del estrés oxidativo, PV y E gingival se observan temprano y fueron progresivamente más significativos comparados con los ND. De acuerdo a estos resultados las modificaciones gingivales no diabéticas se desarrollan naturalmente en función de la edad, en cambio en la senectud asociada con enfermedad diabética la hiperglucemia aumenta progresiva y tempranamente.*

***Palabras clave:*** *Diabetes mellitus, gingiva, periodontits, estrés oxidativo, permeabilidad capilar, edema.*

## INTRODUCTION

According to Van der Velden, the tissues surrounding the periodontium undergo major changes with aging[1]. Aging affects all tissues in the body, producing major anatomical and structural changes, and is associated to a decline in the control of tissue homeostasis with progressive deterioration and loss of ability to repair[2,3]. Diabetes mellitus (DM) is a well-known metabolic disorder due to which chronic inflammatory reactions affect and/or

modulate the structures and function of different tissues in patients[4,5]. DM occurs in various forms; however, all forms of DM are characterized by hyperglycemia and abnormal glucose metabolism, resulting in a deficit in insulin production or insulin action. When the prevalence of diabetic disease is associated to aging, there is an increase in susceptibility to a number of autoimmune and infectious diseases. Diabetes has been suspected of contributing to the deterioration of oral and systemic health because there is higher prevalence of infections in diabetics than in non-diabetics. Moreover, DM and periodontal disease are common chronic diseases -especially in the elderly- and are related to each other. Old age alone is not considered to be a risk factor for the development of gingival-periodontal pathology[6,7], but alterations in the gingival tissue may be caused by nutritional factors, metabolic factors and/or systemic diseases which determine gingival-periodontal alterations[8,9]. When the local oral system is modified, there may be gingival-periodontal inflammatory alterations according to age, while in the associated diabetic condition, old age fosters early production of inflammatory cytokines as an effect of hyperglycemia. Considering old age and diabetic status, our hypothesis is that individuals with gingival-periodontal disease may be at high risk of developing other systemic inflammatory diseases beyond gingival-periodontal disease.

## MATERIALS AND METHODS

Male Wistar rats weighing 200-220 g were obtained from the Animal Facility at Instituto Malbran (Buenos Aires, Argentina). Animals were placed in boxes with controlled room temperature (23±2 º C) and a 12-hour light cycle. They were fed *ad libitum* on standard pellets and water. All experiments were conducted in agreement with the Ethics Committee of Universidad Argentina John F Kennedy (Code 621).

### *Treatment of animals*

The animals were divided into 2 groups: 1) non-diabetic (ND) and 2) diabetic (D) by streptozotocin (STZ) (50 mg/kg). Five control animals and 6 diabetic animals were used for each experimental time. They were euthanized at 4, 8, 12, 17 and 25 weeks from the beginning of the experiment. Diabetic status was confirmed as from 76 hours after STZ injection and throughout the experimental period by means of an automatic glycemia analyzer (Contour TS, Bayer Diagnostic, BA, Argentina). Gingival tissue was obtained at the level of lower incisors and molar zone, to be used in biochemical determinations, vascular permeability and edema.

### *Experimental procedure*

Control and diabetic rats were anesthetized ip with urethane (200mg/kg). Extracted tissue was placed in 50 mM tris-HCl buffer (Ph 7) at 4 ºC, dried on filter paper and homogenized in 10 mM cold Tris-HCl buffer pH 7.2. Samples were centrifuged at 10.000 rpm for 30 minutes. The supernatant was used for biochemical determinations.

### *Biochemical determinations*

Protein concentration was determined using Bradford's method[10]. Lipid peroxidase (MDA) concentrations were determined following Okhawa et al [11]. Lipid peroxidase levels were measured by thiobarbituric acid (TBA) reaction. Tissue supernatant (50 ul) was added to tubes containing 2 ul butylated hydroxytoluene (BHT) in methanol. Then 50 ul 1 M phosphoric acid were added and finally 100 ul 0.8% TBA aqueous solution. The mixture was incubated for 60 min at 60 ºC. Absorbance was measured at 532 nm. TBA levels were expressed in nmol/mg P. The following reagents were used to determine superoxide dismutase (SOD): 0.3mM xanthine oxidase, 0.6 mM diethylenetriamine-penta acetic acid (DETAPAC), 150 uM nitroblue tetrazolium (NBT), 400 nM sodium carbonate and 1g/L BSA. The principle of the method is based on the inhibition of NBT reduced by the superoxide radicals produced by the xanthine oxidase/xanthine system. For the assay, 10 ul supernatant were added to 200 ul NBT diluted in 1.9 ml 50mM tris_HCl, pH 8.0, 0.1 mM DETAPAC and 0.1 mM hypoxanthine. Finally, 20 ul xanthine oxidase were added in 20 sec. It was incubated at 25 ºC for 20 min. At the end of the reaction, 0.8 mM cupric chloride was added and it was read at 560 nm. SOD activity was expressed in U/100mg[12].

### *Vascular permeability and tissue edema*

Similar groups of control rats (n=5) and diabetic rats (n=6) were prepared with the same time sequence to measure vascular permeability and determine gingival edema, following Simard et al[13]. Animals in both groups, not anesthetized, were

injected with Evans Blue (EB) (20 mg/kg) through the tail vein. After 20 min the animals were euthanized, the thoracic cavity opened and 15 ml saline solution –heparin perfused through the right ventricle. Extracted tissues were divided into two portions and weighed. The first portion was desiccated at 60 ºC for 24 hs, while the second portion was immersed in 2 ml formamide for 24 h at 24 ºC to extract the dye (EB). Dye concentration was measured at 620 nm against an EB standard. The second portion was used to determine tissue edema, calculated by the difference between wet weight and dry weight, which reflects the amount of water (g) retained and calculated per ml (1g=1ml).

Statistical analysis

Statistical data were analyzed as means ± standard error of the mean (SEM). Analysis of variance (ANOVA) was accompanied by the Student-Newman-Keuls test for multiple comparisons, which was used to evaluate at a significance of $p<0.05$.

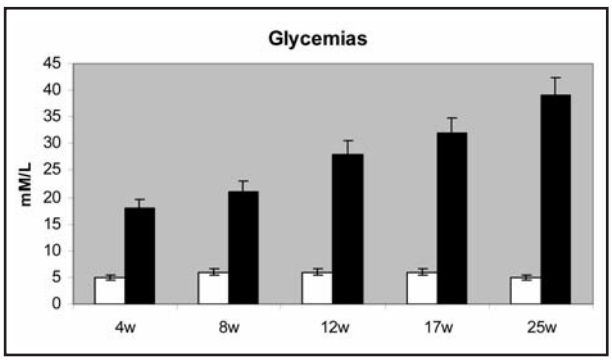


*Fig. 1: Changes in blood glucose levels (mmol/L) in non-diabetic rats (White bars) and diabetic rats (Black bars) from 4 to 25 weeks. Data represent mean ±SEM of 5-6 rats. (Black bars vs. white bars) p< 0.05.*

## RESULTS

Blood glucose level was found to increase progressively in diabetic animals compared to non-diabetic animals from week 4 to week 25 (Fig. 1). At 4 weeks of diabetes, the increase was greater than 300%, while at 25 weeks it was 6.5 times higher than in controls ($p<0.05$). MDA concentrations in gingival tissue at 4 and 25 weeks were also significantly higher in D than in ND, with values of 53% at weeks 4 and 25 of diabetes ($p<0.01$) (Fig. 2). On the other hand, superoxide dismutase (SOD) was significantly lower in diabetic rats than in controls at week 4 (-53.1 %) and week 25 (-57.6 %) ($p<0.05$ and 0.01) (Fig. 3). STZ induces diabetes associated to marked alterations of vascular permeability over 4 to 25 weeks. Fig. 4 shows that permeability to EB bound to protein increased by over 100% in diabetic rats compared to non-diabetic controls ($p<0.05$). Gingival edema also showed alterations at weeks 4 and 25. Fig. 5 shows the progressive increase in gingival edema in diabetic rats to over 100% compared to non-diabetic rats.

## DISCUSSION

Aging is a continuous, complex process that gradually affects various body tissues. Aging is associated to a progressive decline in the ability to maintain tissue homeostasis and alterations in the composition of the extracellular matrix[14,15]. Aging also causes abnormal functioning as well as qualitative and quantitative modifications and structural changes in morphology[16]. Old age produces aging changes with atrophies and reduction of the junction between epithelium and

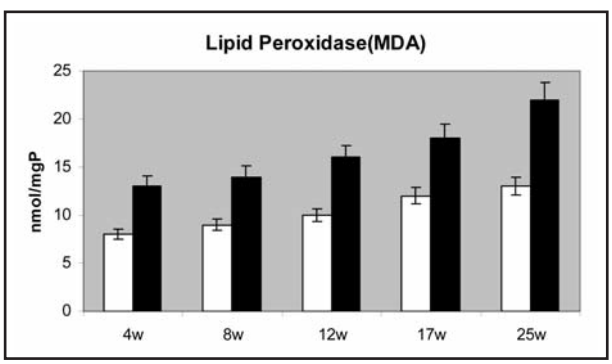


*Fig. 2: Age-related changes in gingival lipid peroxidation (MDA) in non-diabetic rats (white bars) and diabetic rats (black bars) from 4 to 25 weeks. Data represent mean ± SEM of 5-6 rats. (Black bars vs. white bars) p< 0.05.*

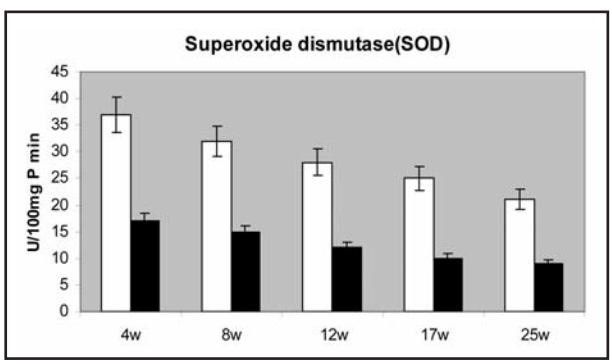


*Fig. 3: Antioxidative enzyme (SOD) activity in gingival tissue in non-diabetic rats (white bars) and diabetic rats (black bars) from 4 to 25 weeks. Data represent mean ± SEM of 5-6 rats (Black bars vs. white bars) p< 0.05.*

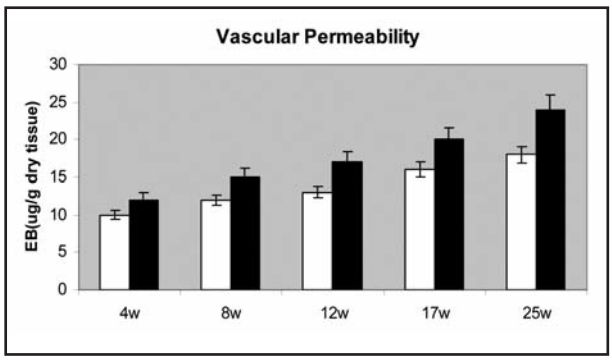


*Fig. 4: Changes in vascular permeability (EB) in gingival tissue in non-diabetic rats (white bars) and diabetic rats (black bars) from 4 to 25 weeks. Data represent mean ± SEM in each group( 5-6 rats). Black bars vs. white bars) p< 0.05.*

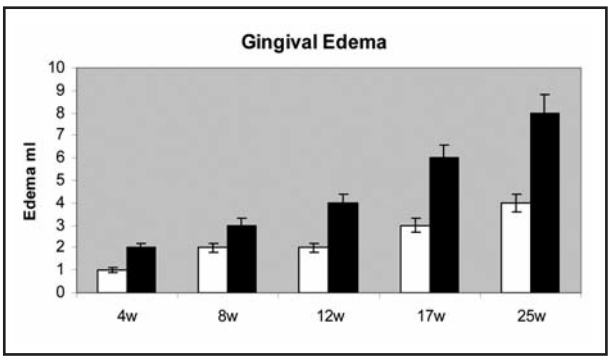


*Fig. 5: Edema induced in gingival tissue of non-diabetic rats (white bars) and diabetic rats (black bars) from 4 to 25 weeks. Data represent mean ± SEM of 5-6 rats in each group (black bars vs. white bars) p< 0.05.*

connective tissue. It has been established that microvascular changes in gums and alveolar mucosa are similar to those in other organs and tissues affected by DM[17]. The association of age produces differential expression of oxidative stress on gingival tissues in non-diabetic animals compared to diabetic animals of the same age. Gingival tissue deteriorates under persistent oxidative stress, inducing an inflammatory reaction due to the presence of microflora in the oral cavity[18]. The loss of homeostatic balance between proteolytic enzymes and their inhibitors and reactive oxygen species (ROS) and the reduction of the antioxidant defense system which protects and repairs living tissues, cells and molecular components, are the main factors in tissue damage. In the aging process, gingival antioxidant defenses decline late, but when aging is associated to diabetes, the two factors contribute to early amplification of the response on tissue damage. Glucose intolerance is a major risk factor for endothelial inflammation in diabetic subjects, compromising microvasculature and altering wound healing[19]. Natural aging in ND rat models shows that old age produces alterations in lipid peroxidation (MDA) as one of the main free radicals. It is interesting to note that our results showed that SOD, as an antioxidant defense system, declines significantly in ND rats as from week 12, regardless of their glycemia status. Vascular permeability and edema showed a significant late increase in ND rats. Together, these results document the fact that phenomena related to aging produce modifications in the oxidative stress of the gingival microvascular system. Other evidence in animal models confirms that high levels of lipid peroxidation and oxidative DNA reduce the synthesis of collagen in the gums of diabetic rats[20,21]. Previous studies have shown antioxidant reduction in diabetic condition[22] and reduction of antioxidant defenses[23]. Age associated to differential expression of inflammation in ND gingival tissue may specifically reflect the influence of age status by changes dependant on global age. Diabetic hyperglycemia measured over long-term diabetes is associated to damage and complications in several organs[24]. Moreover, early hyperglycemia (4 weeks) induces early oxidative stress in gums and increases vascular permeability and edema. The regulation of vascular permeability in normal conditions or pathophysiological conditions such as diabetes is related to the release of certain agents (NO, eicosanoids, bradykinin, free radicals or ROS) which affect microvascular homeostasis. There is an important relationship between levels of hyperglycemia and progression of gingivitis in diabetic patients[25]. Natural aging reduces defense capacity and develops inflammatory problems. Diabetes and aging produce immunosenescence, and chronic periodontal disease exhibits a two-way relationship centered by a local increase and a systemic inflammatory response with severity and rapid progression of the gingival-periodontal tissue, attributed to the possible bacterial increase and host contribution[24]. Hyperglycemia induces a pre-inflammatory state in microvasculature and increases vascular permeability and edema. Tissue and/or organ homeostasis in health is maintained by the continuous process of microcirculation, defined as the exchange between blood and tissue

fluids. This process includes the fluid and its spatial distribution, capillary pressure and permeability of the wall and the potential exchange area. If oxidative stress is altered in diabetic condition, one of the earliest signs of inflammation with increase in fluid filtration from capillaries to tissue, microfiltration and capillary vasodilation increase[26]. These assessments are consistent with hyperfiltration producing alterations in the microcirculation of gingival tissue in diabetic patients[27]. Hyperglycemia causes intravascular accumulation of polyols, leading to an increase in osmolarity with intramural water retention[28]. Moreover, the potential role of an immune receptor or molecular signal in the gingival tissue during the inflammatory process is also suggested as important in aging and progression of diabetic disease. The increase in the expression of C5a and TREM in old age contributes to elevated gingival-periodontal inflammation because these receptors participate actively in the amplification of the host inflammatory response[29,30].

To conclude, this study showed that with age, the activity of free radicals, vascular permeability and edema increase more significantly and earlier in diabetics than in non-diabetics. Aging and diabetes have greater impact on the gingival tissue of diabetics than non-diabetics. In both cases, oxidative stress is present, but long periods of diabetic disease produce early modifications and serious damage, with declining capacity to maintain tissue homeostasis.

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# Oral health-related quality of life in Colombian children with Molar-Incisor Hypomineralization

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***ABSTRACT***
*The aim of this study was to assess the impact of Molar-Incisor Hypomineralization (MIH) on Oral Health-Related Quality of Life (OHRQoL) in schoolchildren from a public educational institution in Bucaramanga, Colombia. Eighty-eight 7- to 10-year-olds took part in the study; of whom half had MIH. The translated and adapted version of the Child Perceptions Questionnaire (CPQ 8-10) was applied by means of an interview. The dependent variable was OHRQoL and explanatory variables were presence and severity of MIH, sex, age, socioeconomic status and social security. Frequencies and proportions were calculated for qualitative variables, and measures of central tendency, dispersion and position were calculated for quantitative variables. Chi-square, Fisher's Exact Test, Mann-Whitney U Test and Kruskal-Wallis tests were used, as appropriate. A p-value $<0.05$ was considered statistically significant. Parents or caregivers of participating children signed informed consent, and children signed an assent. A statistically significant difference was found for age groups ($p<0.001$), socioeconomic status ($p=0.015$) and social security ($p=0.045$) according to the presence of MIH. Likewise, statistically significant differences were found for each of the four domains of the questionnaire and for the overall CPQ 8-10 score ($p<0.0001$) according to the presence of MIH. The presence of the Molar-Incisor Hypomineralization may have negative impact on the Oral Health-Related Quality of Life of the participating children.*

***Key words:*** *Quality of Life, Dental enamel hypoplasia, Oral health, Tooth demineralization.*

## Calidad de vida relacionada con la salud oral en niños Colombianos con Hipomineralización Inciso- Molar

**Resumen**
*El objetivo de este trabajo fue evaluar el impacto de la Hipomineralización Inciso Molar sobre la Calidad de Vida Relacionada con la Salud Oral (CVRSO) en escolares vinculados con una institución educativa pública de Bucaramanga, Colombia. Ochenta y ocho menores de 7 a 10 años hicieron parte del estudio, la mitad presentaba HIM; a todos se les aplicó la versión traducida y adaptada del Child Perceptions Questionnaire (CPQ 8-10) mediante entrevista. La variable de salida fue la CVRSO y las variables explicatorias, la presencia y severidad de HIM, el género, la edad, el estrato socioeconómico y la seguridad social. Se calcularon frecuencias y proporciones para las variables cualitativas, y medidas de tendencia central, dispersión y posición para las cuantitativas. Se utilizaron las pruebas Chi², Test Exacto de Fisher, U. de Mann Whitney y Kruskal-Wallis según fuera apropiado. Un valor de $p<0,05$ fue considerado estadísticamente significativo. Los padres o cuidadores de los menores participantes firmaron un consentimiento informado y los niños y niñas, un asentimiento. Se encontró una diferencia estadísticamente significativa en los grupos de edad ($p<0,001$), el estrato socioeconómico ($p=0,015$), y la seguridad social ($p=0,045$) según la presencia de HIM. Así mismo, se obtuvo una diferencia estadísticamente significativa en cada uno de los cuatro dominios del cuestionario y en el puntaje global del CPQ 8-10 ($p<0,0001$) de acuerdo con la presencia de HIM. Según las percepciones de los participantes al responder al CPQ 8-10, se podría sugerir la presencia de la Hipomineralización Inciso Molar influye de forma negativa sobre la Calidad de Vida Relacionada con la Salud Oral en los niños participantes.*

***Palabras clave:*** *Calidad de vida, Hipoplasia del esmalte dental, Salud bucal, Desmineralización dental.*

## INTRODUCTION

Quality of Life (QoL) is defined as the perception of wellbeing and subjective, personal manifestation of feeling well within the cultural and social context in which one lives. According to the World Health Organization (WHO) it is influenced in a complex way by physical health, psychological status, social relationships and relationship with essential elements

in the environment, among others[1]. Oral health also affects QoL, with major impact on physical, psychological and social aspects. This is especially true for children, who are undergoing physical, mental and social growth, which is why some papers claim that oral diseases can have negative impact on children's QoL, in contrast to children who do not have any oral pathology[2,3].

Developmental Defects of Enamel (DDE) have been reported to impact QoL because they affect both aesthetics and function. These effects include Molar-Incisor Hypomineralization (MIH), defined as a hypomineralized lesion of the enamel as a result of different causes, mainly affecting permanent first molars and frequently associated to similar lesions on upper and/or lower permanent incisors, which causes deterioration and destruction of affected teeth because the enamel is fragile, and depending on the severity, may cause teeth to be lost[4,5]. MIH on permanent incisors compromises aesthetics and MIH on first molars alters the eruption guide for other molars, and hence, occlusion[4,6].

Masticatory function is also altered, since depending on how severely the enamel is affected and the forces applied during mastication, dental wear and fractures may cause dentin to be exposed, with subsequent tooth sensitivity[7]. This leads to the child brushing their teeth less and thereby having inappropriate hygiene, leading to greater susceptibility to carious lesions and increasing deterioration of affected teeth[8,9]. MIH also creates a dental clinical problem because it is difficult to eliminate dental sensitivity, and it causes marginal degradation of restorations due to lack of adequate adhesion between tooth structure and restorative material[4,10].

In 2003, Weerheijm et al. proposed the criteria used by the European Academy of Paediatric Dentistry (EAPD), identifying lesions according to: presence or absence of demarcated opacity, posteruptive breakdown, atypical restoration, premature extraction of first molars due to MIH, failure of eruption of a molar or incisor[11]. In 2006, Mathu-Mujuy and Wright[12] classified MIH as mild, moderate or severe. Many other classifications have been developed considering the severity, size, depth and extent of hypomineralization[13].This lack of uniformity for diagnosing the lesion has meant that the results of studies are not consistent and not comparable epidemiologically[6]. Considering this situation, reports on prevalence of MIH differ widely among populations. Table 1 shows the values reported in some studies[7,14-22].

As mentioned above, MIH affects Oral Health-Related Quality of Life (OHRQoL). Dantas-Neta et al. (2016) evaluated perception of OHRQoL in 594 schoolchildren and their parents by applying the *Child Perceptions Questionnaire* (CPQ 11-14) and the *Parental-Caregiver Perceptions Questionnaire* (P-CPQ). They found that there was a negative impact in the domains of "oral symptoms" [RR 1.30 CI 95% 1.06 – 1.60] and "functional limitation" [RR 1.42 CI 95% 1.08 – 1.86] in schoolchildren with severe MIH compared to those without MIH[23]. Arrow applied the *Parental Perceptions Questionnaire* (PPQ) to parents of 522 children and found no association between OHRQoL and Developmental Defects of Enamel (DDE) in first

**Table 1: Evaluation criteria and prevalence of MIH.**

| Author, year | Country, city | Sample | Criterion | Prevalence |
|---|---|---|---|---|
| Murrieta-Pruneda et al., 2016[14] | Mexico, Mexico City | 435 | EAPD | 13.9% |
| Escobar et al., 2015[15] | Colombia, Medellin | 1075 | EAPD | 11.2% |
| Oyedele et al., 2015[16] | Nigeria, Ile Ife | 469 | EAPD | 17.7% |
| Ng et al., 2015[17] | Singapore | 1083 | EAPD | 12.5% |
| De Lima et al., 2015[18] | Brazil, Teresina | 594 | EAPD | 18.4% |
| Bhaskar & Hedge, 2014[19] | India, Udaipur | 1173 | EAPD | 9.5% |
| Garcia-Margarit et al., 2013[20] | Spain, Valencia | 840 | EAPD | 21.8% |
| Biondi et al., 2012[21] | Argentina, Buenos Aires | 1098 | DDE Index | 15.8% |
| Da Costa-Silva et al, 2010[7] | Brazil, Botelhos | 918 | EAPD | 19.8% |
| Calderara et al., 2005[22] | Italy, Lissone | 227 | Weerheijm et al., 2001 | 13.7% |

permanent molars[24]. In this regard, it is important to mention that Arrow did not discriminate these defects in MIH and that it is not a good idea to use parents' answers as a proxy because their view may be based on external factors unrelated to what the child feels[25].

Since few studies have been published on the influence of Molar-Incisor Hypomineralization on Oral Health-Related Quality of Life in children, the aim of this study was to evaluate this relationship by applying the Colombian version of the *Child Perceptions Questionnaire* (CPQ 8-10) to children attending a public school in the city of Bucaramanga (Colombia).

## MATERIALS AND METHODS

An analytical observational cross-sectional study was performed, with non-probability sampling of 88 7 to 10 year-old schoolchildren from a public educational institution in the city of Bucaramanga. Bucaramanga is the capital of the department of Santander, located in north-east Colombia, and considered in the July-September 2016 quarter to be the city with lowest unemployment in the country[26].

The sample was calculated using the OpenEpi software version 3.1 with 97% confidence and 5% type I error based on a population of 928 students, and expected prevalence of 5.4% according to a study performed in the city of Medellín (Colombia) [27,28]. Participants were selected by convenience sampling to ensure equitable, proportional representation, with half the sample with MIH and the other half without MIH. Schoolchildren in this age range (7 to 10 years) were included because their permanent first molars and incisors have erupted. Children with systemic compromise, physical or mental disability, severe malocclusions, presence of fixed orthodontic appliances, teeth with cavities, fillings in first molars and incisors, and teeth with enamel developmental defects other than MIH (enamel fluorosis, amelogenesis imperfect) were excluded.

Output variable was Oral Health-Related Quality of Life evaluated by the CPQ 8-10. Explanatory variables were presence and severity of MIH, sex, socioeconomic level (tool to classify housing according to the National Statistics Administrative Department in Colombia) and type of social security.

### Clinical examination

Dental clinical examination was performed at the school nurse's office by an examiner previously calibrated by an expert (Cohen's Kappa coefficient = 0.68). Children brushed their teeth, after which presence/absence (yes/no) of MIH and its severity were evaluated following the criteria of Mathu-Muju and Wright[12]. MIH was considered present when at least one affected molar was found according to the guidelines proposed by the EAPD[11]. It is important to note that dental hypersensitivity was not investigated. Inspection was performed using mouth mirror, gauze for drying, tongue depressor and very good lighting. Children without MIH were selected as controls.

### Evaluation of Oral Health-Related Quality of Life

The version of the Child Perceptions Questionnaire (CPQ 8-10) created by Jokovic et al.[29,30]and translated and adapted to Colombian Spanish was used. It consists of 25 questions divided into four domains: "oral symptoms" (five items), "functional limitation" (five items), "emotional wellbeing" (five items) and "social wellbeing" (10 items). Answer options are arranged on a Likert scale with five categories: 0 = never, 1 = once or twice, 2 = sometimes, 3 = often, and 4 = nearly every day. The CPQ 8-10 was applied in an interview. The closer the score was to zero, the better oral health-related quality of life was considered to exist.

In addition, socio-demographic information was collected by means of a questionnaire sent to children's parents or caregivers.

### Statistical analysis

The information collected was entered in duplicate to an Excel database to be validated subsequently in EpiData 3.1. The fully refined database was exported to the Stata IC 12.0 statistical package[31-33]. Univariate analysis was used to calculate central tendency values and dispersion for quantitative variables. Frequency tables were made for categorical variables. Bivariate analysis was used to analyze presence of MIH with relation to sex, age, socioeconomic status and social security by means of Chi-square or Fisher's exact test. The distribution of each domain in the questionnaire was reviewed and the mean score for each domain and total questionnaire score were calculated to be associated

with presence of MIH and sex using Mann-Whitney's test. The Kruskal-Wallis test was used to establish association between questionnaire domains and MIH degree of severity. A value of $p<0.05$ was considered statistically significant.

**Ethical considerations**

This study was classified as "*research with minimum risk*" according to Resolution 8430 of October 1993 which establishes the scientific, technical and administrative standards for health research in Colombia[34]. In addition, it was approved by the Research Ethics Committee of Universidad Santo Tomás. Authorization was requested from the school, and participants' parents signed an informed consent after receiving an explanation of the aim and procedure of the study. Children were asked for assent to participate. The principles of autonomy, beneficence, justice and non-maleficence were observed.

## RESULTS

Half of the 88 children in the sample had MIH. Forty-seven (47; 53.4%) were female. Average age was 8.6 ± 1.2 years [CI 95% 8.4 – 8.9]. Average age was 8.8 ± 1.2 years for males and 8.5 ± 1.1 years for females; with no statistically significant difference for age according to sex (p=0.3167). Table 2 shows the demographics of the study population according to presence of MIH. A statistically significant difference ($p<0.001$) was found according to age group, with a higher proportion of 7- to 8-year-olds having MIH.

Average CPQ 8-10 score for participants with MIH was17.4 ± 14.1 [CI 95% 13.1-21.7] (Median = 12.5),ranging from 2 to 57. Average overall score for the questionnaire in children without MIH was 4.3 ± 4.1 [CI 95% 3.1 – 5.6] (Median = 4.0), ranging from 0 to 22. There was a statistically significant difference between groups ($p<0.0001$).

Table 3 shows Median (Me) and Interquartile Range (IQR) for the scores in each dimension and for the overall questionnaire according to presence of MIH. Median score was 2.0 or higher for all domains when children had MIH.

Median score for the full questionnaire according to presence of MIH and sex was higher in females, regardless of presence of MIH. However, the difference was not statistically significant (Fig. 1).

For MIH severity, 24 (54.6%) of the children had moderate severity (demarcated opacities on the occlusal/incisal third without breakdown, post-eruptive loss of enamel or carious lesions limited to one or two zones, without participation of cusps). There were 16 (33.4%) cases of isolated opacities without loss of dentin in these areas (mild). There was no statistically significant difference in CPQ 8-10 scores according to severity (p=0.4420) (Table 4).

**Table 2: Sample demographics according to presence of MIH (n=88).**

| Variables | With MIH n (%) | Without MIH n (%) | P |
|---|---|---|---|
| **Sex** | | | 0.831[a] |
| Female | 23 (52.3) | 24 (54.5) | |
| Male | 21 (47.7) | 20 (45.5) | |
| **Age** | | | <0.001[b] |
| 7 | 14 (31.8) | 6 (13.6) | |
| 8 | 14 (31.8) | 5 (11.4) | |
| 9 | 3 (6.8) | 18 (40.9) | |
| 10 | 13 (29.6) | 15 (34.1) | |
| **Socioeconomic status** | | | 0.015[b] |
| 1 | 4 (9.1) | 13 (29.6) | |
| 2 | 28 (63.6) | 15 (34.1) | |
| 3 | 11 (25.0) | 13 (29.6) | |
| 4 | 1 (2.3) | 3 (6.8) | |
| **Social security** | | | 0.045[b] |
| Subsidiated | 31 (70.5) | 21 (47.7) | |
| Contributive | 10 (22.7) | 13 (29.6) | |
| Prepaid medical care | 3 (6.8) | 10 (22.7) | |

a: Chi-square test. b: Fisher's exact test
Statistical significance p<0.05

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| Subsidiated | 31 (70.5) | 21 (47.7) | |
| Contributive | 10 (22.7) | 13 (29.6) | |
| Prepaid medical care | 3 (6.8) | 10 (22.7) | |

a: Chi-square test. b: Fisher's exact test
Statistical significance p<0.05

**Table 3: Median and interquartile range for scores in each domain and full questionnaire according to presence of MIH.**

| DOMAIN (items) | With MIH Me (IQR) | Without MIH Me (IQR) | P |
|---|---|---|---|
| Oral symptoms (5) | 5.0 (3.0) | 1.0 (1.0) | <0.0001 |
| Functional limitation (5) | 2.0 (4.5) | 0.0 (1.0) | 0.0003 |
| Emotional wellbeing (5) | 4.0 (6.5) | 0.0 (1.0) | <0.0001 |
| Social wellbeing (10) | 2.0 (5.5) | 0.0 (2.0) | 0.0005 |
| Total CPQ (25) | 12.5 (17) | 4.0 (3.5) | <0.0001 |

Me: Median. IQR: Interquartile range.
Wilcoxon Rank-sum (Mann-Whitney) Test.

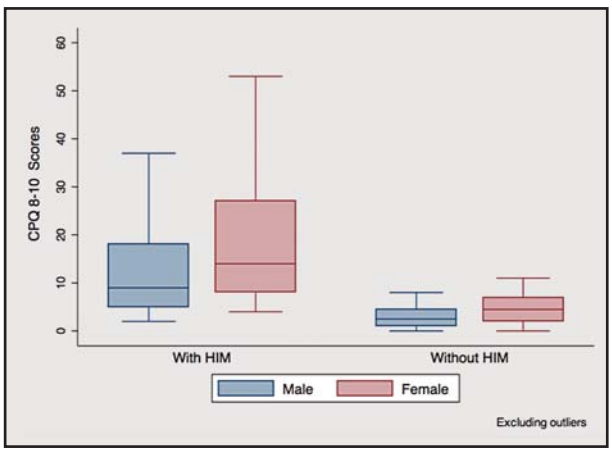


*Fig. 1: Mean and interquartile range for CPQ 8-10 scores according to presence of MIH and sex.*

## DISCUSSION

Presence of Molar-Incisor Hypomineralization affected Oral Health-Related Quality of Life in the children who participated in the study. This is in agreement with Oyedele et al.[8] who report that children with MIH presented a series of associated entities such as dental caries, dentin hypersensitivity and aesthetic compromise, which have a negative influence on OHRQoL.

Dantas-Neta et al. report that severe MIH was found to have a negative impact on OHRQoL when the *Child Perceptions Questionnaire* (CPQ 11-14) was applied to a population of 594 11- to 14-year-old schoolchildren; with Risk Ratio (RR) 1.30 [CI 95% 1.06 – 1.60] in the domain "oral symptoms" and RR

1.42 [CI 95% 1.08 – 1.86] in the domain "functional limitation"[23]. It is important to note that participant age was higher in that study than in ours, considering that it has been suggested that untreated MIH worsens with age due to plaque accumulation, hypersensitivity, enamel breakdown and dental caries[4]. Vargas-Ferreira and Ardenghi[35] also found association between the dimension "functional limitation" in CPQ 11-14 and enamel defects. In contrast, Arrow[36] applied the CPQ 11-14 to children with enamel defects in first molars and found no effect on OHRQoL, although there was association with presence of dental caries. We found that MIH affected females more than males, considering that the CPQ 8-10 score were higher for females. This has also been reported by other authors[23,37]. Girls are considered to be more concerned with their personal appearance and self-perception[36].

Socioeconomic status and type of social security revealed a statistically significant difference between participants with and without MIH. A higher proportion of children with MIH had low socioeconomic status and used the social security system subsidized by the Colombian State. Dantas-Neta et al[23] related low socioeconomic status with children's difficulty to access to oral hygiene products and information, as well as timely dental care, with a negative impact on Oral Health-Related Quality of Life. These variables are therefore considered to be confounding because they are directly related to the OHRQoL, as reported.

As mentioned above, one of the difficulties in comparing results is the variation in methods used to identify hypomineralization, as it is included in the Defects in Development of Enamel (DDE) classification[38-40]. In addition, there is influence of age difference between populations evaluated, the ways in which clinical examination is performed, and recording methods[6]. It should be noted that the European Academy of Paediatric Dentistry suggests taking into account its recommendations to determine presence of MIH[11].

In participants with MIH, severity did not differ statistically between groups with relation to the four domains of the questionnaire and overall score, possibly because very few participants (less than 10%) presented degree of severity 3. Nevertheless, the median score for the whole questionnaire for this group was slightly lower than the score for the group with severity 2 (Me=14.0 vs. Me=15.5).

This study has some limitations. Participants were selected for convenience, so the results found cannot be generalized. In addition, the population study was from a public educational institution that did not include all socioeconomic levels, and participation of children with dental caries lesions was restricted. Among the strengths of the study, it is one of the few studies evaluating OHRQoL in children with MIH. Moreover, the clinical examination was performed carefully by a calibrated examiner, and children's age was appropriate for evaluation of MIH[6].

According to the results, it may be concluded that presence of MIH in 7- to 10-year-olds has negative impact on all dimensions of OHRQoL as reflected by the CPQ 8-10.

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# Factors associated to apical overfilling after a thermoplastic obturation technique – Calamus® or Guttacore®: a randomized clinical experiment

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***ABSTRACT***

*The aims of this study were to analyze whether age, tooth type and sex are related to radiographically visible extrusion of filling material as an unintended outcome in teeth undergoing endodontic treatment with a thermoplastic obturation technique (Calamus® or GuttaCore®) and to determine whether the presence of such overfilling is associated to postoperative pain. We selected 120 teeth with diagnoses involving vital pulp and indication for endodontic treatment. Biomechanical preparation was performed using the Protaper Next® system with X2 or X3 master apical file. Teeth were randomly divided into 2 groups (n=60). Teeth in Group 1 were filled with the Guttacore® system and teeth in Group 2 were filled with single cone technique and Calamus® obturation system. Thermoplastic obturation techniques were found to cause overfilling in 53.33% of the total cases (64 teeth) (56.66% in Guttacore® group and 50% in the Calamus® group). Anterior teeth were found to be associated to presence of overfilling (p= 0.024) (OR = 4.35). Of the 120 teeth treated, 10 (8.33%) presented postoperative pain and radiographically visible overfilling. The association between presence of extruded filling material and mild/moderate pain was statistically significant with p = 0.002.*

*To conclude, endodontic thermoplastic obturation with Guttacore® and Calamus® systems are significantly associated with the probability of filling material extrusion, and overfilling is associated to postoperative pain. Anterior teeth are 4 times more likely to be overfilled with the obturation techniques tested.*

***Key words:*** *Gutta-percha; Pain; Root Canal Filling Materials.*

# Factores asociados con la presencia de sobreobturación apical posterior a una técnica de obturación termoplástica Calamus® o Guttacore®: experimento clínico aleatorizado

***RESUMEN***

*Los objetivos de esta investigación fueron: Analizar si factores como la edad, el tipo de diente y el género están asociados con la extrusión de material obturador radiográficamente visible como desenlace imprevisto en dientes sometidos a tratamiento endodóntico con una técnica de obturación termoplástica (Calamus® o GuttaCore®) y determinar si la presencia de esta sobreobturación está asociada con dolor posoperatorio. Se seleccionaron 120 dientes con diagnósticos que implicaban pulpa vital y con indicación de tratamiento de endodoncia. La preparación biomecánica se llevó a cabo con el sistema ProtaperNext® con lima apical principal X2 o X3. Los dientes se dividieron aleatoriamente de la siguiente forma (n=60): El grupo 1 se obturó con el sistema Guttacore® y el grupo 2 se obturó con una técnica de obturación de cono único y el sistema de obturación Calamus®. Se observó quelas técnicas de obturación termoplástica generaron sobreobturación en 53.33% del total (64 dientes), 56.66% se presentaron en el grupo de obturación con Guttacore® y 50% en el grupo de la técnica con el sistema Calamus®. Se encontró que los dientes anteriores presentaron asociación con la presencia de sobreobturación (p= 0.024) (OR=4.35). De los 120 dientes tratados, 10 (8.33 %) presentaron dolor posoperatorio y sobreobturación radiográficamente visible. La asociación entre la presencia de extrusión de material de obturación y dolor leve-moderado fue estadísticamente significativa con un valor p= 0.002.*

*Como conclusión las técnicas de obturación termoplástica en endodoncia Guttacore® y el sistema Calamus® están asociadas significativamente con la probabilidad de extrusión de material obturador y dicha sobreobturación está asociada con dolor posoperatorio, los dientes anteriores presentan 4 veces más riesgo de sobreobturación con las técnicas de obturación utilizadas.*

***Palabras clave:*** *Gutapercha; Dolor; Materiales para obturación del conducto radicular.*

## INTRODUCTION

The aim of root canal obturation is to prevent pathogenic microorganisms from invading and recolonizing the root canal by after its preparation and biomechanical cleaning by sealing it hermetically, both apically and laterally, with biocompatible materials[1, 2]. Three-dimensional obturation techniques helped by sealers are used to reduce the presence of voids in the obturation and achieve appropriate fit and adherence to root canal walls [1, 3-5].

The concept of 3-D root canal filling with warm gutta-percha condensation technique was introduced by Schilder in 1967[6] and has developed over time with the advent of devices facilitating it[1, 3, 4]. These new techniques enable root canals to be filled with thermoplasticized gutta-percha, which favors 3-D filling[7].

Among recently developed techniques for dispensing thermoplasticized gutta-percha inside the root canal with the aim of facilitating and optimizing vertical condensation are Calamus® (Dentsply®, Maillefer®, Switzerland), which works as an integrated system with "Downpack" for the apical third creating an apical plug and "Backfill" for the middle and coronal thirds, providing 3-D filling throughout the root canal[5, 8, 9], and theGuttacore® system, (Dentsply® Tulsa Dental Specialties, Tulsa, OK, USA), which uses a thermally stable cross-linked gutta-percha core which enables the material to preserve its properties during and after the procedure, thereby achieving 3-D obturation[10-12].

Overfilling has been defined as obturation which reaches the radiographic apex and extrudes within the periapical tissue[13] and has been classified as a procedural accident[14].

Extrusion of filling material has also been called "apical puff", and is radiographically visible as a small quantity of sealer protruding through the apical foramen, sometimes with remains of gutta-percha[15, 16].

With regard to the influence of factors such as tooth type or age on presence of apical overfilling, AlRahabi reports that upper molars are the teeth most likely to have accidental apical overfilling, showing that the type of tooth does influence whether or not there is apical extrusion of filling material. However that study considered lateral condensation techniques, not modern thermoplasticized gutta-percha techniques[17]. With regard to age, a higher number of endodontic failures has been reported in patients aged 41 to 50 years, although the cause of endodontic failure has not been specified as overfilling[18].

Tennert et al. report presence of apical extrusion of filling material in 80% with Thermafil® and 42% with warm vertical compaction technique[4]. However, to date there is no study in the literature evaluating presence of overfilling *in vivo* with the technological development of the techniques used by Tennert et al.[4], including thermoplasticized gutta-percha obturation systems such as Calamus® and Guttacore®.

Prognosis for endodontic treatments with overfilling of material (sealer and/or gutta-percha) has been a subject of controversy. Ricucci et al. report that the type of extruded sealer does not affect treatment prognosis and that overfilling has better prognosis if the tooth does not have preoperative apical lesion[19]. Sadaf et al. report low association between overfilling and postoperative pain (3.3%) and between pain and sealer extrusion (26.7%)[20].

Swartz et al.[2] report 63.41% success rate in overfilled teeth, which is significantly lower than the 89.77% observed for teeth with adequate obturation length. Marquis et al.[21] report 86% success rate for cases without extruded sealer and 73% success rate with extruded sealer. In a meta-analysis to determine optimal obturation length, Schaeffer et al. report that teeth without overfilling have significantly better prognosis than overfilled teeth[22].

Similarly, the extrusion of obturation material has been reported as a factor that delays bone repair in teeth with periapical pathology[23]. It has been reported that apical extrusion of sealer can damage the nerve structures in the periapical tissue, altering the sensitivity of the nerves involved[24-27].

The Thermafil® system, which is the predecessor of Guttacore®, has been reported to have higher risk of extrusion of filling material compared to the cold lateral condensation technique, and this extrusion is associated with the occurrence of postoperative pain 24 to 48 hours after the procedure[28]. This might be explained by apical overfilling stimulating and activating sensory neurons, which are related to the manifestation of acute postoperative pain in the treated tooth.

According to the above, postoperative pain may be associated to overfilling in root canal treatment; however, there is no report on how these symptoms are related to extrusion of filling material as a result of thermoplastic techniques such as Calamus® and

Guttacore®. Neither are there any reports on the association between overfilling with Calamus® and Guttacore® techniques and factors such as age, tooth type or sex.

The aims of this study were thus to analyze whether age, tooth type and sex are associated with radiographically visible extrusion of filling material as an unintended outcome in teeth subjected to endodontic treatment with thermoplastic obturation (Calamus® or Guttacore®) and to determine whether the presence of overfilling is associated to postoperative pain.

## MATERIALS AND METHODS

This was a randomized clinical experiment. It was approved by the Research and Ethics Committee of Universidad el Bosque (Bogotá, Colombia), during an Institutional Ethics Committee session on December 4, 2014.

The study considered patients who visited the post-graduate endodontics clinic at Universidad El Bosque (Bogotá Colombia) with indication for root canal treatment between March 1, 2015 and January 30, 2016. (Fig.1)

Patients were informed and invited to participate in the research project. They participated after completing the regulatory clinical history used at Universidad El Bosque (Bogota, Colombia) and completing the informed consent for the clinical study.

Sample size was calculated probabilistically using the asymptotic normal method with type I error 0.05 and type II error 0.20. Sample size was determined as 60 teeth per group, including a 20% dropout rate.

The study analyzed 120 teeth in patients aged 18 to 60 years of both sexes who had been referred to the post-graduate endodontics clinic at Universidad El Bosque (Bogota, Colombia).

Inclusion criteria were teeth with vital pulp with indication for endodontic treatment, with formed apex, and for which during biomechanical preparation, maximum master apical file diameter was X2 or X3 (Protaper Next®).

Exclusion criteria were teeth diagnosed with apical periodontitis, teeth with open apex or which during biomechanical preparation presented a master apical file larger than X2 or X3 (Protaper Next®).

Biomechanical preparation protocol for teeth using the Protaper Next® system included the following steps: local anesthetic block with 2% lidocaine and 1:80,000 epinephrine; opening and shaping the access cavity with high-speed handpiece, round diamond bur and endo-Z bur; locating root canal entrance with endodontic probe. The operating field was completely isolated with rubber sheet. Root canal apical patency was verified with a size 10 file, working length was determined with a Root ZX II® apex locator (J. Morita®) and confirmed with a conductometry radiograph.

Biomechanical preparation was performed with the Protaper Next® system, and for the selected teeth it was confirmed that they presented master apical file X2 (025.06) or X3 (030.07) by continuing with pecking motion until working length was reached, irrigating constantly with 5.25% sodium hypochlorite. Lastly, the final irrigation protocol was performed, which consisted of drying the canal with sterile paper points, irrigating with 17% EDTA for one minute, drying the canal again with sterile paper points and irrigating with 5.25% sodium hypochlorite, and drying the canal once again with sterile paper points. The manufacturer's instructions were followed for root canal obturation: working length was confirmed, the canal was coated with 1:1 Top Seal® cement[29]

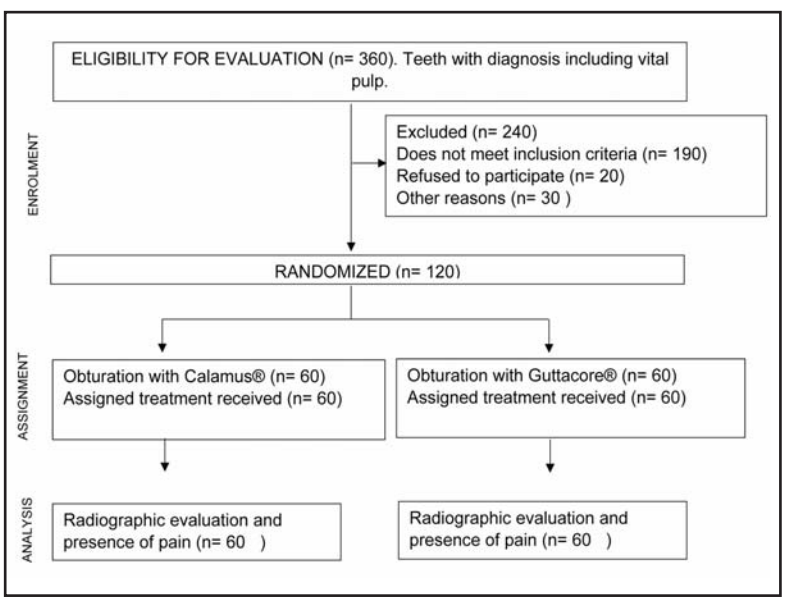


*Fig. 1: Distribution and randomization of the groups.*

with a Protaper Next® system (Denstply®) X2 or X3 paper point to working length[30].

Group 1 was filled with the Guttacore® system (Dentsply®) and Group 2 was filled with a hybrid obturation technique using Protaper Next® single cone (Dentsply®) and obturation technique with Calamus® thermoplasticized gutta-percha (Dentsply®), following the manufacturer's instructions. Lastly, a final radiograph was taken.

In order to standardize the digital periapical radiographs (initial, conductometry, conometry and final radiograph) a mold was made with heavy silicone on which to position the sensor and hold it in fixed position, allowing reproducibility in the angle of the radiographs.

Presence of overfilling or extrusion of filling material was evaluated in the final radiograph by an endodontics specialist (JN) with 16 years' experience, who was blinded to the filling procedure performed on each patient.

All patients had a follow-up visit 24 hours after the endodontic procedure, in order to establish whether there was postoperative pain. If there was, it was managed with occlusal adjustment and analgesic[31]. Intensity and presence of pain were measured on a visual analog scale (VAS) with values ranging from 1 mm to 170 mm, classified as follows: 0 mm to 54 mm mild pain, 54 mm to 114 mm moderate pain and 114 mm to 170 mm severe pain[32].

In order to ensure that the process would be unpredictable, subjects were assigned randomly to the study groups by means of numbers generated using the STATA® software RALLOC command and sealed in opaque, sequentially numbered envelopes. Following diagnosis and signature of informed consent, envelopes were opened and the procedure performed at the post-graduate Endodontics Clinic at Universidad del Bosque (Bogotá, Colombia) by post-graduate residents under teacher supervision. Under no circumstances was patient assignment to one of the experimental groups known prior to endodontic treatment. The endodontist who read and evaluated the radiographs was blinded.

**Statistical analysis**

Pearson's chi-square test was used for differences between proportions. Subsequently, bivariate tests were performed to determine relationships between dependent variables (presence of overfilling) and independent variables (age, sex, tooth type and filling system). The quantitative-qualitative nature of the study variables was taken into account.

Multivariate analysis was adjusted according to socio demographic variables, confounding variables and possible interaction, in order to model the effect of different risk factors on presence of overfilling and determine whether potential differences between experimental groups are maintained after controlling the various factors associated to the presence of the study event. As the outcomes were dichotomic (presence or absence of overfilling or extrusion of filling material), a logistic regression analysis was performed.

A p-value $< 0.05$ was determined in all cases. All statistical analyses were performed on STATA©.

The information obtained was entered directly into an EXCEL© electronic database. Names were kept separate from the rest of the information, which was stored under unique identifying numbers. STATA© files were created to analyze all the information in the registers and individual results.

**Table 1: Baseline characteristics of patients and teeth in the study. There is no significant difference in distribution of patients and teeth between groups.**

| | Calamus® (n=60) | Guttacore® (n=60) | Total (n=120) | P value = |
|---|---|---|---|---|
| **Age (years)** | 51.43±11.72 | 50.7±10.27 | 51.06±10.98 | 0.258 |
| **Sex** | | | | 0.073 |
| Female | 38 (63.33%) | 48 (80%) | 86 (71.67) | |
| Male | 22 (36.66%) | 12 (20%) | 34 (28.33) | |
| **Tooth type** | | | | 0.086 |
| Anterior | 18 (30%) | 8 (13.33%) | 26 (21.67%) | |
| Premolars | 17 (28.33%) | 21 (35%) | 38 (31.67%) | |
| Molars | 25 (41.67%) | 31 (51.67%) | 56 (46.67%) | |

## RESULTS

Table 1 shows the baseline characteristics of the study subjects and their distribution into two experimental groups. Pearson's chi-square test found no significant difference between groups, i.e. the distribution of age (p = 0.258), sex (p = 0.073) and tooth type (p = 0.086) are similar in both experimental groups, and therefore did not affect the results of the study.

Tables 2 and 3 show presence of extruded filling material with the Calamus® and Guttacore® techniques. Both techniques produced extruded sealer. Extruded sealer was present in 53.33% (64 teeth) overall. There was extrusion in 56.66% of teeth treated with Guttacore® and 50% of teeth treated with Calamus®, with no significant difference between groups (p = 0.583), although frequency was higher for Guttacore® (Table 3).

Out of the total patients treated with either of the two thermoplasticized gutta-percha techniques (Calamus® or Guttacore®), 8.33% (10 teeth) presented mild/moderate postoperative pain, while 91.6% presented no pain. No statistically significant difference in postoperative pain was found between filling techniques (p = 0.509), although it was higher for Calamus® (Table 4).

Since all teeth presenting pain also presented extruded filling material, when Pearson's chi-square test (Fisher's exact test) was applied, it found significant association between presence of extruded filling material and mild/moderate pain (p = 0.002) (Table 5).

For this study, teeth were classified as anterior, premolars and molars[17, 33]. Significant association was found between presence of extruded filling material and anterior teeth (p = 0.024). There was higher incidence of overfilling (76.92%) in anterior teeth than in premolars (47.37%) or molars (46.43%) (Table 6).

Logistic regression analysis on the factors associated to presence of extruded filling material shows anterior teeth as a variable with statistically

**Table 2: Presence of apical extrusion with Calamus® and Guttacore®.**

| Extrusion of filling material | Frequency | Percentage |
|---|---|---|
| Yes | 64 | 53.33% |
| No | 56 | 46.67% |
| Total | 120 | 100% |

**Table 3: Apical puff with Calamus® and Guttacore®. There is no significant difference between obturation techniques (P = 0.58).**

| | Presence of apical puff | | | | | |
|---|---|---|---|---|---|---|
| Technique | Yes | | No | | Total | |
| | N | % | N | % | n | % |
| CALAMUS® | 30 | 50% | 30 | 50% | 60 | 100% |
| GUTTACORE® | 34 | 56.66% | 26 | 43.33% | 60 | 100% |
| TOTAL | 64 | 53.33% | 56 | 46.66% | 120 | 100% |

**Table 4: Mild/moderate postoperative pain. There is no significant difference in presence of pain between obturation techniques (P = 0.509).**

| | Postoperative pain | | | | | |
|---|---|---|---|---|---|---|
| Technique | Yes | | No | | Total | |
| | N | % | N | % | n | % |
| CALAMUS® | 6 | 10% | 54 | 90% | 60 | 100% |
| GUTTACORE® | 4 | 6.67% | 56 | 93.3% | 60 | 100% |
| TOTAL | 10 | 8.33% | 110 | 91.6% | 120 | 100% |

**Table 5: Significant association (Pr = 0.002) between presence of extruded filling material and mild/moderate postoperative pain.**

| | Apical puff | | |
|---|---|---|---|
| Pain | No | Yes | Total |
| No | 56 (50.91%) | 54 (49.09%) | 110 (100%) |
| Yes | 0 (0.0%) | 10(100%) | 10(100%) |
| Total | 56(46.67%) | 64(53.33%) | 120(100%) |

**Table 6: Presence of apical puff according to tooth type. There is significant association between anterior teeth and extruded filling material (P = 0.024).**

| | Apical puff | | |
|---|---|---|---|
| Tooth | No | Yes | |
| Anterior | 6 (23%) | 20 (76.92%) | 26 (100%) |
| Premolars | 20(52.63%) | 18(47.37%) | 38(100%) |
| Molars | 30(53.57%) | 26(46.43%) | 56(100%) |
| Total | 56(46.67%) | 64(53.33%) | 120(100%) |

significant association in the raw model (OR 3.70; P = 0.01). In the model adjusted with the variables sex, age and filling system, the same factor (anterior teeth) was the only one with statistically significant association (p = 0.01) with OR 4.35, which means that the risk of extruded filling material from a root canal in an anterior tooth is 4 times higher than in a premolar or molar.

## DISCUSSION

This randomized clinical experiment was conducted to evaluate what factors could influence the presence of accidental apical overfilling or extrusion of sealer. Following randomization, a statistical verification confirmed that factors were distributed between groups in similar proportions, enabling dismissal of the possibility of results being affected by any imbalance. This is important to note because apical foramen diameter varies according to age[34] and differs between anterior and posterior teeth[35], so confirmation of similar distribution between groups provides reliability to the results.

Overall, with the Calamus® and Guttacore® obturation techniques used in this study there was extrusion of filling material in 53.33%. This value differs from Tennert et al.[4], who report 80% extrusion of material with Thermafil® and 42% with a vertical condensation filling technique[4]. In addition, the present study found no significant difference in extrusion of filling material between the two techniques. The difference between Tennert et al. and the current study may be due to study methodological design. Tennert et al. conducted a retrospective study with little control over inclusion criteria, whereas the current study was prospective, with randomized distribution into groups, and controlled both the procedures performed and the inclusion criteria.

De Chevigny et al.[36] found 53% extrusion of filling material in a sample grouping the Toronto study phases 1 to 4, which is in agreement with the 53.33% reported in the current study. However, De Chevigny et al. [36] used lateral condensation and vertical condensation filling techniques, so clearly, filling material extrusion or overfilling may occur regardless of the filling technique and without the possibility of being predicted with total certainty[14], although the outcome is generally functional[19, 21, 36], so that it may be considered an accident with good prognosis.

Said good prognosis may be owed to the periapical tissue's tolerance to the cytotoxicity of the filling material (gutta-percha and sealer)[37, 38], although if filling material extrudes and affects neighboring anatomical structures, it may lead to consequences which could cause patient discomfort[16, 24-27, 39]. Here, it is important to stress that it is not possible to predict how much filling material (sealer or gutta-percha) will extrude or how far it will reach[14, 39], in addition to which extruded material can only be detected radiographically after the obturation has been completed. It is therefore important to endeavor to keep the obturation material within the root canal, limiting extrusion to what it really is: an accident with good prognosis, not an aim of the obturation.

Wong et al.[40] conducted a meta-analysis observing overfilling and postoperative pain in teeth with root canals filled with Thermafil® system, finding postoperative pain in 24%, higher than the 8.33% found in the current study. Wong et al.[40] claim that postoperative pain was due to the extrusion of filling material[40]. This explanation may also apply in the current study, which found that all teeth presenting postoperative pain also had extruded filling material.

AlRahabi[17] report average overfilling of 44.15% in molars (62.5% in upper molars and 25.8% in lower molars), similar to the overall 46.43% found for molars in the current study. However, for anterior teeth, AlRahabi, report 16.25% (20% for upper incisors and 12.5% for lower incisors), which is much lower than the 76.92% found in the current study. Differences in treatment protocols may explain this inconsistency between results. These differences include that the current study determined working length with an apex locator (Root ZX II® J Morita®.) and confirmed it radiographically, while AlRahabi[17] only took a conductometry radiograph, and that in AlRahabi the root canal treatments were performed by undergraduate fourth or fifth year students, while in the current study they were performed by postgraduate students of endodontics. The statistically significant association found between extrusion of filling material and anterior teeth may be explained by two reasons: (a) as has been reported previously, it is easier to fill anterior than posterior teeth,[14, 17] and (b) the difference in the diameter of the apical foramen between anterior and posterior teeth[35]. Statistically significant association between extrusion of filling material and anterior teeth has not

been reported previously in the literature, so anterior teeth should be taken into account as a risk factor for extruded filling material. Special care should thus be taken to maintain a stable apical limit during biomechanical preparation of anterior teeth to prevent extrusion of filling material.

Within the limitations of this study, it may be concluded that the endodontic thermoplastic filling techniques with Guttacore® and the Calamus® system are significantly associated to the probability of filling material extrusion. In this regard, there is no difference between techniques and overfilling is associated with mild/moderate post-operative pain. Anterior teeth are 4 times more likely to be overfilled than premolars and molars with these filling techniques.

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# Inflamatory response in pregnant women with high risk of preterm delivery and its relationship with periodontal disease. A pilot study

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***ABSTRACT***

*Periodontal disease and its inflammatory response have been related to adverse outcomes in pregnancy such as preterm birth, preeclampsia and low birth weight. This study analyzed systemic inflammatory response in patients with high risk of preterm delivery and its relationship to periodontal disease. A pilot study was conducted for a case and control study, on 23 patients at risk of preterm delivery and 23 patients without risk of preterm delivery as controls. Exclusion criteria were patients who had received periodontal treatment, antibiotic or antimicrobial agents within the past three months, or with infections or baseline diseases such as diabetes or hypercholesterolemia. All patients underwent periodontal assessment, laboratory tests (complete blood count, lipid profile, baseline glycemia) and quantification of cytokines (IL-2, IL-4, IL-6, IL-10, TNF-α and INF-γ). Higher levels of pro-inflammatory cytokines (IL-2, IL-4, IL-6, IL-10, TNF-α and INF-γ) were found in patients with chronic periodontitis than in patients with gingivitis or periodontal health. These cytokines, in particular IL-2, IL-10 and TNF- α, were higher in patients at high risk of preterm delivery. Patients with high risk of preterm delivery had higher severity of periodontal disease as well as higher levels of the pro-inflammatory markers IL-2, IL-4, IL-6, IL-10, TNF- α and INF-γ.*

***Key words:*** *Chronic periodontitis, Pregnancy, Preterm birth, Preterm labor, cytokines.*

# Respuesta inflamatoria en pacientes embarazadas con alto riesgo de parto pretérmino y su relación con la enfermedad periodontal. Estudio piloto

**RESUMEN**

*La enfermedad periodontal y su repuesta inflamatoria ha sido relacionada con desenlaces adversos del embarazo como el parto pretérmino, preeclampsia y bajo peso al nacer. La presente investigación analizó la respuesta inflamatoria sistémica en pacientes embarazadas con alto riesgo de parto pretérmino y su relación con la enfermedad periodontal. Se realizó un estudio piloto de casos y controles, en el cual se contó con 23 pacientes que presentaban riesgo de parto pretérmino como casos y 23 pacientes sin riesgo de parto pretérmino como controles. Fueron excluidas las pacientes que hubieran recibido tratamiento periodontal, antibióticos o antimicrobianos en los últimos tres meses, que tuvieran infecciones, o enfermedades de base como diabetes o hipercolesterolemia. A todas las pacientes se les hicieron valoración periodontal, exámenes de laboratorio (cuadro hemático, perfil lipídico, glucemia basal) y cuantificación de citocinas (IL-2, IL-4, IL-6, IL-10, TNF- α e INF-γ). En las pacientes con periodontitis crónica se encontraron niveles más elevados en las citocinas proinflamatorias (IL-2, IL-4, IL-6, IL-10, TNF- α e INF-γ) en comparación con las pacientes con gingivitis o sanas periodontales. Estas citocinas se encontraron más elevadas en las pacientes con alto riesgo de parto pretérmino, en especial la IL-2, IL-10 y TNF- α. Las pacientes con alto riesgo de parto pretérmino presentaron mayor severidad de la enfermedad periodontal y adicionalmente niveles aumentados de los marcadores pro inflamatorios IL-2, IL-4, IL-6, IL-10, TNF- α e INF-γ.*

***Palabras clave:*** *periodontitis crónica, embarazo, nacimiento prematuro, parto prematuro, citocinas.*

## INTRODUCTION

Periodontal disease is a chronic infectious disease around the teeth which triggers a local and systemic inflammatory response[1]. The bacterial challenge generates an inflammatory infiltrate of polymorphonuclear neutrophils, monocytes, B-lymphocytes and T-lymphocytes with release of cytokines such as IL-1, IL-6 IL-8, IL-10 and PGE2, among others, which upon entering the bloodstream can reach the uterus and pass through the fetal placental barrier [2-4]. Normal labor is controlled by inflammatory signaling. As the pregnancy progresses, PGE2, TNF-α and IL-1β levels increase to critical levels, which induce the rupture of the amniotic sac, followed by the beginning of uterine contractions and delivery [5,6] This immune process, which is self-regulated up to the ninth month, may be modified by external stimuli such as periodontal infection, which by triggering a systemic inflammatory response during pregnancy, could induce labor earlier than necessary. Preterm birth is defined as birth at fewer than 37 weeks gestational age [7]. Global incidence of preterm birth is about 9.6%, which is equivalent to 12.9 million preterm babies [7-9]. It is a frequent problem which even affects high-income countries and has a strong impact on maternal and child morbidity and mortality, affecting both families and healthcare systems[10,11].

The aim of this study was to analyze the systemic inflammatory response in pregnant patients with high risk of preterm delivery and its relationship with periodontal disease.

## MATERIALS AND METHODS

A pilot test was conducted for a case and control study. The sample consisted of 46 patients – 23 at risk of preterm delivery and 23 without that risk as controls.

Risk of preterm delivery was diagnosed following the Clinical Practice of the Obstetrics and Gynecology Service at Hospital San Ignacio[12], which define preterm delivery as follows:

1. Risk of preterm delivery: patients with uterine activity without cervical changes. 2. Preterm labor, initial phase: patients with regular uterine activity and cervical changes less than 4 cm y 3. Preterm labor, advanced phase: patients with uterine activity with cervical changes greater than 4 cm.

The patients who took part in the study as cases were hospitalized and at risk of phase 1 and 2 preterm labor. The patients in the control group were pregnant women who attended their normal prenatal checkup as outpatients at the same hospital and Javesalud. Exclusion criteria for both groups were having received periodontal treatment, antibiotics or antimicrobial agents within the past three months, or having active infections or baseline diseases such as diabetes or hypercholesterolemia.

According to Colombia's Ministry of Health[13] and the CIOMS[14], this study was classified as investigation with minimum risk and was approved by the research ethics committees of the School of Medicine (FM-CIE 841215) and the School of Dentistry (CIEFOUJ OD 0131) of Universidad Javeriana. Before enrolling in the study, each patient signed an informed consent.

Blood samples were taken to determine full blood count, glycemia, total cholesterol, triglycerides, cHDL, cLDL and quantification of the inflammatory markers IL-2, IL-4, IL-6, IL-10, TNF-α and INF-γ using the BD Cytometric Bead Array Human Th1/Th2 Cytokine Kit II. The Beckton Dickinson FACSCanto II flow cytometer acquired 50,000 events and the data were analyzed using FCAP Array software. Concentrations were expressed in pg/ml. Periodontal disease was diagnosed based on the Armitage classification, 1999.[15] (Fig. 1).

To analyze the information, a description was made of the demographics, results of periodontal evaluation and systemic inflammatory markers using means, medians, ranges, standard deviations and 95% confidence intervals. For the bivariate analysis, the Chi-square test was used to establish associations and the ANOVA test to establish differences between the averages for the variables of interest. In addition, the Kolmogorov-Smirnov test was performed to establish whether the inflammatory markers had normal behavior. Variables in which behavior was not normal were compared using the U-Mann Whitney test. A difference of $p < 0.05$ (two-tailed) was considered significant.

## RESULTS

### General characteristics

Forty-six patients were analyzed. Average age was 25.91 ± 6.4 years and average gestational age was 29.46 ± 4.3 weeks. For periodontal status, 50.0% had chronic periodontitis, 45.7% had gingivitis associated to biofilm and 4.3% periodontal health. Upon describing according to groups, average age for both groups was 26 years, and gestational age was 31 weeks for the group with high risk and 28 weeks for the group without risk ($p = 0.466$).

### Oral clinical characteristics

No statistically significant difference was found for number of teeth or O'Leary's plaque index. The group with high risk of preterm delivery included a larger number of patients diagnosed with chronic periodontitis than the group without risk (Table 1).

### Cytokine quantification

Patients in the group with high risk of preterm delivery had higher levels of cytokines than patients without risk, with significant statistical differences for TNF-α, IL-10 and IL-2 (Table 2). For relationship between cytokines and periodontal diagnoses, patients with chronic periodontitis tended to have higher levels of pro-inflammatory cytokines than patients with gingivitis or periodontal health. Although the trend was towards higher values of cytokines in patients with periodontitis, it should be noted that there was no significant statistical difference in cytokine concentration according to diagnosis. Since the variables did not have normal behavior, a Kruskall Wallis test was used, which showed no significant difference in distribution between groups (Table 3).

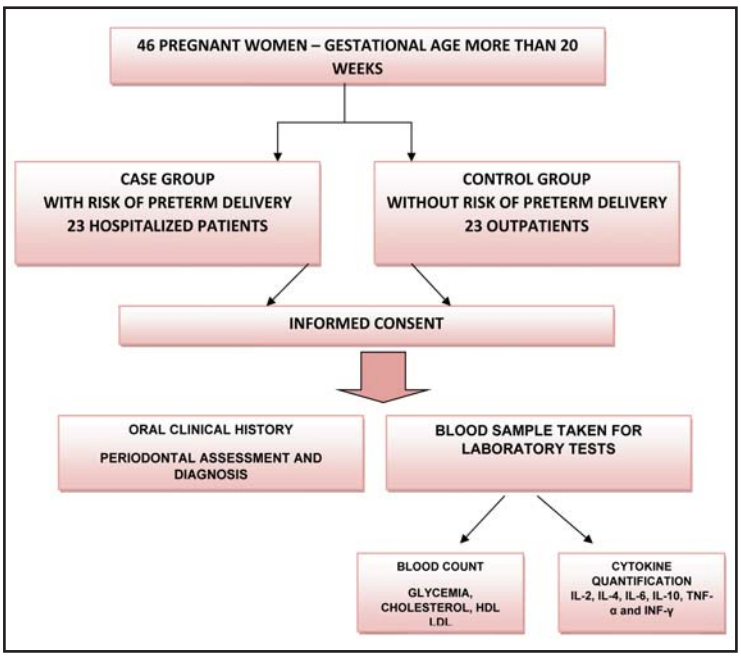


*Fig. 1: Flow diagram showing patient enrollment and testing. The flow diagram shows patients enrolled in the study, with the subgroups formed for Cases (pregnant women with risk of preterm delivery) and controls (pregnant women without risk of preterm delivery). All patients signed informed consent, underwent periodontal clinical assessment and had a blood sample taken for full blood count, glycemia, total cholesterol, HDL, LDL and cytokine quantification.*

**Table 1: Clinical characteristics of pregnant women with high risk and without risk of preterm delivery.**

| Clinical characteristics | High risk | Without risk |
|---|---|---|
| Number of teeth | 28.0 | 27.9 |
| Plaque index % | 61.9 | 45.7 |
| Patients with chronic periodontitis | 14 | 9 |
| Patients with gingivitis | 9 | 12 |
| Healthy patients | 0 | 2 |

**Table 2: Cytokine quantification in pregnant women with high risk and without risk of preterm delivery.**

| Cytokines | High risk | Without risk | P value |
|---|---|---|---|
| IFN-γ | 21.74 pg/ml | 0.10 pg/ml | 0.241 |
| TNF-α | 36.63 pg/ml | 0.02 pg/ml | 0.010 |
| IL-10 | 21.30 pg/ml | 0.34 pg/ml | 0.012 |
| IL-6 | 17.20 pg/ml | 2.06 pg/ml | 0.429 |
| IL-4 | 53.79 pg/ml | 0.11 pg/ml | 0.355 |
| IL-2 | 20.93 pg/ml | 0.19 pg/ml | 0.013 |

Statistically significant p-value < 0.05.

## DISCUSSION

Current evidence suggests that preterm delivery originates mainly from ascending infections from the vagina or uterine cervix, or blood-borne propagation

**Table 3: Relationship between cytokines and periodontal diagnosis.**

| Cytokine | Periodontal diagnosis | | | |
|---|---|---|---|---|
| | Healthy | Gingivitis | Chronic periodontitis | P-value |
| IFN-γ | 0.00 pg/mL | 0.05 pg/mL | 21.79 pg/mL | 0.147 |
| TNF-α | 0.00 pg/mL | 0.06 pg/mL | 36.59 pg/mL | 0.459 |
| IL-10 | 0.00 pg/mL | 0.40 pg/mL | 21.27 pg/mL | 0.053 |
| IL-6 | 1.11 pg/mL | 1.62 pg/mL | 17.68 pg/mL | 0.155 |
| IL-4 | 0.00 pg/mL | 0.16 pg/mL | 53.76 pg/mL | 0.699 |
| IL-2 | 0.00 pg/mL | 0.38 pg/mL | 20.78 pg/mL | 0.213 |

Statistically significant p-value < 0.05. Kruskal-Wallis test was used, enabling three variables to be compared at the same time.

from non-genital sources. Maternal periodontitis is a non-genital source of microorganisms which are known to routinely enter the bloodstream and to have potential to influence the health of the fetoplacental unit[7].

This study evaluated systemic inflammatory response in pregnant patients with and without risk of preterm delivery and its relationship with periodontal disease. The results showed that patients with high risk of preterm delivery had higher levels of cytokines IL-2, IL-4, IL-6, IL-10, TNF-α and INF-γ. These findings are in agreement with Inglis et al. [16], Gursoy et al. [17], von Minckwitz et al. [18] and Turhan et al. [19], which associate increased systemic inflammatory marker levels with adverse events in pregnancy.

Inflammatory cytokines play a key role in the mechanisms that trigger labor. This study found increased quantities of IL-6 and IL-10 in patients with risk of preterm delivery, in agreement with Arntzen et al. [20], Romero et al. [21] and Wommack et al. [22]. The current study also found that the group with risk of preterm delivery included a larger number of patients diagnosed with chronic periodontitis than the group without risk. This is in agreement with Offenbacher et al. [23], who report for the first time a relationship between maternal periodontal disease and preterm birth.

Upon analyzing presence of cytokines with relation to periodontal status, it was found that the group of patients with periodontitis tended to have higher levels of cytokines IL-2, IL-4, IL-6, IL-10, TNF-α and INF-γ; in agreement with Kelso[24] and Zadeh et al.[25], who relates periodontal infection to a systemic inflammatory response, reporting an increase in IL-5, IL-6, IL-4, IL- 10 IL-13 IL-3 and TNF-α in patients with periodontitis. Similarly, Perunovik et al. [26] found that women with preterm delivery had significantly more periodontitis and higher levels of IL-6 and PGE2 in crevicular fluid than the term delivery group.

Studies in recent years have focused on the effect of periodontal treatment on the reduction of pro-inflammatory markers and its relationship with preterm delivery. Offenbacher et al.[27] report that periodontal treatment showed a reduction in preterm delivery rate by 3.8 times, and a decrease in IL-1β and IL-6. Penova-Veselinovic,[28] found that in the group with periodontal treatment there was a significant reduction in levels of IL-1β, IL-10, IL-12p70 and IL-6 compared to controls. Da Silva et al.[29] report in a meta-analysis that non-surgical periodontal therapy during pregnancy reduced the level of inflammatory biomarkers in gingival crevicular fluid and serum, without influence on the level of inflammatory biomarkers in umbilical cord blood and without reducing the occurrence of adverse gestational outcomes.

However, in contrast to the above, studies such as Michalowicz et al. [30], Fiorini T et al.[31] and Pirie M et al.[32] report that non-surgical periodontal treatment in pregnant women did not reduce systemic inflammation markers.

Considering that preterm delivery is a public health issue, preventive measures should be taken, including promoting periodontal health in pregnant women. Unfortunately, as reported by Duque et al.[33] in 2011, physicians' knowledge and attitudes regarding periodontal disease and general health status are still deficient. Greater efforts are therefore needed to raise awareness among the different groups of scientists and specialists regarding the importance of maintaining good periodontal health during the gestation period, given the biological plausibility for etiology of preterm delivery shown by periodontal disease.

Within the limitations of the current study, it may be concluded that patients with high risk of preterm delivery presented greater severity of periodontal disease as well as increased levels of the pro-inflammatory markers IL-2, IL-4, IL-6, IL-10, TNF- α and INF-γ, with statistically significant differences for IL-2, IL-10 and TNF-α.

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# Risk factors for early childhood caries experience expressed by ICDAS criteria in Anapoima, Colombia. A cross-sectional study

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***ABSTRACT***

*The aim of this study was to assess whether caries risk, nutritional status, access to dental care and socio-behavioral factors are associated to two caries experience outcome variables using the Epidemiologic International Caries Detection and Assessment System (ICDASepi), which includes initial enamel caries lesions: 1- The presence of ICDAS-epi caries experience (dmf-ICDASepi ≥ 1), and 2- Having an ICDAS-epi caries experience (dmft-ICDASepi) higher than national figures for the same age. The sample included 316 eight- to 71-month-old children from the municipality of Anapoima, Colombia. Assessments included: prevalence and mean of caries experience using the ICDASepi system without compressed air-drying of teeth surfaces (dmf-ICDASepi), caries risk and nutritional status. Caregivers completed an eleven-item questionnaire assessing oral health-related social determinants, practices and quality of life (OHRQoL), and children's access to dental care. Data were analysed using the Wilcoxon-rank-sum test, the test, the Fisher-exact test, and bivariate-linear and non-conditioned logistic-multivariate regression models. Prevalence and mean number of teeth with dmft-ICDASepi were 65.2% and 3.5±4.13, respectively. Nutritional status outside the normal status, lower educational level of caregivers and age were significantly associated with dmf-ICDASepi≥ 1. OHRQoL, access barriers to miss and to attend dental care, operative-treatment or emergency being the reason to attend dental care, high caries risk, and age were significantly associated with a higher-than-national dmft-ICDASepi. The significant associations found between early childhood caries experience and other variables represent oral-health inequalities in early childhood in Anapoima, Colombia.*

***Key words:*** *Dental caries; dental care; nutritional status; health services accessibility; quality of life.*

## Factores de riesgo de experiencia de caries temprana expresada con criterios ICDAS en niños de Anapoima, Colombia. Estudio de corte transversal

***RESUMEN***

*El objetivo de este estudio fue evaluar en la primera infancia la asociación entre el riesgo de caries, el estado nutricional, el acceso a la consulta odontológica y los factores socio-comportamentales y dos variables de desenlace de experiencia de caries usando el Sistema epidemiológico Internacional de Detección y Valoración de Caries (ICDASepi), que incluye lesiones de caries en el esmalte: 1- La presencia de experiencia de caries ICDAS-epi (ceod-ICDASepi ≥ 1) y, 2- Tener una experiencia de caries ICDASepi (ceod-ICDASepi) mayor que los datos nacionales correspondientes para la misma edad.La muestra fue de 316 niños del municipio de Anapoima, Colombia, de 8 a 71 meses de edad. Las valoraciones incluyeron: prevalencia y promedio de experiencia de caries usando el sistema ICDASepi sin secado de las superficies de los dientes con aire comprimido (dmf-ICDASepi); riesgo de caries y estado nutricional. Los cuidadores respondieron un cuestionario de once ítems que valoraba en relación con salud oral, determinantes sociales, prácticas y calidad de vida y, el acceso de los niños a la consulta odontológica. Los datos fueron analizados con la prueba de suma de rangos de Wilcoxon, la prueba de χ2, la prueba exacta de Fisher y, modelos de regresión logística multivariada tipos bivariantelineal y no condicionada. La prevalencia de experiencia de caries (ceod-ICDASepi) fue de 65.18% y el promedio de dientes con ceod-ICDASepi de 3.5 ± 4.13. El estado nutricional por fuera de rangos de normalidad, el bajo nivel educativo de los cuidadores y la edad se asociaron significativamente con ceo-ICDASepi ≥ 1. Se encontró asociación estadísticamente significativa entre tener un ceod-ICDASepi mayor que el promedio nacional y, calidad de vida relacionada con salud oral, barreras de acceso para perder y asistir a consulta odontológica, tratamiento operatorio o urgencia como motivo de consulta odontológica, alto riesgo de caries y edad. Las asociaciones estadísticamente significativas encontradas en este estudio entre la experiencia de caries de infancia temprana y demás variables representan inequidades en salud oral en la primera infancia en Anapoima, Colombia.*

***Palabras clave:*** *Caries dental, Atención odontológica, Estado nutricional, Accesibilidad a los servicios de salud, Calidad de vida.*

## INTRODUCTION

Over half the children in Latin America are affected by the most severe form of caries (cavitation/dentinal caries lesions)[1]. Studies have shown significant association between caries in children and caries experience, oral health-related quality of life (OHQoL), socio-economic determinants, access to dental care, oral health care practices, diet and nutritional status[2-6].

In Colombia, the recent National Oral Health Study (NOHS) reported conventional caries experience (with cavitation/dentinal caries lesions) (WHO basic reporting codes) as well as ICDAS (International Caries Detection and Assessment System) caries experience with the Epidemiologic modification (no compressed air available) (ICDASepi). In addition to the conventional caries experience, it reported initial caries lesions on enamel with no cavitation (ICDAS Initial: codes 1-2)[7] (Table 1). Prevalence of conventional caries experience (dmft) in 5-year-old children was 62% with mean dmft of 2.8 (dt: 1.9), increasing to 89% and 6.8, respectively, when initial caries lesions were included (dmft-ICDASepi)[8]. The same study reported inequalities and barriers to access to healthcare.

With regard to the nutritional status of Colombian children, national data show that young children (up to the age of 6 years) present2.5% acute malnutrition and 12% chronic malnutrition, with rates higher than 20% for subgroups in rural areas[9].

In its effort to achieve better general and oral health-care, for more than three decades the Colombian General Social Healthcare Security System (abbreviated SGSSS in Spanish) has developed better general/oral healthcare legislation and programs: Statutory Law 1751 of 2015[10], which guarantees the fundamental right to health; Law 1438 of 2011[11], which guarantees healthcare; Law 100 of 1993[12], which extends coverage to the entire population; the positioning of oral health as a third priority in public healthcare[13]; the 2010 Primary Healthcare[14], and reinforcement of healthcare through the programmes "De 0 a Siempre" (From 0 to Always) and "Soy generación sonriente" ("I belong to a smiling generation") implemented by the Ministry of Health and Social Protection[15].

A four-university team recently launched the Colombian Chapter of the Alliance for a Cavity-Free Future (2011)[16], which is a worldwide initiative seeking caries-free cohorts. Thus, El Bosque University agreed with Anapoima-Cundinamarca's municipal government and the community to jointly construct an early-childhood oral/general health model, which is part of an ongoing implementation study.

Taking into account the importance of better general and local understanding of the associations between risk factors and caries experience, the aim of this study was to assess, in young children, how caries risk, nutritional status, access to dental care and socio-behavioral factors are associated to two outcome variables: (1) ICDASepi caries experience ≥ 1, and (2) ICDASepi caries experience higher than national figures for the same age.

## MATERIALS AND METHODS

This was across-sectional study conducted on children 8 to 71 months old from Anapoima, Cundinamarca, Colombia, with IRB (UEB-2011-149), following the STROBE Guidelines. Anapoima Municipality is located in Cundinamarca State in the western Andes range, approximately 87 Km southwest of Bogotá (Colombia's capital city), at 650-950 masl and temperatures 22-28 ºC. Population is 13,659 (urban area 5,709)[17]. Sample size was based on a related prospective study which reported that there were 1,200 children under 6 years of age in Anapoima (type-I error: 0.05 probability, 0% expected population proportion, 5% population-proportion distance), resulting in a sample size of 272 children. A 20% over-sample was added to prevent for drop-outs in the future program, resulting in a total sample of 326 children. The municipal government runs a program to improve nutrition, through which once a month, families with children are invited to receive food supplements. Participation rate is higher than 80%. In connection with this program, radio announcements were broadcast, inviting children to participate in an oral and nutritional examination. The children were examined at the healthcare centre in order of attendance until the sample size was completed. Informed consent was obtained from their caregivers. Subjects were thus selected from a random sample representative of the study population.

Exclusion criteria were: children whose parents did not agree to participate, children whose behaviour prevented examination, and children whose teeth had not yet erupted.

All participants received an oral-hygiene toolkit and referral to a dentist if needed.

***Data collection***

Assessments were conducted from November to December 2012. Clinical assessments included caries, caries risk and nutritional status. Five dentists trained and calibrated in the ICDAS caries assessment system (inter/intra-examiner reproducibility Kappa values ≥ 0.7) assessed caries with the ICDASepi system under epidemiological field conditions, at the Anapoima health centre, after assisted toothbrushing, with portable units, headlamp, mirror, drying cotton (no compressed air available) and WHO ball-ended probes. Filled (f) and missing (m) surfaces were also included in the examination. The caries component ("d") for the conventional caries experience was the same as the one used in the 2015 NOHS[8], namely Moderate and Extensive (dentinal) lesions (Conventional caries experience: dmfs/t).The ICDASepicaries experience also included Initial epicaries lesions (dmfs/t-ICDASepi)[7] (Table 1).

Individual caries risk was assessed with the Cariogram® software[18],setting country risk as high, group risk as standard, and assessing eight of ten factors (excluding salivary buffer capacity and microbiological tests) clinically and by interviewing caregivers, and using national data for mean number of teeth with caries experience for each child's age[8]. Each individual's caries risk was classified as low (very low – low), intermediate, or high (high-very high).

Nutritional status was assessed at the same session, in line with WHO guidelines as adopted for Colombia[19].Height and weight were measured by 4 trained physicians with achild meter and electronic table scale in children ≤ 2 years of age and with a height rod and upright scale in children > 2 years of age. Nutritional status was calculated by means of the height to weight indicator in children under 5 years old and by means of the body mass index (BMI) age indicatorin 5-year-olds. Children's nutritional status was classified as undernutrition, underweight, normal, overweight or obese.

***Access to oral healthcare and socio-behavioural aspects***

An eleven-question survey, modified from nationally validated tools, was applied in the same session to parents/caregivers with regardto the children's oral health-related access to dental care (3 questions); social determinants (2 questions)[8]; practices (3 questions)[20], andquality of life (3 questions)[21].

**Statistical analysis**

For the general description of the variables, central tendency measures (means) and dispersion (standard deviation) were used in quantitative variables, with previous confirmation of normality distribution by means of the Shapiro–Wilk test. In case of non-normal distribution, variables were described using medians and interquartile ranks. For the comparison between subjects with and without ICDAS-epi caries experience, as well as the group of individuals with a higher-than-national vs. the lower/equal mean for ICDAS-epi caries experience, the Z-test was used for mean differences in continuous variables with normal distribution or non-parametric statistics (Wilcoxon rank sum test). The Z-test for difference in

**Table 1: Clinical ICDASepi Caries Categories, and ICDASepi and Conventional Caries Experience.**

| | ICDASepi Caries merged categories | | Correspondent Caries Experience | |
|---|---|---|---|---|
| **Score** | | **Definition** | **ICDASepi** | **Conventional** |
| s | Sound (ICDAS 0) | No evidence of visible caries | No caries experience: dmf-ICDASepi= 0 | No caries experience: dmf = 0 |
| Initial-epi | ICDASepi 1/2 | Initial/distinct visual change in enamel observed with no available air-drying | | |
| Moderate | ICDAS 3,4 | Localised enamel break down or dentine-underlying shadow | dmf-ICDASepi ≥ 1 | dmf ≥ 1 |
| Extensive | ICDAS 5,6 | A distinct cavity in opaque or discoloured enamel with visible dentine | | |

proportions was used for dichotomous qualitative variables, and for multiple qualitative variables when the expected values in each cell were ≥5,$x^2$; otherwise, the Fisher exact test was used.

The association between socio-behavioural aspects (access to oral healthcare, social determinants, practices, and quality of life), and the study outcomes (caries experience and higher-than-national-mean caries experience) was initially estimated in a bivariate way using OR (odds ratio), followed by a $x^2$ test. In order to adjust such association according to the presence of confounding and interaction variables, adjusted ORs were obtained by means of a non-conditioned logistic regression multivariate model. For the selection of the regression variables, the stepwise technique was used, with an entry probability of 0.1 and an exit probability of 0.25, from a complete model with the variables described in the literature as effect modifiers, as those statistically different between groups and the possible interactions that could occur.

The crude and adjusted odds ratio was estimated using the logistic regression model (1) and (2), respectively:

$$p(y|x) = \frac{1}{1 + e^{-(\beta_0 + x\beta_1)}} \quad (1)$$

$$p(y|x_1, x_2, \ldots, x_n) = \frac{1}{1 + e^{-(\beta_0 + x_1\beta_1 + x_2 2 + , \ldots, x_n\beta_n)}} (2),$$

where $p(y|x)$ and $p(y|x_1, x_2, \ldots, x_n)$ are the probability of presenting the outcome given the explanatory variables $x$ or $x_1, x_2, \ldots, x_n$ for crude and adjusted models, respectively, and the OR are $e^{\beta 1}$ for both models.

The reliability of each of these models was evaluated using the Deviance and the Hosmer-Lemeshov goodness to fit test.

In order to correct the type I error by multiple comparisons test, a corrected type I error $\alpha$ was calculated using the Bonferroni correction, where $\alpha = \frac{\alpha}{K}$, being $\alpha = 0.05$ the type I error rate and the number of null hypotheses; all statistical tests whose were $p \leq \alpha$ considered to be statistically significant.

The rest of the statistical tests were considered significant at a p-value<0.05. The statistical analysis was conducted using STATA software (Version 12 SE; Stata Corporation, College Station, Texas).

## RESULTS

In total, 316 eight- to 71-month-old children were included in the assessment of both study outcomes: presence of dmfs-ICDAS-epi ≥ 1, and dmft-ICDASepi mean higher than the national mean for same age group. More than half were girls (56.01%) and of urban origin (55.38%). Caries risk in most children were classified as high (61.07%), followed by low (26.88%) and intermediate (12.02%). Over one third of children were classified outside a normal nutritional status (34.17%), with 24.05% classified as presenting overweight or obesity.

The prevalence of Conventional caries experience (dmf) was of 37.41%, increasing to 65.18% with the dmf-ICDASepi. Statistically significant differences were found between children with and without any level of caries experience (enamel or dentinal) and the variables of age and nutritional status (Table 2).

The mean Conventional caries experience was 1.4±2.5 (dmft) and 2.5±5.3 (dmfs; ds: 2.0±4.5), increasing to 3.5±4.13 (dmft-ICDASepi) and 7.5± 10.21 (dmfs-ICDASepi; ds-ICDASepi: 4.5±6.6).

For the outcome higher-than-national ICDASepicaries experience (dmft-ICDASepi) for same age, Table 2 shows that statistically significant differences were found between children with anequal/lower and higher-than-national mean of dmft-ICDASepi and the variables origin, caries risk and age.

Table 3 shows the analysis of factors associated to presence/absence of ICDASepi caries experience, both with the crude model and with the model adjusted by age, caries risk, and nutritional status. A statistically significant association was found only with the adjusted model regarding access to oral healthcare for the parents' maximum educational level, with children with presence of caries experience reporting 1.9 times more parents' maximum educational level as primary school vs. secondary school (p=0.043).

Table 2 shows the analysis of factors associated to having a higher-than-national mean dmft-ICDASepi, both with the crude model and with the model adjusted by patients' OHRQoL, origin, caries risk, and age. In relation to OHRQoL, statistically significant associations were found for all three risk factors; only with the crude model for the child having presented tooth pain (sometimes/frequently during the last three months vs. never) during the last three months (OR: 2.89; p=0.001); with both the crude (OR: 24.5; p=0.003) and the adjusted

**Table 2: Sociodemographic characteristics, caries risk and nutritional status of subjects according to the presence of dmft-ICDASepi ≥ 1 and of a higher-than-national mean of teeth with dmft-ICDASepi.**

| Variable | Presence of dmft-ICDASepi ≥ 1 | | p value | Higher-than-national mean of dmft-ICDASepi | | p value |
|---|---|---|---|---|---|---|
| | Yes (n=206) | No (n=110) | | Yes (n=85) | No (n=231) | |
| *Sex (n - %)* | | | | | | |
| Girls | 120-58.25 | 57-51.82 | 0.286* | 48-56.47 | 129-55.84 | 0.921* |
| Boys | 86-41.75 | 53-48.18 | | 37-43.53 | 102-44.16 | |
| *Age (months)* | | | | | | |
| Median | 50 | 29.5 | <0.001† | 49 | 43 | 0.0011† |
| IQR | 40-60 | 17-44 | | 38-61 | 28-55 | |
| *Origin (n - %)* | | | | | | |
| Urban | 107-51.94 | 68-61.82 | 0.092* | 36-42.35 | 139-60.17 | 0.005* |
| Rural | 99-48.06 | 42-38.18 | | 49-57.65 | 92-39.83 | |
| *Caries risk (n-%)* | | | | | | |
| Low | 47-22.82 | 38-34.55 | 0.059* | 6-7.06 | 79-34.20 | <0.001* |
| Moderate | 24-11.65 | 14-12.73 | | 6-7.06 | 32-13.85 | |
| High | 135-65.53 | 58-52.73 | | 73-85.88 | 120-51.95 | |
| *Nutritional status (n-%)* | | | | | | |
| Obese | 8-3.88 | 2-1.82 | 0.040‡ | 3-3.53 | 7-3.03 | 0.453‡ |
| Overweight | 51-24.76 | 14-12.73 | | 22-25.88 | 43-18.61 | |
| Normal | 129-62.62 | 79-71.82 | | 54-63.53 | 154-66.67 | |
| Underweight | 13-6.31 | 13-11.82 | | 4-4.71 | 22-9.52 | |
| Undernutrition | 5-2.43 | 2-1.82 | | 2-2.35 | 5-2.16 | |

Abbreviations: dmft-ICDASepi, ICDASepi caries experience including initial caries lesions; IQR, interquartile range.
*Differences calculated by means of $x^2$ test.
† Differences calculated by means of Wilcoxon sum of ranks tests.
‡ Differences calculated by means of Fisher exact test.

(OR: 12.02; p=0.022) models regarding the child having stopped activities (sometimes/frequently vs. never) during the last 3 months due to tooth pain; and with both the crude (OR: 4.8; p=0.002) and the adjusted (OR: 3.85; p=0.013) models regarding the family members having had their sleep interrupted (sometimes/frequently vs. never) during the last 3 months due to child's tooth pain. With the adjusted model, the access to oral healthcare variable regarding access barriers for taking the child to dental care showed statistically significant association with children with a higher-than-national dmft-ICDASepi(OR: 2.98; p=0.043). With regard to children not receiving dental care due to access barriers, strong statistically significant associations were found with both the crude (OR: 6.55; p<0.001) and the adjusted model(OR: 4.49; p=0.011). A statistically significant association was found only with the crude model regarding oral-health practices for operative treatment (OR: 6.8; p<0.01) and emergency (OR: 4.19;p<0.01) as the reason for child's last appointment vs. check-up or prevention.

## DISCUSSION

This study explored whether early childhood caries is associated to caries risk, nutritional status, access to care and socio-behavioural factors in young Colombian children in the municipality of Anapoima, Cundinamarca. Two caries experience outcome variables were established for exploring the associations: one within a more general scenario, where children were split into with/ without caries experience, and the other within a more national scenario intended to gain further insight into the situation of more vulnerable children and their surrounding factors[22], where children were split into with/withouthigher-than-national mean dmft-ICDASepifor same age group. This study found significant associations between early childhood ICDASepi caries experience and nutritional status, OHRQoL, dental care access barriers, caregivers' educational level, caries risk, and age.

The ICDAS caries system was used in this study, as it is compatible with the WHO conventional caries assessment and at the same time broadens the information about disease in a population, enabling

**Table 3: Factors associated with the presence of ICDASepi caries experience (dmft-ICDASepi ≥ 1).**

| Variable | | Presence of dmft-ICDASepi ≥ 1 | | | | | Crude model | | | Adjusted model* | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Yes (n=206) | | No (n=110) | | OR | 95% CI | p value | OR | 95% CI | p value |
| | | n | % | n | % | | | | | | |
| | | *Access to dental care* | | | | | | | | | |
| Parents' maximum educational level † | ≥ high school | 130 | 64.04 | 78 | 71.56 | 1.0 | Reference | 1.0 | Reference | | |
| | Primary school | 67 | 33.00 | 29 | 26.61 | 1.38 | 0.82 - 2.32 | 0.211 | 1.9 | 1.01- 3.55 | 0.0043# |
| | None | 6 | 2.96 | 2 | 1.83 | 1.8 | 0.35 - 9.13 | 0.478 | 1.25 | 0.2 - 7.57 | 0.802 |
| Access barriers to take the child to dental care‡ | No | 140 | 89.74 | 52 | 89.66 | 1.0 | Reference | 1.0 | Reference | | |
| | Yes | 16 | 10.26 | 6 | 10.34 | 0.99 | 0.36 - 2.66 | 0.985 | 0.70 | 0.22 - 2.25 | 0.555 |
| Child missing dental care due to access barriers § | No | 130 | 87.84 | 54 | 94.74 | 1.0 | Reference | 1.0 | Reference | | |
| | Yes | 18 | 12.16 | 3 | 5.26 | 2.49 | 0.70 - 8.81 | 0.156 | 1.12 | 0.26 - 4.70 | 0.871 |
| | | *Social determinants* | | | | | | | | | |
| Monthly income range (n-%) ∏ | > 1 minimum wage | 116 | 58.00 | 64 | 59.81 | 1.0 | Reference | 1.0 | Reference | | |
| | ≤ 1 minimum wage | 84 | 42.00 | 43 | 40.19 | 1.07 | 0.66 - 1.73 | 0.759 | 1.34 | 0.76 - 2.37 | 0.298 |
| Social security health scheme (n-%) ¶ | Contributing / private | 79 | 39.50 | 38 | 35.19 | 1.0 | Reference | 1.0 | Reference | | |
| | Beneficiary | 120 | 60.00 | 70 | 64.81 | 0.81 | 0.5 - 1.32 | 0.407 | 1.21 | 0.68 - 2.16 | 0.898 |
| | None | 1 | 0.50 | 0 | 0 | ** | ** | ** | ** | ** | ** |
| | | *Practices* | | | | | | | | | |
| Reason for child's last visit to the dentist †† | Check-up or prevention | 119 | 78.81 | 49 | 94.23 | 1.0 | Reference | 1.0 | Reference | | |
| | Operative treatment | 20 | 13.25 | 0 | 0 | ** | ** | ** | ** | ** | ** |
| | Emergency | 12 | 7.95 | 3 | 5.77 | 1.41 | 0.38 - 5.20 | 0.606 | 0.459 | 0.10 - 2.04 | 0.307 |
| Who brushes child's teeth? ‡‡ | Parents or combined | 178 | 86.83 | 97 | 92.38 | 1.0 | Reference | 1.0 | Reference | | |
| | Child by him/herself | 27 | 13.17 | 8 | 7.62 | 1.8 | 0.80 - 4.2 | 0.149 | 0.54 | 0.20 - 1.45 | 0.226 |
| How often are child's teeth brushed at night? §§ | Always | 56 | 27.86 | 30 | 29.13 | 1.0 | Reference | 1.0 | Reference | | |
| | Almost always | 99 | 49.25 | 38 | 36.89 | 1.39 | 0.78 - 2.49 | 0.260 | 1.23 | 0.63 - 2.39 | 0.543 |
| | Never | 46 | 22.89 | 35 | 33.98 | 0.70 | 0.37 - 1.31 | 0.271 | 0.66 | 0.31 - 1.40 | 0.283 |
| | | *Oral-health related Quality of life ¶¶* | | | | | | | | | |
| During the last 3 months, in relation with tooth pain, how often | | | | | | | | | | | |
| Has the child had presented it? | Never | 166 | 81.37 | 98 | 89.09 | 1.0 | Reference | 1.0 | Reference | | |
| | Sometimes-frequently | 38 | 18.63 | 12 | 10.91 | 1.86 | 0.93 - 3.74 | 0.078 | 1.01 | 0.43 - 2.35 | 0.971 |
| Has the child stopped his activities? | Never | 196 | 96.08 | 109 | 99.09 | 1.0 | Reference | 1.0 | Reference | | |
| | Sometimes-frequently | 8 | 3.92 | 1 | 0.91 | 4.44 | 0.54 -36.04 | 0.162 | 1.97 | 0.19 -19.61 | 0.561 |
| Have family members had their sleep interrupted? | Never | 193 | 94.61 | 103 | 93.64 | 1.0 | Reference | 1.0 | Reference | | |
| | Sometimes-frequently | 11 | 5.39 | 7 | 6.36 | 0.83 | 0.31 - 2.22 | 0.724 | 0.58 | 0.17 - 1.95 | 0.387 |

*Adjusted by subject's age, caries risk and nutritional status. † Variable available for 203 subjects with and 109 without dmft-ICDASepi ≥ 1. ‡ Variable available for 156 subjects with and 58 without dmft-ICDASepi ≥ 1. § Variable available for 148 subjects with and 57 without dmft-ICDASepi ≥ 1. ∏ Variable available for 200 subjects with and 107 without dmft-ICDASepi ≥ 1. ¶ Variable available for 200 subjects with and 108 without dmft-ICDASepi ≥ 1. **It was not possible to calculate OR for this category given its number of subjects. †† Variable available for 151 subjects with and 52 without dmft-ICDASepi ≥ 1. ‡‡ Variable available for 205 subjects with and 105 without dmft-ICDASepi ≥ 1. §§ Variable available for 201 subjects with and 103 without dmft-ICDASepi ≥ 1. ¶¶ Variables available for 204 subjects with and 110 without dmft-ICDASepi ≥ 1. #Significant at $\alpha' = \frac{0.05}{4} \approx 0.0125$.

**Table 4: Factors associated with the presence of higher-than-national teeth mean ICDASepi caries experience (dmft-ICDASepi).**

| Variable | | Higher-than-national mean dmft-ICDASepi | | | | | Crude model | | | Adjusted model* | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Yes (n=206) | | No (n=110) | | OR | 95% CI | p value | OR | 95% CI | p value |
| | | n | % | n | % | | | | | | |
| | | *Access to dental care* | | | | | | | | | |
| Parents' maximum educational level † | ≥ high school | 53 | 63.86 | 155 | 67.69 | 1.0 | Reference | 1.0 | Reference | | |
| | Primary school | 27 | 32.53 | 69 | 30.13 | 1.14 | 0.66 - 1.97 | 0.627 | 1.08 | 0.59 - 1.97 | 0.78 |
| | None | 3 | 3.61 | 5 | 2.18 | 1.75 | 0.40 - 7.59 | 0.452 | 0.99 | 0.18 - 5.34 | 0.998 |
| Access barriers to take the child to dental care‡ | No | 48 | 80.00 | 144 | 93.51 | 1.0 | Reference | 1.0 | Reference | | |
| | Yes | 12 | 20.00 | 10 | 6.49 | 3.59 | 1.46 - 8.85 | 0.005 | 2.98 | 1.03 - 8.56 | 0.0410# |
| Child missing dental care due to access barriers § | No | 43 | 75.44 | 141 | 95.27 | 1.0 | Reference | 1.0 | Reference | | |
| | Yes | 14 | 24.56 | 7 | 4.73 | 6.55 | 2.48 - 17.28 | <0.001 | 4.49 | 1.41 - 14.31 | 0.011# |
| | | *Social determinants* | | | | | | | | | |
| Monthly income range (n-%) ∏ | > 1 minimum wage | 41 | 50.00 | 139 | 61.78 | 1.0 | Reference | 1.0 | Reference | | |
| | ≤ 1 minimum wage | 41 | 50.00 | 86 | 38.22 | 1.61 | 0.97 - 2.69 | 0.065 | 1.72 | 0.98 - 3.02 | 0.057 |
| Social security health scheme (n-%) ¶ | Contributing / private | 32 | 38.55 | 85 | 37.78 | 1.0 | Reference | 1.0 | Reference | | |
| | Beneficiary | 50 | 60.24 | 140 | 62.22 | 0.91 | 0.54 - 1.54 | 0.751 | 0.96 | 0.54 - 1.69 | 0.898 |
| | None | 1 | 1.20 | 0 | 0 | ** | ** | ** | ** | ** | ** |
| | | *Practices* | | | | | | | | | |
| Reason for child's last visit to the dentist †† | Check-up or prevention | 36 | 63.16 | 132 | 90.41 | 1.0 | Reference | 1.0 | Reference | | |
| | Operative treatment | 13 | 22.81 | 7 | 4.79 | 6.8 | 2.53 - 18.32 | <0.001 | 2.83 | 0.96 - 8.30 | 0.058 |
| | Emergency | 8 | 14.04 | 7 | 4.79 | 4.19 | 1.42 - 12.33 | <0.001 | 1.79 | 0.57 - 5.62 | 0.318 |
| Who brushes child's teeth? ‡‡ | Parents or combined | 71 | 84.52 | 204 | 90.27 | 1.0 | Reference | 1.0 | Reference | | |
| | Child by him/herself | 13 | 15.48 | 22 | 9.73 | 1.69 | 0.81 - 3.54 | 0.159 | 1.49 | 0.63 - 3.52 | 0.353 |
| How often are child's teeth brushed at night? §§ | Always | 19 | 23.17 | 67 | 30.18 | 1.0 | Reference | 1.0 | Reference | | |
| | Almost always | 38 | 46.34 | 99 | 44.59 | 1.35 | 0.71 - 2.54 | 0.348 | 1.16 | 0.58 - 2.32 | 0.655 |
| | Never | 25 | 30.49 | 56 | 25.23 | 1.57 | 0.78 - 3.15 | 0.200 | 1.51 | 0.71 - 3.21 | 0.276 |
| | | *Oral-health related Quality of life ¶¶* | | | | | | | | | |
| During the last 3 months, in relation with tooth pain, how often | | | | | | | | | | | |
| Has the child had presented it? | Never | 60 | 72.29 | 204 | 88.31 | 1.0 | Reference | 1.0 | Reference | | |
| | Sometimes-frequently | 23 | 27.71 | 27 | 11.69 | 2.89 | 1.54 - 5.41 | 0.001 | 1.72 | 0.85 - 3.47 | 0.125 |
| Has the child stopped his activities? | Never | 75 | 90.36 | 230 | 99.57 | 1.0 | Reference | 1.0 | Reference | | |
| | Sometimes-frequently | 8 | 9.64 | 1 | 0.43 | 24.5 | 3.01 -199.3 | 0.003 | 12.02 | 1.43 -100.6 | 0.002# |
| Have family members had their sleep interrupted? | Never | 72 | 86.75 | 224 | 96.97 | 1.0 | Reference | 1.0 | Reference | | |
| | Sometimes-frequently | 11 | 13.25 | 7 | 3.03 | 4.88 | 1.82 - 13.08 | 0.002 | 3.85 | 1.32 - 11.17 | 0.013 |

*Adjusted by subject's origin, caries risk and age. † Variable available for 83 subjects with and 229 without a higher-than-national dmft-ICDASepi. ‡ Variable available for 60 subjects with and 154 without a higher-than-national dmft-ICDASepi. § Variable available for 57 subjects with and 148 without a higher-than-national dmft-ICDASepi. ∏ Variable available for 82 subjects with and 225 without a higher-than-national dmft-ICDASepi. ¶ Variable available for 83 subjects with and 225 without a higher-than-national dmft-ICDASepi. **It was not possible to calculate OR for this category given its number of subjects. †† Variable available for 57 subjects with and 146 without a higher-than-national dmft-ICDASepi. ‡‡ Variable available for 84 subjects with and 226 without a higher-than-national dmft-ICDASepi. §§ Variable available for 82 subjects with and 222 without a higher-than-national dmft-ICDASepi. ¶¶ Variables available for 83 subjects with and 231 without a higher-than-national dmft-ICDASepi. #Significant at $\alpha' = \frac{0.05}{4} \approx 0.0125$.

better understanding of related risk factors and supporting public health measures[7,22-24].Colombia, among other countries, used the ICDAS caries system in its recent National Oral Health Study[8]. This supports the use of the ICDAS caries system in the current study to gain further insight into possible associations of risk factors with caries, including early manifestations of caries, i.e., non-cavitated enamel caries lesions (Initial).

In this population, the prevalence of conventional caries experience was higher than 50% (4-yr. olds: 55%; 5-yr. olds: 53%) but lower than the national values reported in the recent NOHS for 5-year olds (62%)[8], and values reported recently in Bogotá in 4- (59%) and 6-yr. olds (66%)[23].When initial caries lesions were included, the prevalence of ICDAS-epi caries experience in the total population was slightly lower (65.18%) than equivalent modified ICDAS caries experience for the country (NOHS) for same ages (67.05%)[8].

Even though a convenience sample was selected in this study, the high participation rate in the local governmental nutrition programme and call to participate in a free oral and nutritional health examination provided subjects from both affluent and less affluent areas. It is however possible, that children from less needy families were less likely to participate in the programme.

The significant association between ICDASepi caries experience ≥ 1 and malnutrition is in agreement with Gaur and Nayak[5]. Overweight and obesity were more frequent in children with ICDASepi caries experience (29%) than were low-weight risk and malnutrition (9%), representing a public health alert. Recent evidence shows association between high free-sugar daily intake and caries[25].

Aspects of oral health-related quality of life were strongly associated to both outcomes. Sheiham[3] also found an association between caries and decreased quality of life in preschool children. The lack of association in this study between the two outcome variables and social determinants might be due to the facts that the study population is smaller and more homogeneous than in other national municipalities and that formal employment is higher, possibly with higher income levels. Social determinants have been shown to be important in the NOHS[8], and are part of the dental caries 'Global oral health inequalities' task group agenda worldwide[26].

The significant association between caries experience and access to dental care as well as between caries experience and rural/urban origin (borderline) might reflect social inequalities, in agreement with the NOHS[8]. Insurance limitations and a shortage of dentists have been related to low income[26]. The latter might explain the local situation and reinforces the significant association found with the crude model between higher-than-national mean of ICDASepi experience and visiting the dentist for operative treatment/emergency.

The significant associations found between both outcome variables and age, while logical with regard to natural caries history, also refer to a relevant age increase in caries experience in this study. This may reflect problems with dental care at early ages and may be partially explained by Colombia's 2000 legislation, which established that a child's first dental visit should take place at 2 years of age, including only operative treatment of cavitated caries lesions. Since 2011, it has included bi-annual fluoride varnish applications starting at the age of one year, among other oral health promotion and prevention activities[8].This aspect is also supported in this study by the association between a higher-than-national mean number of teeth with ICDASepi caries experience and caries risk. It should however be noted that the Cariogram includes caries experience as one of its factors, with an implicit association effect. When analysing other relevant risk factors in this population using the Cariogram, most children presented high and frequent daily intake of carbohydrates (66.5%), which is common in Colombian diet, and more than half reported not using fluoride toothpaste (56%) even though there is strong evidence that fluoride toothpaste prevents and controls caries as from the eruption of the first teeth[27].

This study and the NOHS[8]show that action is needed to improve oral health in infants and young children. In Anapoima, these actions, among others framed in the current paradigm of caries, are already being conducted with an emphasis on early childhood, together with government, health, education, families and community bodies, within the Colombian Chapter of the Alliance for a Cavity Free Future[16].

This study found significant associations between early childhood caries experience and nutritional status, OHRQoL, dental care access barriers, caregivers' educational level, caries risk, and age, both in terms of any ICDASepi caries experience and of having a higher-than-national mean figures of ICDASepi caries experience, representing oral health inequalities in early childhood in Anapoima, Colombia.

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# Comparison of canal transportation and centering ability of Xp Shaper, WaveOne and Oneshape: A cone beam computed tomography study of curved root canals

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***ABSTRACT***

*The aim of the present study was to investigate the shaping abilities of XP Shaper and compare it with other single file rotary NiTi systems utilizing full rotation and reciprocation motion, by cone beam computed tomography. Mesiobuccal canals of forty-five mandibular first molars, were allocated into three equal groups (n=15) according to the rotary system applied; WaveOne, OneShape and XP shaper. Pre-and post-instrumentation images were obtained at 3mm, 5mm and 7mm from the apex using cone beam computed tomography and assessed to determine canal transportation and centering ability.*

*Data were analyzed using Kruskal-Wallis test to compare the three systems and Friedman's test to compare the root levels. Results showed that WaveOne and OneShape rotary systems produced greatest mean transportation with no statistically significant difference between them, while XP Shaper produced the lowest statistically significant mean transportation. Canal centering ability differed significantly among the three systems used. It was concluded that XP shaper preserved the original canal anatomy better than WaveOne and OneShape rotary systems.*

***Key words:*** *Root canal, Cone beam computed tomography.*

## Comparación del transporte apical y la capacidad de conservación de la anatomía del conducto de XpShaper, WaveOne y Oneshape: Estudio de conductos curvos por tomografía computarizada de haz cónico

***RESUMEN***

*El objetivo del presente trabajo fue investigar la capacidad de tallado apical de XP Shaper y compararla con dos sistemas de NiTi, rotatorio y reciprocante, mediante tomografías computarizadas de haz cónico. Se analizaron los canales mesio vestibulares de cuarenta y cinco primeros molares inferiores. Los dientes fueron divididos en tres grupos experimentales (n=15): WaveOne, OneShape and XP shaper. Se obtuvieron imágenes pre y post instrumentación a 3mm, 5mm y 7 mm del ápice utilizando tomografías computadas de haz cónico para determinar la presencia de transporte apical y la capacidad de conservación de la anatomía original del conducto. Se utilizó el test deKruskal-Wallis para comparar los tres sistemas de intrumentación y el test de Friedman para comparar las mediciones en los tres niveles de raíz. XP Shaper mostró la menor cantidad de transporte apical estadísticamente significativa mientras que WaveOne y OneShape mostraron el mayor transporte apical sin diferencia estadísticamente significativa entre los dos grupos. XP shaper permitió conservar la anatomía del canal original mejor que WaveOne y OneShape.*

***Palabras clave:*** *Conducto radicular; Tomografía computada de haz cónico.*

## INTRODUCTION

The ultimate goal of root canal treatment is to remove infected pulpal remnants, eliminate microorganisms and adequately shape the root canal system[1]. Optimum biomechanical preparation can be achieved through uniform enlargement of the root canal system in all dimensions to permit thorough disinfection while preserving the original curvature without inducing iatrogenic errors[2].

However, endodontic preparation in narrow and curved root canals has always been a challenge, due to the tendency of the prepared canal to deviate from its natural axis[3]. Innovations and techniques are continuously being developed with the aim of reducing the difficulties encountered during endodontic therapy. Nickel-titanium instruments provide satisfactory treatment of curved canals in shorter times through their enhanced properties of shape memory, super elasticity and cutting efficiency[4].

Various single-file systems with different metallurgy and designs have been promoted to prepare the root canals with one instrument using either continuous

rotation or reciprocation motion. WaveOne (Dentsply Maillefer, Ballaigues, Switzerland) and OneShape (Micro Mega, Besancon, France) are representatives of these single file systems. WaveOne rotary system works in a reciprocating motion and is made of a special NiTi-alloy called M-Wire, which is produced by a novel thermal treatment process. The M-Wire provides the instrument with increased flexibility and improved resistance to cyclic fatigue. The Primary WaveOne file with a tip size of 25 has a fixed 8% taper from D1 to D3 and a gradually decreasing percentage tapered design from D4 to D16.

On the other hand, OneShape rotary system is made of a conventional austenite NiTi alloy with a tip size of 25 and a constant 6% taper. The instrument incorporates several cross-sectional designs and variable pitch along its entire length.

The XP Shaper instrument (FKG, LaChaux-de-faund, Switzerland) was recently presented on the market. It is based on the MaxWire adaptive core technology. The MaxWire alloy enables the instrument to change its shape from a fairly malleable and straight shape at room temperature to a more robust shape at body temperature (Fig 1). This transformation causes the instrument to be flexible and straight at room temperature and to have elevated cutting efficiency at body temperature[5].

The purpose of the study was to compare the shaping ability of the new rotary NiTi instrument (XP-Shaper) with other single file NiTi instruments in different motions utilizing reciprocation (WaveOne) and full rotation (One shape) in terms of canal transportation and canal centering ability, using cone beam computed tomography. The null hypothesis was that there would be no difference among the three single- file Ni-Ti rotary systems regarding the analyzed parameters.

## MATERIALS AND METHODS

### Sample selection

A total forty-five human permanent mandibular first molars extracted due to periodontal or prosthodontic reasons were collected from the Department of Oral Surgery, Faculty of Dentistry, Cairo University. Preoperative periapical radiographs were taken to inspect the mesial roots and to determine the angle of root curvature according to Schneider's method[6]. Inclusion criteria were the presence of two separate canals in the mesial root with independent apical foramina, complete root formation, no internal root calcification, no internal or external root resorption, and mesiobuccal canal curvatures between 20° and 35°.

### Sample preparation

The crowns were removed using a water-cooled safe sided diamond disc leaving 3 mm above the cemento-enamel junction. The distal roots were separated from the mesial roots using diamond discs. Root canal patency and the existence of two separate mesial canals were confirmed by simultaneous application of two K-files #10 (Maillefer, Ballaigus, Switzerland) in the canals. Only the mesiobuccal

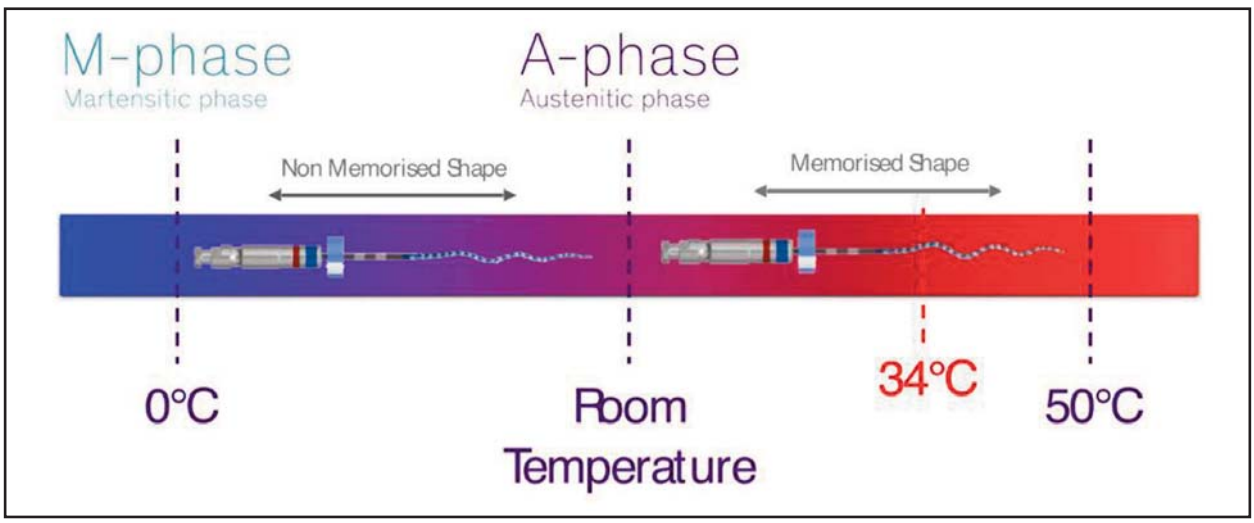


*Fig. 1: Martensitic—austenitic phase transformation of XP Shaper at different temperatures.*

canals were used in our study. The working length of each canal was determined by subtracting 1 mm from the apical foramen.

Before scanning, the roots were fixed by mounting them vertically halfway in transparent auto-polymerizing acrylic resin (Acrostone, Dental & Medical Supplies, Cairo, Egypt) mixed according to the manufacturer's instructions in a silicon mold (10 cm x 10 cm). The root apices were sealed with wax (Wilson, Sao Paulo, Brazil) to preserve the apical foramen from resin penetration. To ensure standardization of the specimens during tomographic scanning, each root was placed in the unset acrylic resin such that its long axis was parallel to the long axis of the mold. In addition, an amalgam filling was inserted into the resin at the bucco-distal line angle of the roots, to enable the orientation of the canal during scanning.

**Pre-instrumentation scanning**

All roots were scanned using cone beam computed tomography (CBCT) (Scanora 3D, Soredex, Palodex group, Finland) at 85 kVp and 15 mA to detect canal shape before instrumentation. For each specimen, three tomograms were chosen according to the distance from the root apex, as follows: 3 mm from the root apex (Representing the apical third), 5 mm from the root apex (Representing the middle third) and 7 mm from the root apex (Representing the cervical third). All scans were assessed using a Software program (OnDemand 3D, Cybermed, South Korea).

**Root Canal Preparation**

A glide path was created using #15 K-file (Maillefer, Ballaigus, Switzerland). Then samples were randomly divided into 3 equal groups (n = 15 canals per group) as follows:

- Group I: The WaveOne group, where roots were mechanically prepared using Primary WaveOne file (size 25) operated by X-smart plus endodontic motor (Dentsply, Tulsa Dental, Tulsa, OK) using the reciprocation pre-set mode.

- Group II: The OneShape group, where roots were mechanically prepared using OneShape file (size 25, taper 0.06) operated in continuous rotation motion using an electric motor with a rotational speed set at 350 rpm and a 5-Ncm torque in a crown-down technique. Both instruments were used with a slow in-and-out pecking motion with an amplitude of about 3 mm. After three pecks, the flutes of the instruments were cleaned and reinserted, and the process was repeated until full working length was reached.

- Group III: The XP-Shaper group, where roots were shaped using the XP-shaper file with the electric motor set at 900 rpm and 1-Ncm torque. The file was inserted into the canal using long gentle strokes (5-7 mm); 5 strokes were applied until working length was reached.

X-smart plus endodontic motor (Dentsply, Tulsa Dental, Tulsa, OK) was employed for root canal preparation of all samples, following the manufacturer's instructions for each instrument. Each instrument was used to prepare only four canals. Freshly prepared 2.6% sodium hypochlorite solution (Clorox, Cairo, Egypt) was used as an irrigant during the instrumentation procedure, placed with 30-gauge needle tips (NaviTip, Ultradent, South Jordan, UT, USA) as deeply as possible into the canal without binding. Apical patency was retained using a #10 K-file. Once the rotary instrument reached the working length and rotated freely, it was removed. Then 10 ml of distilled water were used as a final flush.

**Post-instrumentation scanning**

The root canals were scanned after mechanical preparation using CBCT, similarly to the pre-instrumentation scanning protocol. Pre- and post-instrumentation scans were superimposed using the aforementioned software program to evaluate the degree of transportation as well as the centering ability of the tested instruments. The shortest distance from the periphery of the root (mesial and distal) to the edge of the canal was measured using the length measuring tool on the reconstructed cross-sectional scans.

The degree of canal transportation was calculated according to the formula provided by Gambill *et al*.[7].

Canal transportation (CT) = (M1 - M2) - (D1 - D2)

**where M1:** refers to the shortest distance from the mesial edge of the root to the mesial edge of the un-instrumented canal.

**M2:** refers to the shortest distance from the mesial edge of the root to the mesial edge of the instrumented canal.

**D1:** refers to the shortest distance from the distal edge of the root to the distal edge of the un-instrumented canal.
**D2:** refers to the shortest distance from the distal edge of the root to the distal edge of the instrumented canal (Fig. 2).
Regarding the transportation direction, CT equal to 0 (zero) denoted lack of transportation, a negative value denoted transportation towards the distal direction, and a positive value denoted transportation towards the mesial direction.
Centering ability ratio was calculated using the same values obtained during the measurement of transportation according to the following equation:

Centralization ability ratio = (M1 - M2)/ (D1 - D2) or (D1-D2) /(M1-M2)

The formula was selected in such a manner that the lowest of the results acquired through the difference should be the numerator. A result equal to 1.0 signified perfect centralization. When the value was closer to zero, it denoted that the instrument had lower capacity to maintain itself in the central axis of the canal.

**Assessment of root canal preparation**
Root canals were prepared by one operator, while canal curvatures prior to and after instrumentation were assessed by a second examiner who was blind to all the experimental groups.

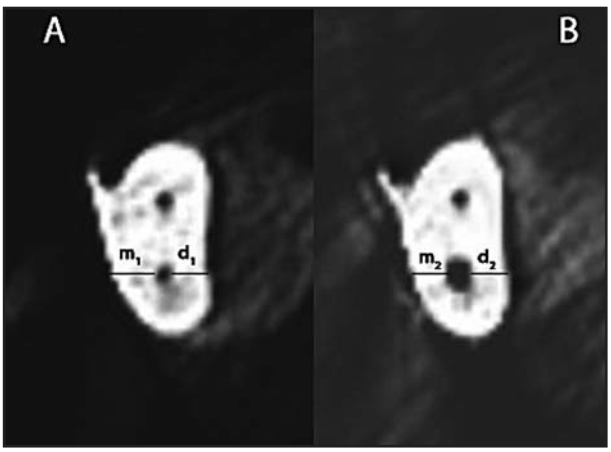


*Fig. 2: (A) Pre-instrumentation and (B) post-instrumentation CBCT images with markings showing points of measurements used for determining canal transportation and centering ratio.*

**Statistical analysis**
Data were presented as mean and standard deviation (SD) values. Kruskal-Wallis test was used to compare the three systems. Friedman's test was used to compare the different root levels. Dunn's test was used for pair-wise comparisons. Fisher's Exact test was used to compare frequency data of the three systems. The significance level was set at $P \leq 0.05$.

## RESULTS

**Canal Transportation**
At 3 and 5 mm from the apex, WaveOne and OneShape had highest mean transportation, with no statistically significant difference between them, while the XP Shaper had significantly lower mean transportation (Table 1).
At 7 mm from the apex, all three groups differed significantly. WaveOne had the highest mean transportation (0.22 ± 0.09), followed by OneShape (0.14 ± 0.11), and finally XP Shaper with the lowest value (0.08 ± 0.06).
With regard to the root canal levels; our results showed that preparing the canal with WaveOne instrument created a statistically significant difference between different levels (*p*-value = 0.035). The highest distal transportation was found at 3 mm from the apex, while the highest mesial transportation was observed at the 7 mm level. However, OneShape and XP Shaper instruments showed no statistically significant difference among the different root levels (*p*-value = 0.061 and 0.175 respectively) (Table 2).

**Centering Ability**
The maintenance of canal curvature was better with One Shape (0.54 ± 0.11) and XP Shaper (0.41± 0.15) than with WaveOne, which had the statistically significant lowest mean centering ratio (0.31 ± 0.12) (Table 3).
Regarding different root canal levels, at the level of 3 mm from the apex, One Shape had the highest mean statistically significant centering ratio (0.51± 0.32), while there was no statistically significant difference between WaveOne and XP Shaper instruments. At the 5 mm level there was no statistically significant difference among the three systems. At 7 mm level, OneShape had the statistically significantly highest mean centering ratio, followed by XP Shaper, and lastly, WaveOne.

**Table 1: Mean, standard deviation (SD) and results of Kruskal-Wallis test for comparison between canal transportation values (mm) after using the three systems.**

| Root level | WaveOne | | One Shape | | XP Shaper | | P-value |
|---|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Mean | SD | |
| 3 mm | 0.14 A | 0.10 | 0.12 A | 0.16 | 0.07 B | 0.06 | 0.023* |
| 5 mm | 0.19 A | 0.13 | 0.21 A | 0.15 | 0.05 B | 0.04 | 0.001* |
| 7 mm | 0.22 A | 0.09 | 0.14 B | 0.11 | 0.08 C | 0.06 | 0.001* |
| Total | 0.18 A | 0.07 | 0.16 A | 0.11 | 0.07 B | 0.04 | <0.001* |

*: Significant at P ≤ 0.05. Different superscripts in the same row denote statistically significant differences.

**Table 2: Frequencies (n), percentages (%) and results of Friedman's test for comparison between direction of transportation among different root levels.**

| System | Direction | 3 mm | | 5 mm | | 7 mm | | *P*-value |
|---|---|---|---|---|---|---|---|---|
| | | n | % | n | % | n | % | |
| WaveOne | Distal | 10 | 66.7 | 6 | 40 | 2 | 13.3 | 0.035* |
| | Mesial | 5 | 33.3 | 7 | 46.7 | 13 | 86.7 | |
| | No transportation | 0 | 0 | 2 | 13.3 | 0 | 0 | |
| One Shape | Distal | 13 | 86.7 | 9 | 60 | 7 | 46.7 | 0.061 |
| | Mesial | 2 | 13.3 | 6 | 40 | 8 | 53.3 | |
| | No transportation | 0 | 0 | 0 | 0 | 0 | 0 | |
| XP Shaper | Distal | 8 | 53.3 | 11 | 73.3 | 6 | 40 | 0.175 |
| | Mesial | 5 | 33.3 | 4 | 26.7 | 9 | 60 | |
| | No transportation | 2 | 13.3 | 0 | 0 | 0 | 0 | |

*: Significant at P ≤ 0.05

**Table 3: Mean, standard deviation (SD) and results of Kruskal-Wallis test for comparison between centering ratio after using the three systems.**

| Root level | WaveOne | | One Shape | | X Shaper | | P-value |
|---|---|---|---|---|---|---|---|
| | **Mean** | **SD** | **Mean** | **SD** | **Mean** | **SD** | |
| 3 mm | 0.29 B | 0.19 | 0.51 A | 0.32 | 0.24 B | 0.34 | 0.016* |
| 5 mm | 0.40 | 0.29 | 0.46 | 0.22 | 0.49 | 0.26 | 0.571 |
| 7 mm | 0.23 C | 0.16 | 0.64 A | 0.07 | 0.51 B | 0.30 | <0.001* |
| Total | 0.31C | 0.12 | 0.54 A | 0.11 | 0.41 B | 0.15 | <0.001* |

*: Significant at P ≤ 0.05. Different superscripts in the same row denote statistically significant differences.

## DISCUSSION

Ever since Schilder supported the concept of preparing the root canal in a funnel shape, while preserving its original curve[8]; ideal cleaning and shaping of the root canal systems has remained a very challenging procedure.

The American Association of Endodontics defined transportation as "removal of the canal wall structure on the outside of the curve in the apical half of the canal due to the files' tendency to restore their original shape during canal preparation"[9]. The inappropriate pattern of dentin removal adversely affects the treatment prognosis, as it causes high risk of straightening the original canal curvature, and increases the rate of debris extrusion and subsequent postoperative discomfort[10].

In the present study, mesiobuccal canals of extracted first mandibular molars were chosen to provide

conditions similar to the clinical situation and to allow realistic evaluation of the instruments' performance[11]. CBCT was used because it allows detailed three-dimensional (3D) observation of the root canal anatomy with high-resolution images, faster acquisition and reconstruction scheme. CBCT is an effective tool for measuring dentin thickness, apical transportation and canal centering [12,13].

Two parameters were selected to assess the shaping ability of the instruments tested in this study: (1) Apical transportation, which can endanger efficient root canal sealing, thereby reducing the treatment outcomes, and (2) maintenance of canal centering, which is basic in preparing curved canals[11].

Results showed no statistically significant difference between WaveOne and OneShape rotary systems at the levels of 3 and 5 mm from the apex, where both instruments produced high mean transportation compared to the XP Shaper instrument. This could be credited to the tip diameter corresponding to a size 25 for both WaveOne and the primary OneShape instruments, comparable to size 17 which XP Shaper initially starts with. At the level of 7 mm from the apex; WaveOne produced the highest statistically significantly mean transportation, followed by OneShape, while the XP Shaper produced the lowest mean transportation.

There is an inverse relationship between instrument tapering and canal transportation[14]. The primary WaveOne instrument has an 8% taper over the first 3 millimeters. This is greater than the other two rotary instruments, which decrease 4.3% and 5.5% respectively. The OneShape instrument has a constant 6% taper along its entire length and the XP shaper possesses an initial 1% taper along its whole length, which expands to a final 4% taper[5,15].

For overall canal transportation, this study showed that the XP Shaper produced the lowest statistically significantly mean transportation, while the WaveOne and OneShape instruments produced highest mean transportation, with no statistically significant difference between them.

The outstanding results of the XP Shaper can be attributed to its Adaptive Core technology, thanks to which it can expand while preserving the original canal anatomy and curvature[5]. The XP Shaper is believed to apply minimal stresses on the dentin walls, and can thus adapt easily to the canal irregularities[16].

Although the results of our investigation cannot be compared directly with those of Azim *et al.*[5] because of the different systems and methodology applied, their results were consistent with ours. They reported that XP Shaper was superior to Vortex Blue in terms of shaping ability, where the file created non-uniform preparation adapting to the complex canal anatomy.

On the other hand, the attitude of the OneShape instrument in the canal could be explained by its asymmetrical cutting edges. When combined with continuous rotation at a relatively high speed (350 rpm), this design feature causes the instrument to progress into the curved canals, creating some stress that might result in the observed apical transportation [15].

Similar results were drawn in by Agarwal *et al.*[17] and Alrahabi and Alkady[18], who found no statistically significant difference between WaveOne and OneShape instruments regarding canal transportation. Likewise, You *et al.* [19] and Capar *et al.* [20] reported similar transportation results for reciprocation motion and conventional continuous rotation technique. However, there have been contradictory results with Saber *et al.*[15] who report that the use of OneShape file caused a significantly greater apical transportation than WaveOne file. Still other studies report that WaveOne system preserved the original canal curvature better than the OneShape system did[21,22].

Our results confirm the increasing tendency of canal transportation as the diameter of the files increases[10]. OneShape and XP Shaper instruments showed no statistically significant difference among the different root levels. On the other hand, WaveOne instrument showed more distal canal transportation at the level of 3 mm from the apex and higher tendency toward mesial transportation at the 7 mm level. It is therefore suggested that instruments with taper greater than 0.06 should not be used for apical enlargement of curved canals.

Sinai reported that aggressive instrumentation in the cervical third of the root canal may lead to strip perforations and subsequent inflammatory complications[23]. Less transportation towards this area can be considered a favorable feature for the Wave One and the XP Shaper instruments. Agarwal *et al.*[17] showed that at 3 mm above the apex, ProTaper and WaveOne groups showed transportation towards the lateral side of the canal curvature, while the OneShape group remained centered, which agrees with the results of the present study. This result differs from previous studies that report

that the apical segment usually has more canal transportation toward the outside of the curve[24].

It should be noted that from a clinical standpoint, Wu *et al*[25] reported that apical transportation of more than 0.3 mm can negatively affect the sealability of the root canal filling material. In the present study none of the tested rotary systems caused more than 0.2 mm apical transportation.

Our results demonstrated that at the level of 3 mm from the apex; OneShape instrument showed the highest mean statistically significant centering ratio and there was no statistically significant difference between WaveOne and XP Shaper instruments. At the 5 mm level; there was no statistically significant difference among the three systems. At the 7 mm level; OneShape showed the statistically significant highest mean centering ratio followed by XP Shaper, and lastly by WaveOne instrument. These findings proved that instruments with constant taper in the apical section had good centering ability compared to instruments with progressive tapers along the cutting surface [26].

With regard to total centering ratio; OneShape showed the statistically significant highest mean centering ratio followed by XP Shaper followed by WaveOne. The superiority of OneShape instrument can be credited to its design, which progressively changes from variable 3-cutting edges at the tip to S-shaped 2 cutting edges near the shaft[27]. The snake-like motion helps preserve the original canal anatomy due to the offset rotation center, causing the file to engage and disengage along the canal wall, thus reducing the stresses between the file and the canal wall[18].

WaveOne instrument showed low centering ability, as it is a relatively large rigid single file with more taper that moves apically until it reaches the working length, creating a piston effect[28].

The findings of this research are consistent with previous results reported by different authors, such as Saleh *et al.*[29] who showed that canals prepared with the F360 and OneShape systems were better centered than those prepared with Reciproc and WaveOne systems, and Agarwal *et al.*[17] who showed that a OneShape group had less transportation and remained more centered than a WaveOne group, although the differences were not statistically significant. However, they are contradicted by Dhingra *et al.*[22] and Tambe *et al.*,[21] who showed the superiority of WaveOne system over OnesShape in terms of centering ability.

It can be concluded that overall, XP Shaper produced less canal transportation than WaveOne and OneShape instruments.

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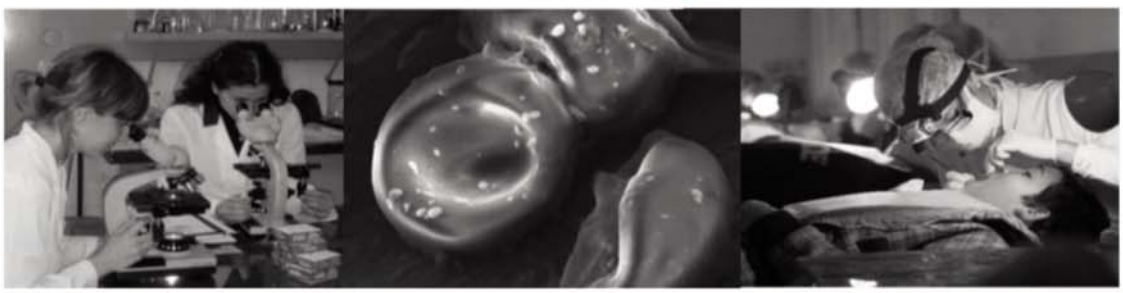

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