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

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

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

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 Submission of a paper to the journal will be taken to imply that it presents original unpublished work, not under consideration for publication elsewhere.

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# PREVALENCE OF SELF-REPORTED HALITOSIS AND ASSOCIATED FACTORS IN ADOLESCENTS FROM SOUTHERN BRAZIL

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***ABSTRACT***

*The aim of this study was to assess the prevalence of self-reported halitosis and associated factors in adolescents from Passo Fundo, Brazil. Additionally, we studied adolescents' concern with their own breath and whether anyone had ever told them that they had halitosis. A cross-sectional observational survey was conducted on 15- to 19-year old high school students from public and private schools in the city of Passo Fundo, Brazil. The random sample consisted of 736 adolescents from 20 schools. An interview with a structured questionnaire was administered. The prevalence of self-reported halitosis and associated factors was analyzed by either the chi-square test or Fisher's exact test. The prevalence of self-reported halitosis was 39.67%. The mother's level of education was associated with the outcome. Gender and behavioral habits were not associated with self-reported halitosis. Halitosis reported by others presented a prevalence of 10.73% and was associated to male gender and oral hygiene habits. Individuals with less toothbrushing frequency and no use of dental floss were associated to halitosis reported by others. It was observed that 88.58% of the participants are concerned with halitosis. It is concluded that there is high prevalence of self-reported halitosis, which is associated with a socioeconomic pattern. Most adolescents report concern with their own breath.*

***Key words:*** *Halitosis; adolescent; prevalence; associated factors.*

## PREVALÊNCIA DE HALITOSE AUTORREPORTADA E FATORES ASSOCIADOS EM ADOLESCENTES DO SUL DO BRASIL

***RESUMO***

*O objetivo do presente estudo foi verificar a prevalência de halitose autorreportada e fatores associados em adolescentes de Passo Fundo, Brasil. Além disso, foram verificados a preocupação dos adolescentes com o seu próprio hálito e se eles já foram avisados por alguém sobre essa condição em qualquer momento de suas vidas. Esse estudo transversal envolveu estudantes do ensino médio, da rede pública e privada, com idades entre 15 e 19 anos, da cidade de Passo Fundo, Brasil. Uma amostra randômica de 736 adolescentes de 20 escolas foi utilizada. Um questionário estruturado foi aplicado. A prevalência de halitose autorreportada e fatores associados foram analisados por qui-quadrado ou teste exato de Fischer. A prevalência de halitose autorreportada foi de 39,67%. O nível educacional da mãe foi associado com esse desfecho. Gênero e hábitos comportamentais não estiveram associados com halitose autorreportada. Apenas 10,37% dos adolescentes reportadam que outras pessoas indicaram a existência desse problema, sendo associada com o gênero masculino e com hábitos de higiene bucal. Os indivíduos que reportaram menor frequência de escovação dentária e ausência do uso de fio dental apresentaram associações com a advertência da halitose por outras pessoas. Foi observado que 88,58% dos participantes são preocupados com o próprio hálito. Concluiu-se que a halitose autorreportada apresenta alta prevalência e está associada com fatores socioeconômicos. Advertência sobre mau hálito por outras pessoas foi relatada apenas por uma minoria, sendo associado com o gênero masculino e hábitos de higiene bucal. A maioria dos adolescentes reportou preocupar-se com o próprio hálito.*

***Palavras chave:*** *Halitose; adolescente; prevalência; fatores associados.*

## INTRODUCTION

Halitosis is a condition in which exhaled air is unpleasantly altered both for patients and for people with whom they relate[1]. It affects millions of people around the world, although its prevalence varies, apparently triggered mainly by lack of oral hygiene and a disorderly lifestyle. It can cause social restrictions, interfere in quality of life, and may be an indicator of important systemic diseases. In most societies where halitosis is prevalent, people seek solutions, usually due to the discomfort or embarrassment to which they are subjected[2]. A lot of money is spent – often unsuccessfully – on breath fresheners[1].

One of the reasons for studying halitosis is its social impact as a result of patients feeling insecure in social, professional and family interactions. It may also influence quality of life and embarrass people relating to the individual with bad breath.

Therapeutic interventions in the health field are basically assessed through two types of outcomes. Surrogate outcomes are defined as a measurement of the disease process, and are usually therapy-centered, whereas true outcomes reflect unequivocal evidence of tangible benefit to patients, and are exclusively patient-centered. Surrogate outcomes are used assuming they represent true outcomes, either separately or together[3].

For example, some studies assess halitosis only through the objective measurement of volatile sulfur compound (VSC) concentrations in the exhaled air. The results of these investigations have a completely different meaning from studies that determine the percentage of individuals who report having halitosis[1].

Both outcomes are meaningful to understanding the issue, but observation through VSC monitors is considered a surrogate outcome, while self-reported perception of halitosis is recognized as a true outcome. Epidemiological observation taking into consideration both types of outcomes provides important information and enables broader understanding of the issue[1].

Adolescence is the transition period between childhood and adulthood, and is characterized by various changes in biological, psychological and social development[4]. Adolescents are subject to several health-related manifestations, including halitosis, which exceeds the biological scope, affecting the social scope and potentially harming the physical and psychological health of affected individuals[5]. Moreover, presence of halitosis may indicate presence of important systemic diseases requiring urgent diagnosis and treatment.

Nevertheless, in Brazil there are few scientific papers on adolescents, and still fewer on adolescent halitosis, which is a highly overestimated and taboo subject. The aim of this study was to assess prevalence of self-reported halitosis and associated factors in adolescents from southern Brazil. Additionally, it assessed subjects' concern with their own breath and whether they had ever been told they had bad breath.

## MATERIAL AND METHODS

### *Study design and location*

In this cross-sectional observational study, we interviewed 15- to 19-year-old adolescents enrolled at public and private high schools in the city of Passo Fundo, in 2012. Passo Fundo is located in the state of Rio Grande do Sul, southern Brazil. The city's population is about 190,000, according to the Brazilian Institute of Geography and Statistics. 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 reported 7,558 students enrolled in regular high school education at 23 schools, divided into 16 public schools and 7 private schools, all in the urban area of the city. Of this total 6,256 students (82.78%) attended public schools and 1,302 (17.22%) attended private schools (Department of Education of the State of Rio Grande do Sul).

### *Ethical considerations*

The Institutional Review Board of the University of Passo Fundo approved this study following authorization from the 7$^{th}$ Regional Office of Education to conduct it at public schools, and after formal approval by the principals of the private schools. All selected students presented the Informed Consent Form signed by parents or legal guardians, and those who were present on the day of the survey were interviewed. If a student was absent on the day of the survey, a second attempt was made to collect data. This study was fully conducted in accordance with the World Medical Association Declaration of Helsinki.

### *Sample*

All 23 high schools in the city were visited by the study coordinator and invited to participate. Twenty schools accepted the invitation (n=20), and 30% of the students from each of them were invited to participate. Participants were selected randomly by draw from the lists of all students aged 15 to 19 years from each participating school, regardless of their school schedule.

The research team visited all classrooms that included selected students, to present the objectives of the study. After the explanation, the selected students received an Informed Consent Form to be signed by their parents or guardians. In case of absence, a later contact was made.

### *Interview*

A structured questionnaire including demographic data, socioeconomic condition, general health behavior, health record, and oral health self-perception was administered using a set of questions from the PCATool-SB Brazil adult version, validated in Brazil[6], in addition to other questions on oral health at this age, such as oral hygiene habits and halitosis. The adolescents were interviewed between April and July 2012 by teams that included an interviewer and a recorder who had been previously trained by the study coordinator to ensure standard procedures. The training consisted of theoretical classes that included literature review on the subject, and reading and explanation of each question in the questionnaire. First, team members were trained, and secondly, high school students not selected to participate in the study were examined. The reproducibility of the interview was verified in 10% of the respondents chosen by draw, revealing an agreement rate of 98%.

***Statistical analysis***

The dependent variable was the prevalence of self-reported halitosis, which was ascertained by asking three questions: "Do you have bad breath?", "Has anyone ever complained to you about your breath?" and "Are you concerned about your breath?". The questions were answered on a Lickert scale response card with the answer choices "never", "rarely", "sometimes", "often", and "always"[7]. The answers were classified as "YES" for "sometimes", "often", and "always"; and as "NO" for "never" and "rarely". The explanatory variables for halitosis in adolescents were divided into two groups (Fig. 1) - one group with sociodemographic factors, and another with behavioral, biological, and oral factors[8].

Ethnicity/skin color was classified as white or non-white. The non-white group included adolescents who referred to themselves as black, yellow, brown or indigenous. Socioeconomic condition was assessed through two factors. The first was mother's level of education, classified into three groups: (1) complete or incomplete higher education, (2) complete or incomplete high school, and (3) complete elementary school at the most. The second factor was the type of school the adolescent attended, using public or private school as an income proxy, with students from public schools being considered to belong to lower income families.

Smoking was classified into three groups: (1) no history of smoking, (2) current smokers and (3) former smokers. Health condition was classified into two groups: (1) no health problem or unaware of health problem and (2) with a health problem that has lasted or will probably last more than one year. Toothbrushing frequency was classified into three groups: (1) more than three times a day, (2) three times a day, and (3) less than three times a day. Use of dental floss and orthodontic treatment were both dichotomized as yes or no.

Data were analyzed with the statistical package SPSS 18 (SPSS Inc., Chicago, United States). Associations between the dependent variable and independent variables were analyzed by chi-square test or Fisher's exact test, and presented by frequency distribution. The significance level applied was 5%.

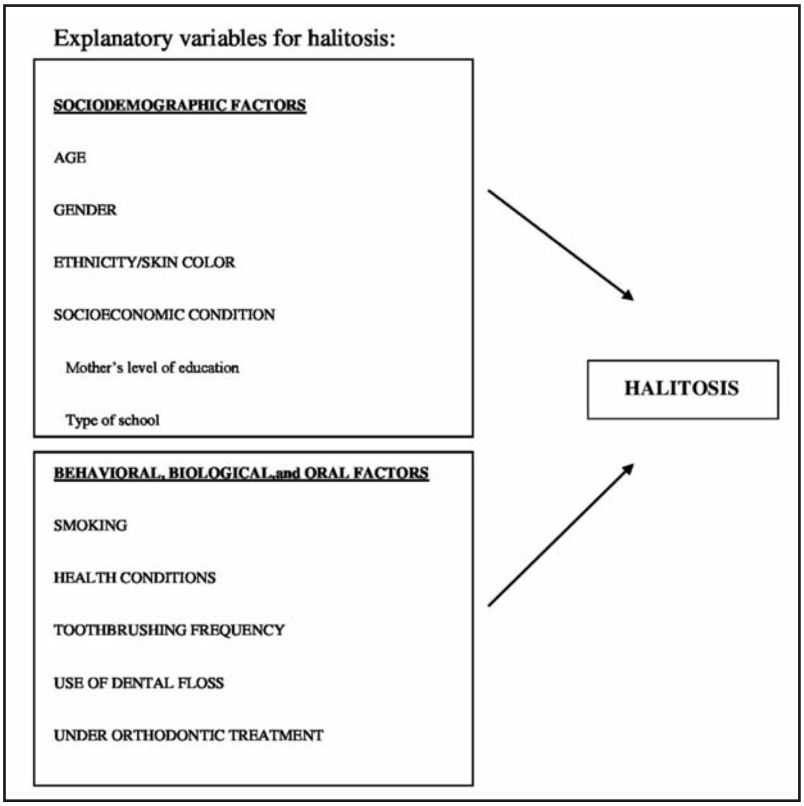


*Fig. 1: The theoretical framework linked to the analysis of the present study.*

## RESULTS

This study addressed 20 schools in the city of Passo Fundo. Participant recruitment is illustrated in the flowchart in Fig. 2.

Results show that 15- to 19-year-old old students in the city of Passo Fundo are predominantly female. Most individuals in this sample are 15, 16 and 17 years old. Regarding sociodemographic characteristics, most individuals are white, study at public schools, and the mother's level of education is low. In relation to health habits, a small percentage of individuals (less than 6%) reported having smoked at some time, more than 85% brush their teeth at least 3 times a day, and about half report using dental floss. About 12% report having health problems (mostly respiratory conditions), and about one third are under orthodontic treatment (Table 1).

Prevalence of self-reported halitosis was 39.67%. Table 2 shows the self-reported halitosis outcome and its association to potentially explanatory exposures. Gender had no statistically significant association to the outcome, although the result was close to reaching significance. Similarly, ethnicity and type of school were not associated to self-reported halitosis. The mother's level of education was associated to the outcome, considering that students whose mothers had less access to formal education presented higher prevalence of self-reported halitosis. Smoking, oral hygiene, health conditions and history of orthodontic treatment were not associated to self-reported halitosis.

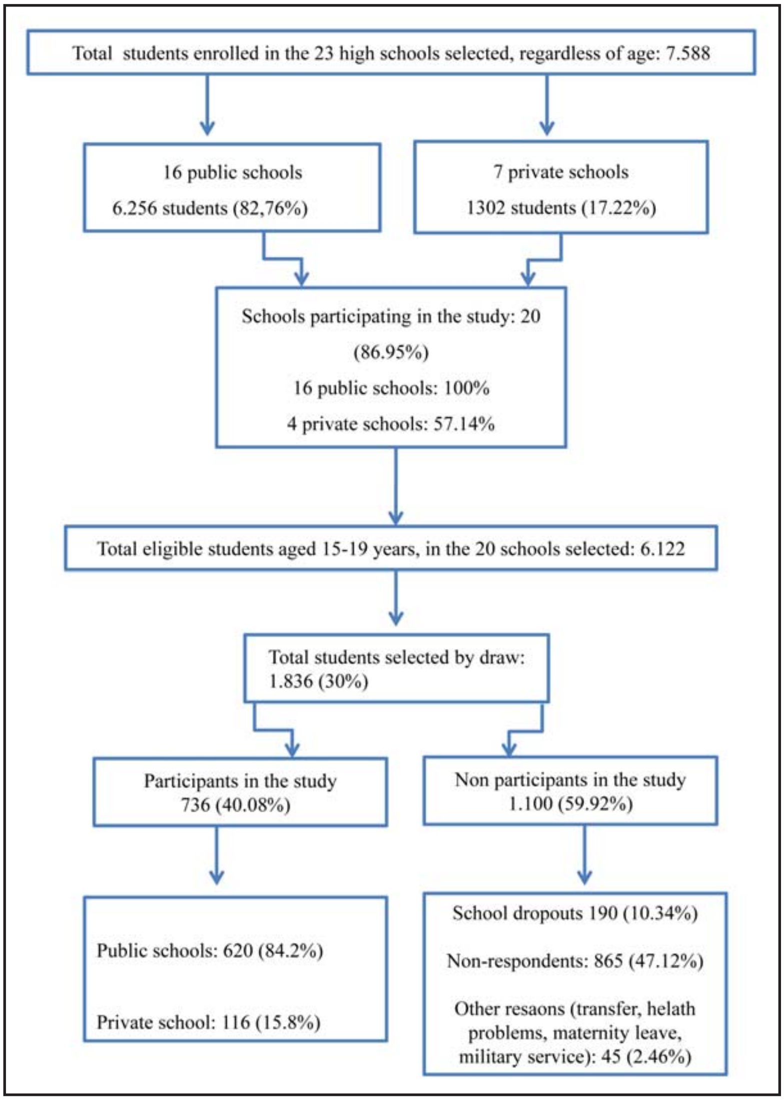


*Fig. 2: Study flowchart.*

Table 3 shows the results for "halitosis indicated by others". For this type of assessment, prevalence of halitosis was 10.73%. Halitosis indicated by others was associated to male gender and oral hygiene habits. Individuals with less toothbrushing frequency and no use of dental floss were associated to indication of halitosis by others.

Concern with bad breath was assessed, showing that 88.58% of participants care about halitosis, but no statistically significant association with the explanatory variables was found. Non-white ethnicity presented a lower percentage of concern, almost reaching statistical significance (Table 4).

## DISCUSSION

The aims of this study were to determine prevalence of self-reported halitosis and associated factors in adolescents from Passo Fundo, Brazil and to measure adolescents' concern with their own breath and whether they had ever been told they had bad breath. A cross-sectional observational study was carried out on high school students aged 15 to 19 years. The sample was similar to the percentage of students enrolled at public and

**Table 1. Description of the study sample.**

| | | N | % |
|---|---|---|---|
| **SOCIODEMOGRAPHIC FACTOR** | | | |
| GENDER | MALE | 323 | 43.9 |
| | FEMALE | 413 | 56.1 |
| AGE | 15 | 236 | 32.1 |
| | 16 | 251 | 34.1 |
| | 17 | 175 | 23.8 |
| | 18 | 58 | 7.9 |
| | 19 | 16 | 2.2 |
| ETHNICITY | WHITE | 511 | 69.4 |
| | NON-WHITE | 225 | 30.6 |
| TYPE OF SCHOOL | PUBLIC | 620 | 84.2 |
| | PRIVATE | 116 | 15.8 |
| MOTHER'S LEVEL OF EDUCATION | Complete or incomplete higher education | 164 | 22.3 |
| | Complete or incomplete high school | 265 | 36 |
| | | | |
| | Finished up to elementary school | 307 | 41.7 |
| **BEHAVIORAL, BIOLOGICAL, AND ORAL FACTORS** | | | |
| SMOKING | SMOKER | 17 | 2.3 |
| | FORMER SMOKER | 26 | 3.5 |
| | NEVER SMOKED | 693 | 94.2 |
| TOOTHBRUSHING FREQUENCY | >3 times a day | 186 | 25.3 |
| | 3 times a day | 435 | 59.1 |
| | <3 times a day | 115 | 15.6 |
| USE OF DENTAL FLOSS | YES | 390 | 53 |
| | NO | 346 | 47 |
| UNDER ORTHODONTIC TREATMENT | YES | 241 | 32.7 |
| | NO | 495 | 67.3 |
| HEALTH PROBLEMS | YES | 91 | 12.4 |
| | NO | 646 | 87.6 |

private schools in the city and the percentage of explanatory variables obtained such as ethnicity and gender was similar to the city's population data (Fig. 2).

All 23 high schools in the city were invited to participate, of which 20 accepted. A simple random sample of adolescents was selected, with a total 30% of students from each school. Of the selected students, 40.08% accepted to participate. Other studies using similar methodology report higher[9] or similar[10]participation rates. Although according to their age group, participants were close to being independent from their parents, the fact that legal restrictions required parents' signature on the consent form may account for the response rate.

Efforts were made to decrease any biases in the study, especially concerning the questionnaire. For this purpose, the research team was trained so that members would act similarly and to reduce any doubts that might arise while administering the questionnaire. Question comprehension was previously assessed among groups of adolescents.

**Table 2. Frequency distribution of exposures regarding self-reported halitosis among adolescents 15 to 19 years old.**

| VARIABLE | | SELF-REPORTED HALITOSIS YES – n (%) | SELF-REPORTED HALITOSIS NO – n (%) | p-value |
|---|---|---|---|---|
| **SOCIODEMOGRAPHIC FACTORS** | | | | |
| GENDER | Male | 117 (15.9%) | 206 (27.9%) | 0.053 |
| | Female | 175 (23.8%) | 238 (32.4%) | |
| ETHNICITY | White | 195 (26.5%) | 316 (42.9%) | 0.119 |
| | Non-white | 97 (13.2%) | 128 (17.4%) | |
| TYPE OF SCHOOL | Public | 252 (34.2%) | 368 (50%) | 0.12 |
| | Private | 40 (5.5%) | 76 (10.3%) | |
| MOTHER'S LEVEL OF EDUCATION | Complete or incomplete higher education | 51 (7%) | 113 (15.3%) | 0.005 |
| | Complete or incomplete high school | 100 (13.5%) | 165 (22.4%) | |
| | Finished up to elementary school | 141 (19.1%) | 166 (22.5%) | |
| **BEHAVIORAL, BIOLOGICAL, AND ORAL FACTORS** | | | | |
| SMOKING | Smoker | 10 (1.4%) | 7 (0.95%) | 0.176 |
| | Former smoker | 8 (1.1%) | 18 (2.44%) | |
| | Never smoked | 274 (37.2%) | 419 (57%) | |
| TOOTHBRUSHING FREQUENCY | >3 times a day | 71 (9.6%) | 115 (15.6%) | 0.30 |
| | 3 times a day | 168 (22.8%) | 267 (36.2%) | |
| | < 3 times a day | 53 (7.2%) | 62 (8.4%) | |
| USE OF DENTAL FLOSS | YES | 149 (20.2%) | 241 (32.7%) | 0.21 |
| | NO | 143 (19.4%) | 203 (27.5%) | |
| UNDER ORTHODONTIC TREATMENT | Yes | 92 (12.5%) | 149 (20.2%) | 0.30 |
| | No | 200 (27.1%) | 295 (40%) | |
| HEALTH PROBLEMS | Yes | 37 (5%) | 54 (7.33%) | 0.45 |
| | No | 250 (34%) | 395 (53.6%) | |

Self-assessment or self-perception of halitosis is a highly relevant outcome, since it involves the individual in the process and makes him/her understand the importance of the condition[11]. Accordingly, it is safe to assume self-perception as a true patient-centered outcome, which is highly recommended in contemporary research in the health field[3].However, organoleptic testing and self-perception both involve a subjective component, which may ultimately be questioned; therefore the additional use of an objective measurement to assess breath, such as the volatile sulfur compounds monitor (halimeter), may be recommendable. Its main limitation is that it does not detect all of the odorivectors present in exhaled air, and indicates only quantitative – not qualitative – values. It only detects some of the components offensive to the human sense of smell, while self-assessment relates to the individual perception of the person affected by halitosis[1,12]. Studies that assess halitosis exclusively through objective measurement of volatile compound concentration in exhaled air

**Table 3. Frequency distribution of exposures regarding halitosis indicated by others among adolescents 15 to 19 years old.**

| | | HALITOSIS INDICATED BY OTHERS | | |
|---|---|---|---|---|
| VARIABLE | | YES | NO | p-value |
| | SOCIODEMOGRAPHIC FACTOR | | | |
| GENDER | Male | 48 (6.5%) | 275 (37.3%) | 0.001 |
| | Female | 31 (4.2%) | 382 (52%) | |
| ETHNICITY | White | 51 (7%) | 460 (62.5%) | 0.19 |
| | Non-white | 28 (3.8%) | 197 (26.7%) | |
| TYPE OF SCHOOL | Public | 63 (8.5%) | 557 (75.6%) | 0.15 |
| | Private | 16 (2.2%) | 100 (13.5%) | |
| MOTHER'S LEVEL OF EDUCATION | Complete or incomplete higher education | 18 (2.4%) | 146 (20%) | 0.36 |
| | Complete or incomplete high school | 23 (3.1%) | 242 (33%) | |
| | Finished up to elementary school | 38 (5.2%) | 269 (36.5%) | |
| | BEHAVIORAL, BIOLOGICAL, AND ORAL FACTORS | | | |
| SMOKING | Smoker | 0 (0%) | 17 (2.3%) | 0.35 |
| | Former smoker | 3 (0.4%) | 23 (3.1%) | |
| | Never smoked | 76 (10.3%) | 617 (84%) | |
| TOOTHBRUSHING FREQUENCY | >3 times a day | 16 (2.2%) | 170 (23%) | 0.000 |
| | 3 times a day | 35 (4.8%) | 400 (54.3%) | |
| | < 3 times a day | 28 (3.8%) | 87 (12%) | |
| USE OF DENTAL FLOSS | YES | 31 (4.2%) | 359 (48.7%) | 0.007 |
| | NO | 48 (6.5%) | 298 (40.4%) | |
| UNDER ORTHODONTIC TREATMENT | Yes | 24 (3.2%) | 217 (29.5%) | 0.36 |
| | No | 55 (7.5%) | 440 (60%) | |
| HEALTH PROBLEMS | Yes | 11 (1.5%) | 80 (11%) | 0.40 |
| | No | 68 (9.25) | 577 (78.4%) | |

provide results which have a completely different meaning from those of studies that verify the percentage of individuals who report having halitosis[1,11].

Both study methods make important contributions to understanding the issue, but observation using VSC monitors is considered a surrogate endpoint, while self-perceived halitosis is recognized as a true outcome. Epidemiological studies with true outcomes provide insight into the real impacts of halitosis. The perception and impact of oral health status on quality of life have only recently become a matter of concern to the academic community, and several authors currently endorse patient-based results. In addition to gathering substantial information, studies aim to measure the impact of oral health disorders on the patient's quality of life[1,12].

This standpoint is important with relation to the concept of health not only as the absence of disease, but also as quality of life, and the concept of healthcare as not merely extending life and

**Table 4. Frequency distribution of exposures regarding concern with bad breath among adolescents 15 to 19 years old.**

| VARIABLE | | CONCERN WITH BAD BREATH YES | NO | p-value |
|---|---|---|---|---|
| **SOCIODEMOGRAPHIC FACTORS** | | | | |
| GENDER | Male | 285 (38.7%) | 38 (5.1%) | 0.44 |
| | Female | 367 (50%) | 46 (6.2%) | |
| ETHNICITY | White | 446 (60.5%) | 65 (8.8%) | 0.057 |
| | Non-white | 206 (28%) | 19 (2.6%) | |
| TYPE OF SCHOOL | Public | 553 (75%) | 67 (9.1%) | 0.15 |
| | Private | 99 (13.4%) | 17 (2.3%) | |
| MOTHER'S LEVEL OF EDUCATION | Complete or incomplete higher education | 140 (19%) | 24 (3.2%) | 0.13 |
| | Complete or incomplete high school | 232 (31.5%) | 33 (4.5%) | |
| | Finished up to elementary school | 280 (38%) | 27 (3.6%) | |
| **BEHAVIORAL, BIOLOGICAL, AND ORAL FACTORS** | | | | |
| SMOKING | Smoker | 16 (2.2%) | 1 (0.13%) | 0.13 |
| | Former smoker | 20 (2.7%) | 6 (0.8%) | |
| | Never smoked | 616 (83.6%) | 77 (10.5%) | |
| TOOTHBRUSHING FREQUENCY | >3times a day | 165 (22.4%) | 21 (2.8%) | 0.83 |
| | 3 times a day | 387 (52.5%) | 48 (6.5%) | |
| | < 3 times a day | 100 (13.5%) | 15 (2%) | |
| USE OF DENTAL FLOSS | YES | 344 (47%) | 46 (6.2%) | 0.41 |
| | NO | 308 (42%) | 38 (5.1%) | |
| UNDER ORTHODONTIC TREATMENT | Yes | 214 (29%) | 27 (3.6%) | 0.50 |
| | No | 438 (59.5%) | 57 (7.7%) | |
| HEALTH PROBLEMS | Yes | 83 (11.2%) | 8 (1%) | 0.24 |
| | No | 559 (76%) | 76 (10.3%) | |

eliminating diseases, but rather, doing so while maintaining the best possible quality[13].

This study showed high prevalence of self-reported halitosis (39.7%), regardless of gender. This agrees with other studies on halitosis conducted with similar methodology on adults. In the United States, (1996) prevalence of self-reported halitosis was 31%[14]; in France (1998) it was 32% among 4815 individuals aged 15 years or older[15]; in Switzerland (2009) it was 32% among 419 adults aged 18 to 94 years[16]. Another Swiss study (2009) on 626 army recruits aged 18 to 25 years found 21.4% prevalence of self-reported halitosis[17]. In Japan (2010) a similar study on 474 university students found 42% prevalence of self-reported halitosis[18]. Our study found similar results in Brazil, revealing halitosis as a condition having high impact.

Our study found a statistically significant association between self-reported halitosis and having a mother with a lower level of education, with 19.1% of the respondents having mothers whose educational level was not higher than elementary school and reporting halitosis. The same has been reported in studies showing that socioeconomic conditions, such as mother's level of education, are strongly associated to poorer oral health conditions[19,20]. Adolescents with poorer oral health conditions, including higher prevalence of cavities and periodontal diseases can be expected to have higher prevalence of bad breath.

Although the difference was not statistically significant, 34.2% of the respondents answered "yes" to self-reported halitosis and attend public schools, while 5.5% answered "yes" and attend private schools.

Behavioral, biological, and oral factors are not statistically associated to self-reported halitosis. Fifty-nine percent of the respondents brushed their teeth three times a day, which is the most common toothbrushing frequency reported in the literature[21,22]. This may account for the absence of association observed in this topic. It has also been suggested that the epidemiological situation of oral health in adolescents may not be directly related to the oral hygiene habits reported.

With regard to smoking, 37.2% of the students reported halitosis and having never smoked. Surprisingly, no association was found in this study between halitosis and smoking, even though it is known that the smell of cigarette may be mistaken for halitosis and exposure to smoking causes deterioration in oral health conditions, especially periodontally, which is related to halitosis.

Another important finding is that 34% of this sample reports suffering from halitosis with no presence of systemic diseases. This supports other studies that showed halitosis mainly as a condition with oral etiology[23].

Subjects with poorer oral health status and behaviors could be expected to be more likely to have halitosis, as would those under orthodontic treatment as a result of the difficulty in performing oral hygiene. However, there was no statistically significant association between these factors in this study. The fact that not much literature is available about halitosis in adolescence makes it difficult to compare data for these topics.

A less frequent approach to measuring halitosis is through third party reporting. This method was used in a study on a population of elderly people in the United States, which found prevalence of 24%[14]. In contrast, our study found that 10.7% of the sample had been told they had bad breath at some time in their lives. This difference may be explained by the age difference or even by cultural differences between populations.

Our study found a statistically significant association between halitosis and toothbrushing frequency, as well as between halitosis and use of dental floss. Of the respondents, 6.5% were male and reported halitosis indicated by others, presenting a statistically significant association. Of the respondents who reported having been told they had bad breath, 4.8% claimed they brushed their teeth three times a day, and 4.2% said they used dental floss. Thus, a relation between supposedly more adequate oral hygiene habits and lower impact of halitosis is clearly seen.

The discrepancy between self-reported halitosis and halitosis indicated by others can be accounted for by the fact that the former reflects self-perception while the latter requires someone else to be willing to point out the presence of halitosis. Other studies also reflect this discrepancy[14,23].

Our study also investigated whether adolescents are concerned with their own breath, finding that 88.7% are. This concern was independent of gender, ethnicity, type of school, mother's level of education, toothbrushing frequency, use of dental floss, orthodontic treatment, or other associated factors, without statistically significant association between the level of concern with halitosis and the explanatory variables. People of all ages, including adolescents, have long been concerned about halitosis. This is reflected by the considerable increase in the sale of mouthwashes, as shown by a study conducted in Brazil,[24] and massive advertising campaigns for breath fresheners[25].

Halitosis is a health condition with high prevalence[1,2,14,17], which may be present from childhood to old age, thus including adolescence. Halitosis has high social impact, affecting family, social, and professional life, and may have an impact on quality of life. A recent study on adolescents showed that those with self-reported halitosis had lower scores of quality of life measured by the OHIP-14[26]. Halitosis may thus be inferred to have an impact on public health. Despite the importance of halitosis as a highly prevalent condition, there are few studies involving adolescents, and few studies in general, since halitosis has historically been treated as an issue of lesser importance in dentistry[1,12,25].

The results of this study should be interpreted in light of the methodological understanding of its capacity to generate evidence. Because it is a cross-sectional study, causality may not be inferred. However, the observation of associated factors provides information on risk indicators, which are essential for establishing preventive strategies, early diagnosis and prompt treatment.

Considering the methodological characteristics and limitations, the results of the present study allow us to conclude that:

- Self-reported halitosis is a prevalent condition in about 40% of adolescents.
- Halitosis reported by others was observed in about 10% of adolescents.
- Almost 90% of adolescents reported concern with their own breath.
- Mother's low level of education was associated to self-reported halitosis.
- Males were more likely to have halitosis reported by others.
- Toothbrushing frequency and the use of dental floss are potential protective factors.

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## REFERENCES

1. Rösing CK, Loesche, W. Halitosis: an overview of epidemiology, etiology and clinical management. Braz Oral Res 2011; 25: 466-471.
2. Eldarrat, AH. Influence of oral health and lifestyle on oral malodour. Int Dent J 2011; 61: 47-51.
3. Hujoel PP. Endopoints in periodontal trials: the need for an evidence-based research approach. Periodontol 2000 2004; 36:196-204.
4. Armfield JM, Mejia GC, Jamieson LM. Socioeconomic and psychosocial correlates of oral health. Int Dent J 2013; 63: 202-209.
5. Seemann R, Conceicao MD, Filippi A Greenman J, Lenton P, Nachnani S, Quirynen M, Roldan S, et al. Halitosis management by the general dental practitioner—results of an international consensus workshop. J Breath Res 2014; 8: 017101doi: 10.1088/1752-7155/8/1/017101
6. Fontanive LT. Adaptação do instrumento Primary Care Assessment Tool – Brasil versão usuários dirigido à saúde bucal. [Master Dissertation] Porto Alegre: Faculdade de Medicina, Universidade Federal do Rio Grande do Sul, 2011. URL: http://hdl.handle.net/10183/53155
7. Vagias W. Likert-type scale response anchors. Clemson: Clemson University [cited 2016 May 20]. URL: https://www.clemson.edu/centers-institutes/tourism/documents/sample-scales.pdf.
8. Corraini P, Baelum V, Pannuti CM, Pustiglioni AN, Romito GA, Pustiglioni FE. Tooth loss prevalence and risk indicators in an isolated population of Brazil. Acta Odontol Scand 2009; 67:297-303.
9. Park YD, Patton LL, Kim HY. Clustering of oral and General health risk Behaviors in Korean adolescents: A national representative sample. J Adolesc Health 2010; 47: 277-281.
10. Brennan DS, Spencer AJ, Roberts-Thomson KF. Tooth loss, chewing ability and quality of life. Qual Life Res 2008; 17: 227-235.
11. Eli I, Baht R, Koriat H, Rosenberg M. Self-perception of breath odor. J Am Dent Assoc 2001; 132: 621-626.
12. Loesche WJ, Kazor C. Microbiology and treatment of halitosis. Periodontol 2000 2002; 28: 256-279.
13. Guyatt GD, Cook DJ. Health status, quality of life and the individual. JAMA 1994; 272: 630-631.
14. Loesche WJ, Grossmann N, Dominguez L, Schork MA. Oral malodour in the elderly. In: van Steenberghe D, Rosenberg M. Bad breath: a multidisciplinary approach. Leuven, Belgium: Leuven University Press, 1996: 181-194.
15. Frexinos, J, Denis P, Allemand H, Allouche S, Los F, Bonnelye G. Descriptive study of digestive functional symptoms in the French general population. Gastroenterol Clin Biol 1998; 22: 785-791.
16. Bornstein MM, Kislig K, Hoti BB, Seemann R, Lussi A. Prevalence of halitosis in the population of the city of Bern, Switzerland: a study comparing self-reported and clinical data. Eur J Oral Sci 2009; 117: 261-267.
17. Bornstein MM, Stocker BL, Seemann R, Bürgin WB, Lussi A. Prevalence of halitosis in young male adults: A study in Swiss army recruits comparing self-reported and clinical data. J Periodontol 2009; 80: 24-31.
18. Yokoyama S, Ohnuki M, Shinada K, Ueno M, Wright FA, Kawaguchi Y. Oral malodor and related factors in Japanese senior high school students. J Sch Health 2010; 80: 346-352.
19. Perera I, Ekanayake L. Social gradient in dental caries among adolescents in Sri Lanka. Caries Res 2008; 42: 105-111.
20. Mendes LGA, Biazevic MGH, Michel-Crosato E, Mendes MOA. Dental caries and associated factors among Brazilian adolescents: a longitudinal study. Braz J Oral Sci 2008; 7: 1614-1619.
21. Colussi PR, Haas AN, Oppermann RV,Rösing CK. Factors associated with changes in self-reported dentifrice consumption in a Brazilian group from 1996 and 2009. Braz Dent J 2012; 23: 737-745.

22. Freddo SL, Aerts DR, Abegg C, Davoglio R, Vieira PC, Monteiro L. Oral hygiene habits and use of dental services among teenage students in a city in Southern Brazil. Cad Saude Publica 2008; 24:1991-2000.
23. Delanghe G, Ghyselen J, Feenstra L, van Steenberghe D. Experiences of a Belgian multidisciplinary breath odour clinic. Acta Otorhinolaryngol Belg 1997; 51: 43-48.
24. Montenegro MM, Flores MF, Colussi PR, Oppermann RV, Haas AN, Rösing CK. Factors associated with self-reported use of mouthwashes in southern Brazil in 1996 and 2009. Int J Dent Hyg 2014; 12: 103-107.
25. Zalewska A, Zantonski M, Jablonka-Strom A, Paradowska A, Kawala B, Litwin A. Halitosis – a common medical and social problem. A review on pathology, diagnosis and treatment. Acta Gastroenterol Belg 2012; 75: 300-309.
26. Broughton JR, TehMaipi J, Person M, Randall A, Thomson WM. Self-reported oral health and dental service-use of rangatahi within the rohe of Tainui. N Z Dent J 2012; 108: 90-94.

# ALVEOLAR DIMENSIONAL CHANGES RELEVANT TO IMPLANT PLACEMENT AFTER MINIMALLY TRAUMATIC TOOTH EXTRACTION WITH PRIMARY CLOSURE

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***ABSTRACT***

*The purpose of this study is to evaluate the dimensional changes that occur in the alveolar ridge after minimally traumatic tooth extraction by means of computed tomography (CT), with special focus on the portion of bone supporting the gingival zenith.*

*Twenty subjects with indication for single-rooted tooth extraction and preserved alveolar walls were selected for this study. After a minimally traumatic extraction, two CT scans were performed; the first within 24 hours post-extraction (TC1) and the second 6 months (TC2) later. A radiographic guide with a radiopaque marker was used to obtain references that enabled accurate measurements over time, in both vertical and horizontal directions. The bone crest immediately apical to the gingival zenith was identified and termed "osseous zenith". The displacement of the osseous zenith in horizontal and vertical direction was analyzed and correlated with several alveolar anatomical variables with the aim of identifying possible predictors for bone remodeling.*

*Dimensional changes that occur in post-extraction sockets within a 6-month period showed significant vertical and horizontal displacement of the osseous zenith ($p<0.001$). Mean vertical resorption was 2.1 ± 1.7 mm, with a median of 1.9 mm and a range of 0.2 to 7.5 mm. Mean horizontal resorption was 1.8 ± 0.8 mm with a median of 1.7 mm and a range of 0.6 to 4.4 mm. However, no correlation was found between the width of the facial alveolar crest and the displacement of the osseous zenith.*

*The results of the present study showed that if the width of the facial crest at the apical-coronal mid-point is less than 0.7 mm, a high degree of displacement of the osseous zenith (> 3 mm) should be expected.*

*The present study suggests that the width of the alveolar crest at its mid-level, rather than crestal width, may be correlated with the displacement of the osseous zenith.*

***Key words:*** *tooth socket- tooth extraction- computed tomography.*

# CAMBIOS DIMENSIONALES EN EL REBORDE ALVEOLAR RELACIONADOS CON LA COLOCACIÓN DE IMPLANTE POSTERIOR A EXODONCIA ATRAUMÁTICA CON CIERRE PRIMARIO

***RESUMEN***

*El objetivo del presente estudio es evaluar los cambios dimensionales que ocurren en el reborde alveolar luego de realizar una exodoncia atraumática mediante tomografía computada, con especial atención en el tejido óseo que soporta el cénit gingival.*

*Se seleccionaron 20 sujetos con indicación de exodoncia de piezas unirradiculares y que presentaban las paredes alveolares conservadas. Luego de llevar a cabo una exodoncia atraumática se realizaron dos tomografías computadas (TC); la primera dentro de las 24 hs post-exodoncia (TC1) y la segunda a los 6 meses (TC2). Se utilizó una guía radiológica con un marcador radiopaco para obtener medidas precisas en el tiempo, tanto en el plano horizontal como vertical. Se identificó la cresta ósea inmediatamente apical al cénit gingival y se definió como "cénit óseo". Se realizó un análisis del desplazamiento del cénit óseo en el plano horizontal y vertical y se correlacionó con diferentes variables anatómicas con el objetivo de determinar posibles predictores del remodelado óseo.*

*Los cambios dimensionales que ocurren 6 meses post-exodoncia mostraron un desplazamiento significativo del cénit óseo ($p<0.001$). La reabsorción vertical promedio fue de 2.1 ± 1.7 mm con una media de 1.9 mm y un rango de 0.2 a 7.5 mm. El promedio de reabsorción horizontal fue de 1.8 ± 0.8 mm con una media de 1.7 mm y un rango de 0.6 a 4.4 mm. No se halló correlación entre el ancho de la cresta vestibular y el desplazamiento del cénit óseo.*

*Los resultados del presente estudio mostraron que si el ancho de la cresta vestibular en el punto medio (en sentido apico-coronal) es menor a 0.7mm se puede esperar un desplazamiento del cénit óseo (>3mm).*

*Este estudio sugiere que el ancho de la cresta alveolar en su punto medio podría predecir el desplazamiento del cénit óseo.*

***Palabras clave:*** *alvéolo dentario, extracción dentaria, tomografía computada.*

## INTRODUCTION

The alveolar healing process following tooth extraction has been studied throughout the history of dentistry, but has commanded more attention over the past two decades due to the advent of dental implants.

Several authors analyzed the physiological healing process that occurs after tooth extraction and described its remodeling process [1-2], where the total volume of the socket is markedly reduced and presents resorption of the facial and lingual walls, as well as of the bone filling in its center[3]. It has been determined that there is more bone resorption on the vestibular than on the lingual or palatal crest of the alveolus[4]. The greatest degree of resorption in post-extraction sockets occurs during the first three to six months after tooth extraction[5]. In one study, bone resorption was reported to be as much as 50% in a vertical direction[6]. Recent systematic reviews agree that there is more bone resorption in a vertical direction than in a horizontal direction[7, 8]. Several clinical trials have demonstrated that socket preservation procedures with bone grafts and guided bone regeneration significantly reduce bone resorption[9,10].

Tools for accurate, reproducible measurements of bone changes are necessary to obtain a clear notion of normal bone remodeling. Various techniques have been utilized to measure alveolar remodeling in humans, such as subtraction radiography, intraoperative models, direct measurement at re-entry surgical procedures, and lately, cone beam volumetric tomography (CBVT)[12,13].

To obtain accurate measurements, however, it is essential to locate the same reference locations in different CBVT studies taken at different points in time. The authors developed a simple, reproducible, effective method that enables the same point in space to be identified in different CT scans with a high degree of accuracy[11].

Many dentists and patients nowadays expect their implant-supported restorations not only to be functionally healthy over time, but also to look like natural dentition. The ability to achieve a biomimetic result, however, has been challenged by the difficulty in obtaining predictable peri-implant gingival morphology. Current understanding and knowledge establish that the stability and precise location of the peri-implant gingival margin is greatly dependent on the position of the underlying bone crest, among other factors. The most apical point in the facial gingival margin has been defined as the gingival zenith, and its location is of paramount importance in the overall esthetic result[12]. Analogously to the gingival zenith, the authors defined the bone crest that gives support to the gingival zenith as the *osseous zenith*, establishing its location as the nearest portion of bone tissue underlying the gingival zenith.

Thus, as esthetic expectations increase, more attention is focused on the remodeling process of the mid-facial bony crest, or osseous zenith. Various studies in animal models demonstrated that regardless of the extraction technique utilized, there is always some degree of resorption of the facial crestal bone[13]. Studies performed on human subjects with CBVT have rarely focused on the displacement of the osseous zenith, and thus we lack information regarding the extent to which this bone crest, which will support the implant facial gingival zenith, moves in apical and lingual directions. There is also insufficient information regarding any predictor of such displacement and alveolar behavior with atraumatic tooth extraction and primary closure with soft tissues.

Osseous zenith displacement may be closely related to the width of the facial crest. It is therefore the aim of this study to determine the displacement of the osseous zenith in vertical and horizontal directions, and to provide a description of dimensional changes in 20 dental alveoli 6 months after atraumatic tooth extraction with primary closure, utilizing a novel measuring technique and CBVT.

Additionally, this report examines the association between several pre-extraction measurements and bone remodeling in an attempt to find variables with predictable value for bone remodeling patterns.

## MATERIALS AND METHODS

Twenty subjects who visited the Periodontology Department at the Buenos Aires University School of Dentistry were enrolled in this clinical tomographic prospective study. The inclusion criteria was being systemically healthy. All subjects were older than 21 years and had extraction indications of one or more single-rooted teeth with preserved alveolar bone, with the intention of replacing the tooth with an implant supported prostheses. The diagnosed indications for tooth extractions were caries, endodontic complications, root fracture,

orthodontic, and prosthetic reasons. All patients in the study accepted the clinical procedures and signed approved informed consent. The project was approved by the FOUBA ethics committee.
Exclusion criteria were blood dyscrasia, pregnancy, human immunodeficiency virus, uncontrolled hypertension, requiring antibiotic prophylaxis, being under antibiotic treatment, uncontrolled diabetes, smokers, or fractured teeth with alveolar wall damage.

*Diagnostic Phase:* A medical, clinical and radiographic history was made for each subject and they all received hygiene instructions, and, if required, periodontal therapy. Study models were obtained to make an acrylic stent to be used as reference marker for taking tomographic measurements. The acrylic guide with a radiopaque element was constructed as previously described[14]. Briefly, an "L" shaped metal wire 20 mm long was bent at 90 degrees in the middle, so that both halves measured 10 mm. The wire marker was included in an acrylic structure adapted to a study cast model, and used as the radiographic stent. Special care was taken to place the wire marker in the center of the facial aspect of the tooth, indicating the gingival zenith and the long axis of the tooth.

*Surgical Phase*: Local anesthesia was applied and careful extractions were performed in all cases in order to preserve the alveolar crests. A periotome was initially used, followed by application of light traction and slight rotational forces with dental forceps. Following extraction, gentle curettage of the socket was performed to remove any remaining soft tissue. Thorough examination of the integrity of the alveolar walls was corroborated clinically. A lingual or palatal flap was elevated and displaced to perform a primary closure without elevating a vestibular flap to obtain primary closure[14]. Simple or mattress sutures and nylon material were used in all cases.

*Post-Surgical Care*: Sutures were removed 15 days after surgery. Subjects were treated with amoxicillin 500 mg every 8 hours for 7 days and ibuprofen 600 mg every 8 hours as needed for pain. Regular hygiene of the treated area was interrupted and replaced with a local antimicrobial mouthwash for 3 days (0.12% chlorhexidine digluconate every 12 hrs.).

*Tomographic Study*: Within 24 hours of the extraction, each patient underwent a computed tomography (TC1) scan with the previously prepared acrylic guide containing the radiopaque reference points in order to standardize measurements at baseline and at 6 months (TC2). The studies were performed with a 3D cone-bean volume CT(Promax 3D, Planmeca, Finland).
Images were analyzed through the Romexis Viewer 2.0.3.R program (Romexis Viewer 2.0.3.R, Planmeca, Finland).

**Initial Tomograms**
Three easily recognizable anatomical landmarks were identified and traced in the initial tomographic image: the osseous zenith, the lingual bony crest and the alveolus apex. Additionally, the long axis of the tooth (LAT) was traced as described previously (Fig. 1).
The osseous zenith represents the bone underlying the gingival zenith and was defined as the most coronal aspect of the osseous crest at the center of facial aspect of tooth. The alveolus apex was identified as the most apical aspect of the alveolus.

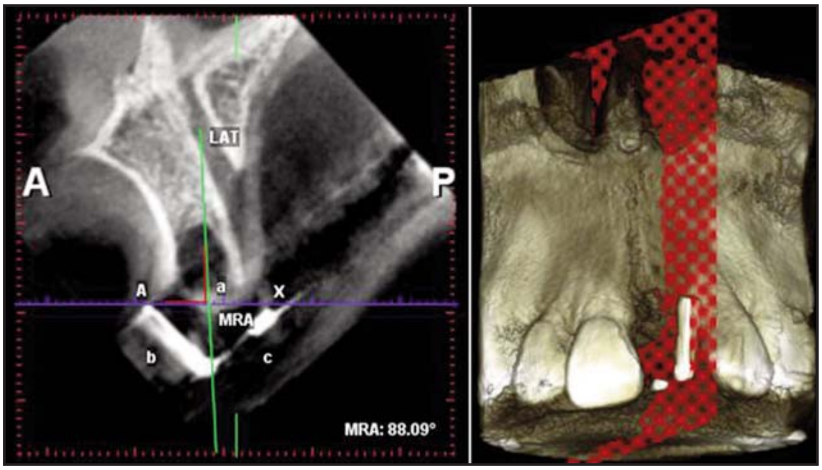


*Fig. 1: The radiopaque element was placed such that both tips and angle of the wire marker were aligned with the long axis of the tooth (LAT) extracted. The radiopaque element of the guide was identified on the sagittal plane and the reference points using Romexis Viewer 2.0.3.R. Planmeca.*

On tomograms taken immediately after tooth extraction, the osseous zenith, the apex of the alveolus and the lingual crest corresponded to, and were labeled as, point B1, point C5 and point D1 respectively.

*Levels of horizontal measurements*: Five parallel levels of horizontal measurement were selected as follows.
Firstly, five points were identified and marked along the LAT and labeled with the letter C. The most coronal point (C1) was a point where a segment originating in the bony crest (osseous zenith, B1) intersected the LAT at a 90º angle. The most apical point (C5) coincided with the alveolus apex. Points C2, C3, and C4 were respectively located along the LAT at a 25%, 50% and 75% of the total distance between C1 and C5.
Concordantly, five points were identified along the buccal bony surface (B1 to B5). While point B1 coincided with osseous zenith, points B2, B3, B4 and B5 were identified as the points were segments originating in the LAT at a 90º from points C2, C3, C4 and C5, respectively, intersected the buccal bony surface. Points D1, D2, D3, D4 and D5 were identified as the points where segments originating in the LAT at a 90º from points C2, C3, C4 and C5, respectively, intersected the lingual bony surface.
Additionally, along these five segments, the width of the bony buccal wall, the width of the lingual wall and the alveolus width were measured.

*Levels of vertical measurements:* Three levels of vertical measurements were selected as follows. Vertical measurements were taken at the level of the osseous zenith (level B) at the center of the alveolus (level C) and at the lingual bony crest (level D). All vertical measurements were taken linearly through these levels, and always parallel to the LAT.

*Levels of interproximal measurements:* The image was 3-dimensionally aligned and reference points were established. The three planes – horizontal (blue), sagittal (red) and frontal (green) – were positioned. A reference point was identified, marked along the LAT and labeled with the letter E1. Measurements were taken from E1 to E2 located at the interproximal bony wall.

Six months after the tooth extractions, a new tomographic image was taken utilizing the radiographic stent. All previously determined reference points and segments were traced over the second tomographic image on the same plane using the "backtrack analysis method". This method was described by Bontá et al. in previous paper[12] and enables reproducibility of measurements.
Additionally, new 6 post extraction measurements were taken in a similar way as the initial measurements and labeled under the prime symbol (\`). Thus, a new set of points B1\` to B5\`, C1\` to C5\`, and D1\` to D5\` were established.

*Horizontal measurements (variations in horizontal measurements)*: Dimensional changes that occurred along the buccal wall in a horizontal direction were measured at the previously mentioned five different horizontal levels, as changes in the segments B1-B1\` through B5-B5\`.

*Vertical measurements (variations in the vertical measurements)*: Vertical resorption of the buccal wall was calculated by the linear vertical displacement of the original bony crest (point C1) as follows. Point C1\` was determined where a line originating in point C1, running apically parallel to the LAT, intersected the buccal bony surface. Similarly, variations in the lingual crest height were determined with points D, D1 and DD.
Accordingly, bone fill of the alveolus was calculated based on the vertical measurement at the center of the alveolus. Thus, point CC' was calculated where segment originating in point C (placed at the AX reference segment) and running apically along the LAT, intersected the bony surface. Bone fill was linearly calculated as the difference between segments C-C1 and C-CC'.

**Statistical Analysis**
Descriptive statistics of variables included the arithmetic mean, median, standard deviation (SD), minimum (Min) and maximum (Max). In the text, group data are presented as mean ± SD. Statistical tests for paired samples were used to compare measurements between pre-operative and post-operative examinations: paired *t* test or Wilcoxon signed-rank test was selected according to whether normality assumption was satisfied or rejected, respectively. Normality assumption was tested

by Shapiro-Wilk test. A value of $P<0.05$ was considered statistically significant.

Receiver operating characteristic (ROC)[15] was analyzed with the aim of finding optimal cut-off points for vestibular plate and alveolus widths, which can help to predict high vertical and horizontal displacements of the osseous zenith. An ROC curve is constructed by plotting sensitivity versus 1-specificity. Sensitivity and specificity are the proportion of positive and negative cases detected, respectively. Vertical and horizontal displacements of the osseous zenith which exceeded the 85th percentile for the sample used in the present study (3.0 mm and 2.6 mm, respectively) were classified as "positive" cases. The rest of the values were considered as "negative" cases. Thus, in the ROC context, the widths of the vestibular plate and the alveolus are the diagnostic variables. The displacements of the osseous zenith in both vertical and horizontal directions, classified as positive (high) or negative according to the above criteria, are the outcome variables. The following criteria for the ROC analyses were set: both low values of vestibular plate width and high values of alveolus width would be related to high values of vertical and horizontal displacements of the osseous zenith. The area under the ROC curve (AUC) and its confidence interval of 95% (95% CI) are reported. AUC is a measure of the accuracy of the diagnostic variable for detecting positive and negative outcomes. The accuracy is minimal when AUC is 0.5 and maximal when AUC is 1.0. One way to interpret the intermediate values is as follows: between 0.5 and 0.7, accuracy is low; between 0.7 and 0.9, moderate; more than 0.9, high. The $P$ value was calculated for the null hypothesis that AUC equals 0.5[16]. When this $P$ value was less than 0.05 (significant result), optimal cut-off points were chosen using the Youden index[17]. Briefly, optimal cut-off points selected by this method maximize the sum of sensitivity and specificity. Sensitivity and specificity for optimal cut-offs points are reported.

Analyses were performed with statistical software. (Infostat version 2013[18] - used for descriptive statistics, paired $t$ test, Wilcoxon signed-rank test and Shapiro-Wilk test, R version 3.0.2[19], pROC[20] and verification packages[xxi] - used for ROC analyzes).

## RESULTS

Reference landmarks were determined at baseline and in final evaluations performed on each subject included in the study. The measurements were taken in a predictable manner, by recording dimensional changes that occur in post-extraction sockets within a 6-month period.

Table 1 summarizes the results at the pre-operative measurements of the width of the vestibular plate, palatal and alveolus width at levels 1 to 5, as well as the alveolar height.

ROC analyses were performed for each of the five levels where both vestibular plate and alveolus widths were measured. Only at level 3 were significant results obtained for the prediction of high displacement of the osseous zenith in both directions. Thus, in order to simplify the presentation of results, reporting of further analyses was restricted only to level 3.

At level 3, mean widths of the vestibular plate and palatal plate and alveolus width were 1.0 ± 0.5 mm (range: 0.4 to 2.1 mm), 3.7 ± 1.5 mm (range: 0.9 to 6.8 mm) and 4.2 ± 0.6 mm (range: 3.2 to 5.2 mm), respectively. Alveolus height was 8.5 ± 2.2 mm (range: 6.0 to 14.3 mm).

Vestibular plate horizontal remodeling is shown in Table 2. LAT-VP values decreased significantly between pre-operative and post-operative examinations for all five measured levels ($p<0.001$).

At level 3, pre-operative and post-operative LAT-TV values were 3.0 ± 0.5 mm (range: 2.2 to 4.3 mm) and 1.5 ± 1.8 mm (range: -5.5 to 4.2 mm), respectively; and difference between them was -1.5 ± 1.6 mm (range: -7.8 to -0.1 mm).

Vertical and horizontal displacement of the osseous zenith was also significant ($p<0.001$) (Fig. 2). Mean vertical resorption was 2.1 ± 1.7 mm, with a median of 1.9 mm and a range of 0.2 to 7.5 mm. Mean horizontal resorption was 1.8 ± 0.8 mm with a median of 1.7 mm; and a range of 0.6 to 4.4 mm.

ROC analyses were performed with the aim of finding a variable which can help predict vertical and horizontal displacement of the osseous zenith. Table 3 shows the results of ROC analysis. AUC values for the prediction of high vertical and horizontal displacement of the osseous zenith by the width of the vestibular plate (at level 3) were 0.82 (95% IC: 0.63 to 1.00) and 0.88 (95% IC: 0.73 to 1.00), respectively. In both cases, the AUC

**Table 1. Width of plate (vestibular and palatal faces) and alveolus at the five measured levels and height of alveolus in the 20 examined cases on preoperative examinations.**

| | Mean | Median | SD | Min | Max |
|---|---|---|---|---|---|
| Vestibular plate width (mm) | | | | | |
| level 1 | 0.9 | 0.9 | 0.3 | 0.4 | 1.8 |
| level 2 | 1.0 | 0.9 | 0.4 | 0.5 | 1.8 |
| level 3 | 1.0 | 0.9 | 0.5 | 0.4 | 2.1 |
| level 4 | 1.1 | 1.0 | 0.7 | 0.2 | 3.2 |
| level 5 | 1.9 | 1.8 | 1.1 | 0.5 | 4.7 |
| Palatal plate width (mm) | | | | | |
| level 1 | 1.3 | 1.4 | 1.0 | 0.3 | 4.1 |
| level 2 | 2.5 | 3.0 | 1.4 | 0.6 | 5.3 |
| level 3 | 3.7 | 3.6 | 1.5 | 0.9 | 6.8 |
| level 4 | 4.9 | 5.3 | 1.9 | 1.5 | 8.2 |
| level 5 | 6.8 | 6.9 | 2.2 | 3.3 | 10.3 |
| Alveolus width (mm) | | | | | |
| level 1 | 5.8 | 5.7 | 1.0 | 4.1 | 7.7 |
| level 2 | 4.9 | 4.8 | 0.7 | 3.5 | 5.8 |
| level 3 | 4.2 | 4.2 | 0.6 | 3.2 | 5.2 |
| level 4 | 3.4 | 3.4 | 0.6 | 2.1 | 4.8 |
| level 5 | 1.3 | 1.3 | 0.4 | 0.6 | 2.0 |
| Alveolus height (mm) | 8.5 | 8.3 | 2.2 | 6.0 | 14.3 |

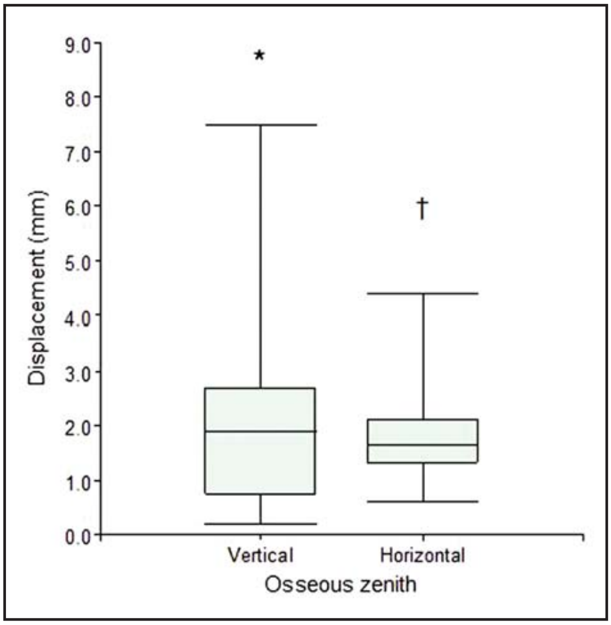


*Fig. 2: Boxplot diagrams for vertical and horizontal displacements of the osseous zenith in the 20 examined cases, with the boxes representing 25th and 75th percentiles, including the median and whiskers showing minimum and maximum. *Significant vertical displacement (p< 0.001, Wilcoxon signed-rank test). †Significant horizontal displacement (p < 0.001, paired t test).*

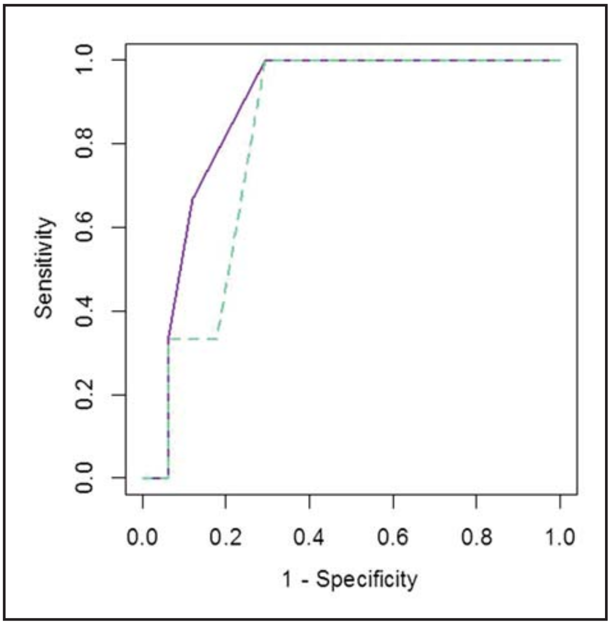


*Fig. 3: ROC curves for the prediction of high displacement of the osseous zenith in both vertical (green dashed) and horizontal (purple solid) directions by vestibular plate width (measured at level 3) using the 20 examined cases.*

differed significantly from 0.5 (P <0.05). Thus, AUC values indicate significant and moderate accuracy for these two evaluations. The two ROC curves are shown in Fig. 3.

A vestibular plate width of 0.7 mm (at level 3) was the optimal cut-off point obtained for prediction of high vertical and horizontal resorption of the osseous zenith. Thus, if table width is less than or

**Table 2. Changes in LAT-TV buccal plate (BP) at the five measured levels between preoperative and postoperative examinations.**

| Level | Level LAT-TV (mm) | | | | | | |
|---|---|---|---|---|---|---|---|
| | n | Mean | Median | SD | Min | Max | *P* Value |
| 1 | | | | | | | |
| Preoperative (Pre) | 20 | 3.5 | 3.4 | 0.6 | 2.6 | 5.3 | |
| Postoperative (Post) | 13 | 0.7 | 1.0 | 1.3 | -1.4 | 3.0 | <0.001 * |
| Difference (Post - Pre) | 13 | -2.9 | -2.3 | 1.2 | -5.1 | -1.5 | |
| 2 | | | | | | | |
| Preoperative (Pre) | 20 | 3.3 | 3.2 | 0.6 | 2.6 | 5.0 | |
| Postoperative (Post) | 20 | 1.2 | 1.5 | 2.3 | -7.5 | 4.7 | <0.001 † |
| Difference (Post - Pre) | 20 | -2.2 | -1.7 | 2.1 | -10.5 | -0.3 | |
| 3 | | | | | | | |
| Preoperative (Pre) | 20 | 3.0 | 2.9 | 0.5 | 2.2 | 4.3 | |
| Postoperative (Post) | 20 | 1.5 | 1.9 | 1.8 | -5.5 | 4.2 | <0.001 † |
| Difference (Post - Pre) | 20 | -1.5 | -1.1 | 1.6 | -7.8 | -0.1 | |
| 4 | | | | | | | |
| Preoperative (Pre) | 20 | 2.8 | 2.6 | 0.8 | 2.2 | 4.9 | |
| Postoperative (Post) | 20 | 1.6 | 1.7 | 1.7 | -4.7 | 3.9 | <0.001 † |
| Difference (Post - Pre) | 20 | -1.2 | -0.8 | 1.5 | -7.1 | -0.1 | |
| 5 | | | | | | | |
| Preoperative (Pre) | 20 | 2.7 | 2.3 | 1.1 | 1.3 | 6.0 | |
| Postoperative (Post) | 20 | 1.8 | 1.9 | 1.7 | -4.3 | 4.1 | <0.001 † |
| Difference (Post - Pre) | 20 | -0.9 | -0.5 | 1.5 | -6.5 | 0.6 | |

**Table 3. Analysis of receiver operating characteristic (ROC) for the prediction of high displacements of the osseous zenith by vestibular plate and alveolus widths (measured at level 3) using the 20 examined cases.**

| | Displacement of the osseous zenith in vertical direction | | | | | Displacement of the osseous zenith in horizontal direction | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Diagnostic variable | AUC (95% IC) | *P* Value | Optimal cut-off point | Sensitivity | Specificity | AUC (95% IC) | *P* Value | Optimal cut-off point | Sensitivity | Specificity |
| Vestibular plate width (level 3) | 0.82 (0.63 to 1.00) | 0.044 * | 0.7 mm † | 1.00 | 0.71 | 0.88 (0.73 to 1.00) | 0.021 * | 0.7 mm ‡ | 1.00 | 0.71 |
| Alveolus width (level 3) | 0.58 (0.26 to 0.90) | 0.355 | NC | NC | NC | 0.75 (0.34 to 1.00) | 0.101 | NC | NC | NC |

* P <0.05, AUC significantly different from 0.5.
† Vestibular plates widths ≤0.7 mm predict osseous zenith displacement in vertical direction >3.0 mm, with sensitivity 1.00 and specificity 0.71.
‡ Vestibular plates widths ≤0.7 mm predict osseous zenith displacement in horizontal direction >2.6 mm, with sensitivity 1.00 and specificity 0.71.

equal to 0.7 mm, a vertical resorption greater than 3.0 mm and a horizontal resorption greater than 2.6 mm are predicted; in both cases with sensitivity 1.00 and specificity 0.71.

ROC analysis for the prediction of high values of vertical and horizontal displacement of the osseous zenith by the alveolus width returned an AUC of 0.58 (95% IC: 0.26 to 0.90) and 0.75 (95% IC: 0.34 to 1.00), respectively. Since in both cases the AUC did not differ significantly from 0.5, the level of prediction was not considered useful and optimal cut-off points were not calculated.

## DISCUSSION

The resorption process responsible for dimensional changes following tooth extraction has been studied in different animal models[21,23] and in human subjects[26-27]. Different methods have been published in the literature for identifying and measuring dimensional changes that occur in post-extraction sockets, with or without the application of biomaterials. Dimensional and contour changes in post-extraction sockets have been described by serial study cast measurements[28,29], lateral cephalograms[30], radiographic analyses[31],[32] and direct measurements of the ridge following surgical re-entry procedures[33].

Schropp et. al.[7] used study models and standardized intraoral and subtraction radiography to evaluate 46 patients over a 12-month period. They reported loss of volume in the horizontal dimension in the range of 5 to 7mm within the first 12 months. This corresponded to approximately 50% of the original width of the alveolar bone. Bidimensional intraoral radiographs allowed only mesiodistal measurements and there were limitations in the application of subtraction radiography techniques. Fickl et al. evaluated dimensional changes in post-extraction sites in five beagle dogs by scanning models obtained before treatment, and at 2 and 4 months post-extraction. They concluded that the exposure of the buccal bone had a detrimental effect on the resorption process occurring after tooth extraction. In the presented animal model, 0.7mm additional volumetric shrinkage could be observed. The authors described limitations when trying to observe changes in the different alveolar ridge walls[34]. Nevins et al. presented a method for measuring alveolar ridges by performing CT scans in 36 patients immediately after extraction, at 30 days and at 90 days post-extraction[35]. Nineteen extraction sockets received bovine demineralized bone graft, and seventeen sockets received a non-osteogenic material. The majority of the sockets treated with the bone graft showed a loss smaller than 20% of the buccal plate. In contrast, 71% of the control sockets showed a loss of more than 20% of the buccal plate. The nasal floor was used as the anatomical reference but there was no precise reference to enable the location of the same point horizontally in subsequent scans. In general, the articles described agree that bone resorption inevitably occurs after tooth extraction and that bone grafting of the socket diminishes the effect, but is incapable of preventing it completely. The methods for measuring described in these articles present varying degrees of reproducibility and accuracy according to the technique applied.

The method used in this study previously proved to be simple, reproducible and effective, enabling the identification of the same point in space in different CT scans with a high degree of accuracy[12]. This measurement system enabled measurement of morphological changes in alveolar ridges after extraction and accurate determination of the displacement of the osseous zenith in the apical and lingual direction.

ROC curves were used to analyze our results statistically. ROC analysis was developed in the mid-twentieth century to optimize signal detection by radars[36]. Biomedical applications of this method were soon found, and today it is used as a predictive tool for decision-making in different fields of health sciences[37]. In this context, ROC curves are currently used in dentistry, including periodontology[38]. The importance of this statistical technique is that it minimizes the probability of error when a clinical decision between two opposite options must be made[39]. In the present study, ROC analysis provided a simple way to help predict, with significant and acceptable accuracy, the displacement of the osseous zenith based on the width of the vestibular plate. More specifically, our results suggest that low values (< 0.7mm) of the width of the vestibular plate at level 3 (mid-alveolar height) would predict high displacement of the osseous zenith in both horizontal and vertical directions.

Hard tissue modeling and remodeling following tooth extraction were studied in the dog model by Cardaropoli et al[40].; and Araujo and Lindhe[41]. They demonstrated that the socket was first occupied by a coagulum, which was subsequently replaced by granulation tissue, provisional connective tissue and woven bone. This immature hard tissue was later replaced with lamellar bone and marrow. During healing, the height of the buccal bone wall was substantially reduced. In addition, about 30% of the marginal portion of the alveolar process of the extraction site was modeled and lost.

The patterns of bone remodeling were variable regarding the extent of vertical and horizontal resorption of the facial wall and alveolar fill.

The utilization of an accurate method to evaluate alveolar remodeling enables identification of dimensional changes that occur in the alveolar ridge after extraction. With this methodology[12], it was possible to observe, and precisely measure in human subjects −throughout time and in a non-invasive manner− changes and bone morphology in alveolar ridges in all three spatial dimensions

The present study confirms significant resorption of the facial wall showing more resorption towards the coronal bony crest. Lingual bony wall showed little resorption.

The portion of bone providing support to the gingival zenith, termed in this study "osseous zenith", showed significant displacement towards the lingual and apical direction (median vertical: 1.9 mm - median horizontal: 1.7 mm).

With regard to the limitations of this study, we can say that a larger number of cases should be evaluated in order to confirm these results.

In conclusion, physiological changes that occur in the alveolus after dental extraction make the pursuit of the highest esthetic goals difficult and unpredictable. Final bone morphology determines the optimal position in which an implant should be placed as a tooth replacement, and thus greatly affects the ultimate esthetic results. Understanding and predicting the amount and pattern of bone remodeling enables the clinician to elaborate adequate treatment alternatives to prevent, revert or compensate for bone resorption. Atraumatic extractions and the displaced flap for alveolar primary closure produced greater bone resorption in the vestibular crest than in the palatal-lingual crest in both vertical and horizontal direction

The present study suggests that if the width of the facial crest at the apical-coronal mid-point is less than 0.7 mm, a high degree of displacement of the osseous zenith (> 3 mm) should be expected. The width of the alveolar crest at its mid-level, rather than the crestal width, may be correlated with the displacement of the osseous zenith.

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## REFERENCES

1. Atwood DA. Some clinical factors related to the rate of resorption of residual ridges. J Prosthet Dent 2001; 86: 119-125.
2. Johnson K. A study of the dimensional changes occurring in the maxilla after tooth extraction. Part I. Normal healing. Aust Dent Journal 1969; 8: 241-244.
3. Boyne PJ. Osseous repair of the postextraction alveolus in man.Oral Surg, Oral Med and Oral Pathol 1966; 21: 805-813.
4. Barone A, Aldini N, Fini M, Giardino R., Calvo-Guirado JL, Covani U. Xenograft versus extraction alone for ridge preservation after tooth removal: a clinical and histomorphometric study. J of Periodontol 2008; 79: 1370-1377.
5. Pietrokovski J, Massler M. Alveolar ridge resorption following tooth extraction. J Prosthet Dent 1967; 17: 21-27.
6. Amler MH. The time sequence of tissue regeneration in human extraction wounds.Oral Surg, Oral Med and Oral Pathol 1969; 27: 309-318.
7. Schropp L, Wenzel A, Kostopoulos L, Karring T. Bone Healing and Soft Tissue Contour Changes Following Single-Tooth Extraction: A Clinical and Radiographic 12-Month Prospective Study. Int J Periodontics Restorative Dent 2003; 23: 313-323.
8. Tan WL, Wong TL, Wong MC, Lang NP. A systematic review of post-extraction alveolar hard and soft tissue dimensional changes in humans. Clin Oral Implants Res 2012; 23: 1-21.
9. Van Der Weijden F, Dell´Acqua F, Slot DE. Alveolar dimensional changes of post-extraction sockets in humans: A systematic review. J Clin Periodontol 2009 ;36: 1048-1058.
10. Chen ST, Wilson TG Jr, Hämmerle CH. Immediate or early placement of implants following tooth extraction: review of biologic basis, clinical procedures, and outcomes. Int J Oral Maxillofac Implants 2004; 19: 12-25.

11. Artzi Z, Tal H, Davan D. Porous bovine bone mineral in healing of human extraction sockets. Part 1: Histomorphometric evaluation at 9 months. J Periodontol 2000; 21: 1015-1023.
12. Bontá H, Galli F, Caride F, Carranza N. A method for measuring post-extraction alveolar dimensional changes with volumetric computed tomography. Acta Odontol Latinoam 2012; 25: 186-192.
13. Chu SJ, Tan JH, Stappert CF, Tarnow DP. Gingival zenith positions and levels of the maxillary anterior dentition. J Esthet Restor Dent. 2009; 21:113-20.
14. Fickl S, Zuhr O, Wachtel H, Stappert CF, Stein JM, Hurzeler MB. Dimensional changes of the alveolar ridge contour after different socket preservation techniques. J Clin Periodontol 2008; 35: 906-913.
15. Bonta H, Carranza N, Caride F, Rojas M, Galli F. Lateral sliding flap to assist with anterior maxillary dental implant esthetics. J of Impl and Advanced Clin Dent.2015;7(9):11-21. http://jiacd.com/wp-content/files_mf/1448304877JIACDNov15.pdf
16. Bewick V, Cheek L, Ball J. Statistics review 13: receiver operating characteristic curves. Crit Care 2004; 8:508-512.
17. NCAR. Research Application Program 2012.verification: Forecast verification utilities. R package version 1.35. URL: http://CRAN.R-project.org/package=verification..
18. Youden WJ. Index for rating diagnostic tests. Cancer 1950; 3: 32-35.
19. Di Rienzo JA, Casanoves F, Balzarini MG, Gonzalez L, Tablada M, Robledo CW. Info Statversion 2013. Grupo InfoStat, FCA, Universidad Nacional de Córdoba, Argentina. URL: http://www.infostat.com.ar.
20. R Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. 2013. URL:: http://www.R-project.org/.
21. Robin X, Turck N, Hainard A, Tiberti N, Lisacek F, Sanchez JC, Müller M. pROC: an open-source package for R and S+ to analyze and compare ROC curves. BMC Bioinformatics 2011;12,77. DOI: 10.1186/1471-2105-12-77.
22. Akobeng AK. Understanding diagnostic tests 3: Receiver operating characteristic curves. Acta Pediatr 2007; 96: 644-647.
23. Kuboki Y, Hashimoto F, Ishibashi K. Time-dependent changes of collagen crosslinks in the socket after tooth extraction in rabbits. J Dent Res 1988; 67: 944-948.
24. Araújo MG, Linder E, Wennström J, Lindhe J. The influence of Bio-Oss Collage non healing of an extraction socket: An experimental study in the dog. Int J Periodontics Restorative Dent. 2008; 28: 123-135.
25. Carmagnola D, Adriaens P, Berglundh T. Healing of human extraction sockets filled with Bio-Oss. Clin Oral Implants Res 2003; 14: 137-143.
26. Lekovic V, Kenney EB, Weinlaender M, Han T, Klokkevold P, Nedic M, Orsini M. A bone regenerative approach to alveolar ridge maintenance following tooth extraction. Report of 10 cases. J Periodontol 1997;68: 563-570.
27. Lekovic V, Camargo PM, Klokkevold PR, Weinlaender M, Kenney EB Dimitrijevic B, Neduc M. Preservation of alveolar bone in extraction sockets using bioabsorbable membranes. J Periodontol 1998; 69:1044-1049.
28. Lam RV. Contour changes of the alveolar processes following extractions. J Prosthet Dent. 1960; 10: 25-32.
29. Watt DM, Likeman PR. Morphological changes in the denture bearing area following the extraction of maxillary teeth. Br Dent J 1974; 136: 225-235.
30. Atwood DA, Coy WA. Clinical, cephalometric, and densitometric study of reduction of residual ridges. J Prosthet Dent 1971; 26, 280-295.
31. Barone A, Aldini NN, Fini M, Giardino R, Calvo-Guirado JL, Covani, U. Xenograft versus extraction alone for ridge preservation after tooth removal: a clinical and histomorphometric study. J Periodontol 2008; 79:1370-1377.
32. Atwood DA. A cephalometric study of the clinical rest position of the mandible. Part II. The variability in the rate of bone loss following the removal of occlusal contacts. J Prosthet Dent 1957; 7: 544-552.
33. Carlsson GE, Persson G. Morphologic changes of the mandible after extraction and wearing of dentures.A longitudinal, clinical, and x-ray cephalometric study covering 5 years. Odontol Revy 1967;18: 27-54.
34. Fickl S, Zuhr O, Wachtel H, Stappert CF, Stein JM, Hurzeler MB. Dimensional changes of the alveolar ridge contour after different socket preservation techniques.J Clin Periodontol 2008; 35: 906-913.
35. Nevins M, Camelo M, De Paoli S, Friedland B, Schenk R K, Parma-Benfenati S, Simion M, Tinti C et al. A study of the fate of the buccal wall of extraction sockets of teeth with prominent roots.Int J Periodontics Restorative Dent 2006; 26: 19-29.
36. Zweig MH, Campbell G. Receiver-operating characteristic (ROC) plots: a fundamental evaluation tool in clinical medicine. Clin Chem 1993;39: 561-577.
37. Fawcett T. An introduction to ROC analysis. Pattern Recognit Lett 2006; :861-874.
38. Nummikoski PV, Steffensen B, Hamilton K, Dove SB. Clinical validation of a new subtraction radiography technique for periodontal bone loss detection. J Periodontol 2000;71: 598-605.
39. Swets JA, Dawes RM, Monahan J. Better decisions through science. Sci Am 2000;283: 82-87.
40. Cardaropoli G, Araujo M, Lindhe J. Dynamics of bone tissue formation in tooth extraction sites. An experimental study in dogs. J Clin Periodontol 2003; 30, 809-818.
41. Araújo MG, Sukekava F, Wennström JL, Lindhe J. Ridge alterations following implant placement in fresh extraccion sockets: An experimental study in the dog. J Clin Periodontol 2005; 32: 645-652.

# LOWER INCISOR INCLINATION REGARDING DIFFERENT REFERENCE PLANES

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***ABSTRACT***

*The purpose of this study was to assess the degree of lower incisor inclination with respect to different reference planes. It was an observational, analytical, longitudinal, prospective study conducted on 100 lateral cephalograms which were corrected according to the photograph in natural head position in order to draw the true vertical plane (TVP). The incisor mandibular plane angle (IMPA) was compensated to eliminate the variation of the mandibular plane growth type with the formula "FMApx.– 25 (FMA) + IMPApx. = compensated IMPA (IMPACOM)". As the data followed normal distribution determined by the Kolmogorov-Smirnov test, parametric tests were used for the statistical analysis, T-test, ANOVA and Pearson coefficient correlation test. Statistical analysis was performed using a statistical significance of $p < 0.05$. There is correlation between TVP and NB line (NB) (0.8614), Frankfort mandibular incisor angle (FMIA) (0.8894), IMPA (0.6351), A-po line (Apo) (0.609), IMPACOM (0.8895) and McHorris angle (MH) (0.7769). ANOVA showed statistically significant differences between the means for the 7 variables with 95% confidence level, P=0.0001. The multiple range test showed no significant difference among means: APo-NB (-0.88), IMPA-MH (0.36), IMPA-NB (-0.65), FMIA-IMPACOM (0.01), FMIA-TVP (0.18), TVP-IMPACOM (0.17).*

*There was correlation among all reference planes. There were statistically significant differences among the means of the planes measured, except for IMPACOM, FMIA and TVP. The IMPA differed significantly from the IMPACOM. The compensated IMPA and the FMIA did not differ significantly from the TVP. The true horizontal plane was mismatched with Frankfort plane in 84% of the sample with a range of 19°. The true vertical plane is adequate for measuring lower incisor inclination.*

***Key words:*** *Orthodontics, Incisor, reference standards.*

## INCLINACIÓN DEL INCISIVO INFERIOR RESPECTO A DIFERENTES PLANOS DE REFERENCIA

***RESUMEN***

*El propósito del estudio fue concordar el grado de inclinación del incisivo inferior medido con los diferentes planos de referencia. Estudio observacional, analítico, longitudinal y prospectivo en 100 radiografías laterales que fueron corregidas con base en la fotografía en posición natural de la cabeza, para trazar el plano vertical verdadero (PVV). Se compensó el ángulo del plano incisivo mandibular (IMPA) para eliminar la variación del plano mandibular respecto al tipo de crecimiento con la fórmula "FMApx. – 25 (FMA) + IMPApx. = IMPA compensado (IMPACOM)". Los datos tuvieron una distribución normal de acuerdo a la prueba Kolmogorov-Smirnov, por lo tanto se aplicaron pruebas paramétricas, T para medias de una muestra, coeficiente R de Pearson y ANOVA. El análisis estadístico fue realizado con una significancia estadística de $p < 0.05$.*

*Existe correlación entre el PVV con la línea NB (NB) (0.8614), ángulo de Frankfort incisivo mandibular (FMIA) (0.8894), IMPA (0.6351), línea Apo (APo) (0.609), IMPACOM (0.8895) y ángulo de McHorris (MH) (0.7769). De acuerdo al análisis de ANOVA existen diferencias estadísticamente significativas entre las medias de las 7 variables con un nivel de 95% de confianza p=0.0001. Mediante la prueba de múltiples rangos para determinar diferencias entre medias, se demostró que no existen diferencias significativas entre: APo-NB (-0.88), IMPA-MH (0.36), IMPA-NB (-0.65), FMIA-IMPACOM (0.01), FMIA-PVV (0.18), PVV-IMPACOM (0.17).*

*Todos los planos de referencia presentaron correlación entre ellos. Las mediciones derivadas de éstos tuvieron diferencias estadísticamente significativas, a excepción de FMIA, IMPACOM Y PVV. Existen diferencias estadísticamente significativas entre el ángulo IMPA y éste con su compensación. No se encontraron diferencias significativas entre el IMPACOM y PVV. El plano de Frankfort y el PHV no son coincidentes en un 84%, con un rango de 19 grados.*

***Palabras clave:*** *Ortodoncia, incisivo, estándares de referencia*

## INTRODUCTION

In orthodontics, the position of the lower incisor is considered essential to reaching an appropriate diagnosis and designing a treatment plan. It even defines certain aspects such as whether extractions are needed and what type of anchorage should be used.[1-3] Lower incisor position has a significant functional effect. It must resist incisal overeruption and provide harmony and functionality to the temporomandibular joint, providing an appropriate anterior guide and protrusive movements, which are crucial to mutually protected organic occlusion.[4] It also affects stability, because when the incisors are within the cortical bone, rather than outside it or at the biological limit, there is less risk of relapse.[5]

Tweed advocated placing the mandibular incisors upright to achieve balance and harmony in the lower facial third[6] Angle held that lower lip curvature is determined by incisor position[7], and many authors have emphasized that the effect of lower incisor position is crucial to dental and facial aesthetics.[8-10]

Lower incisor position was formerly considered so important that the course of the treatment plan was based only on its initial and final position, considered the key to successful treatment.[8-13]

Although it is currently known that a correct diagnosis must consider multiple factors in addition to the lower incisor, there is no doubt regarding its major effect in terms of function, stability and aesthetics. [1-3, 9, 14]

It is important to assess the position of the lower incisor not only on all three planes of space in relation to the mandibular alveolar bone and chin, but also in relation to the vertical and horizontal composition of the face.[15] Unlike the vertical and transversal plane, the anteroposterior plane has a very restricted field anatomically, therefore, in order to achieve optimal incisor position, it is important to assess its angulation. Excessive inclination can cause recession of the gingival margin or bone dehiscence, and insufficient angulation can cause fenestration.[16-18]

The lower incisor has thus been the object of much attention for many years. Many authors have tried to establish an optimal position, taking as reference different planes involving both hard and soft tissues.

With the implementation of radiography in the dental field, several cephalometric analyses have emerged. They have been extraordinarily useful to diagnostic procedures by enabling accurate assessments of skeletal and dental relationships occurring in different types of malocclusion.[19] For cephalometric study, the vertical and horizontal true planes have also been implemented, which are obtained from the Natural Head Position (NHP).[20-23]

There are currently multiple reference planes to measure incisor angulation; however, they are all affected by the other anatomical structures of each patient, so that their interpretation is highly variable. The bases of all cephalometric analysis were proposed years ago, and despite further contributions from different studies, current research establishes that a valid cephalometric reference should include the following features: reliability, intraindividual reproducibility, low inter-individual variability and average orientation close to the vertical or horizontal true plane.[20]

Tweed first highlighted the importance of the lower incisors, emphasizing the need to position them vertically on the medullary bone of the mandible body in order to achieve balance and harmony in the inferior facial third.[6, 24-26] After many years of observing the changes accomplished with respect to tooth position and their effects on facial aesthetics, he introduced his concept of "diagnosis triangle".[24]

Downs related incisor inclination to its upper antagonist, the mandibular plane and the occlusal plane. In addition to the use of the Frankfort mandibular plane, he introduced the A-Po line as a reference. However, unlike Tweed, he highlighted the importance of positioning the incisors in relation to the occlusal plane, and not necessarily in relation to the mandibular plane.[27-29] Schudy confirmed this and reported that the lower incisor is more consistently related to the occlusal than the mandibular plane in all types of facial morphology[30]. Knösel et al. found that third order angulation of fixed orthodontic appliances dictates incisal inclination, which refers to a perpendicular to the occlusal plane, and incisor inclination presents no correlation with craniofacial cephalometric measurements. [31, 32]

Steiner's cephalometric analysis associated the lower incisor to the middle facial third, and suggested individualizing the position of the lower incisor according to the relative prominence of the chin and the patient's maxillary-mandibular discrepancy. To this end, he proposed the line NB, formed by the union of Nasion and point B, as a reference.[33]

Tweed reaffirmed the standards of the proposed angles in his diagnostic triangle, and took the FMA angle variation (formed by the mandibular plane and the Frankfort horizontal plane) from 16 to 35 degrees with a standard value of 25; the IMPA angle (lower incisor with respect to mandibular plane) from 80-85 with norm of 90, and the FMIA angle (lower incisor with regard to Frankfort Horizontal plane) ideally as 65, though in rare cases it requires 75 degrees to achieve better facial balance.[34,35]

An important consideration when applying these measurements is the compensation that IMPA requires with regard to FMA. As the inclination of the mandibular plane with regard to Frankfort plane increases or decreases, the measure of lower incisor with respect to the mandibular plane will also vary but in the opposite direction. In other words, for each degree by which the FMA exceeds the norm of 25°, the mandibular incisors must be positioned a lower number of the norm of 90°, and vice versa, in case of a decrease of FMA, the mandibular incisors must be positioned a higher number of the IMPA's norm of 90°.[35]

Another outstanding author, Ricketts, established that the APo line represents a relationship of bony bases which can be affected by movements of the lower incisor, the APo line itself, or both, which should be considered when estimating the direction and degrees of desired movement in order to functionally place anterior teeth. His norm of incisal inclination differs from Downs', which is 21±5 degrees.[36-38]

McHorris proposed using the CI angle, formed by the radius of the mandibular hinge axis and the longitudinal axis of the lower incisor, at the intersection with the incisal edge of the latter. He acknowledged that the CI line was not affected by the mandibular position relative to the maxilla or the cranial base. He also pointed out the importance of condylar position in harmony with the teeth, because since they are at the service of the condyles in both static and dynamic relationship, there is more harmonious occlusion.[39, 40.]

In 1950 there was an important introduction: the natural head position, defined as an innate, physiological, reproducible head position, obtained when the patient is relaxed, sitting or standing, with the horizontal visual axis at eye level. It is known as the self-balance position of the head or natural orientation.[41] Also, this position offers the advantage of the cephalometric evaluation being based on the same standards as the clinical evaluation.[20- 23,42]

It is difficult to define a reference plane to set the ideal position of the lower incisor because there are multiple factors that affect and modify it. In addition, cephalometric analyses were obtained from dentures considered ideal, with the confluence of skeletal and dental features that usually do not occur in malocclusions, therefore they have been the subject of criticism and disagreement in the orthodontic field.[43]

The aim of this study was to match the degree of inclination of the lower incisor measured with different reference planes under the hypothesis that there is agreement among them. The reference planes considered were: (1) the Frankfort horizontal plane (FH) (which gives rise to FMIA angle), (2) the Nasion-Point B line (NB), (3) the Point A-Pogonion line (APo), (4) the mandibular plane (PM), (which gives rise to IMPA angle), (5) the McHorris plane (MH), and (6) the true vertical plane (TVP).

## MATERIAL AND METHODS

Prior authorization was obtained from the Division of Postgraduate Studies of the School of Dentistry of the Meritorious Autonomous University of Puebla (FEBUAP) for the biological and methodological requirements. An observational, analytic, longitudinal, prospective study was conducted. Using a non-probabilistic convenience sample, 100 lateral cephalograms were analyzed from patients who visited the orthodontic clinic in 2014 and met the selection criteria. Selection criteria were: fully erupted dentition up to first molar; with initial panoramic and cephalogram radiographs with sufficient sharpness to identify the lower incisors and other anatomical structures that are part of the reference planes; without previous orthodontic or orthopedic treatment; without supernumerary teeth, prosthetic dentures or missing teeth, and without craniofacial deformities or syndromes. A profile photograph of the patients in lateral head position (NHP) was taken in order to draw a true vertical and the radiograph was corrected based on this photograph. The lower incisor was measured taking as reference the NB, APo, IMPA, FMIA, TVP and McHorris plane. The FMA angle (Frankfort plane and mandibular plane) was also measured, to establish the patient's mandibular growth type.

To standardize the researchers, 10 radiographs were taken randomly and retraced two weeks later to avoid memory bias. The measurements were compared to those from the first trace and Pearson's R coefficient test was performed to determine measurement error. No statistically

significant difference was found between the two measurements.
Subsequently, all radiographs were measured using cephalometric paper, 0.05mm pencil and an Ormco brand protractor.
IMPA compensation was performed as described by Tweed[34,35] with the formula proposed by the researchers (Fig 1). Statistical analysis was conducted using SPSS version 20 and STATGRAPHICS Centurion XVI.II, with a statistical significance of $p< 0.05$. The data had normal distribution, which was determined with the Kolmogorov-Smirnov test, therefore parametric tests were applied, *T* test for sample means, Pearson's R coefficient and ANOVA.

**RESULTS**

A total 100 lateral cephalograms were included. Pearson's R coefficient test showed correlation between lower incisor inclination and all planes studied, including the true vertical plane (Table 1). Comparison of the 7 variables with ANOVA showed statistically significant differences between means with a 95% level of confidence p=0.0001 (Table 2). A multiple range test was used to determine in which groups these differences are significant. It is important to mention that there were no differences between the TVP, IMPACOM and FMIA (Table 3). The Student-T test for means was applied to determine the differences between the means of the studied planes and the TVV, finding no statistically significant difference between the TVP and the FMIA and IMPACOM planes (Table 4). Finally, Student-T test for means

**FMApx.-25+IMPApx.=IMPACOM**

- FMApx. = FMA angle patient.
- 25= FMA angle standard.
- IMPApx.= IMPA angle patient.
- IMPCOM= IMPA compensated

*Fig. 1: Formula to compensate the IMPA.*

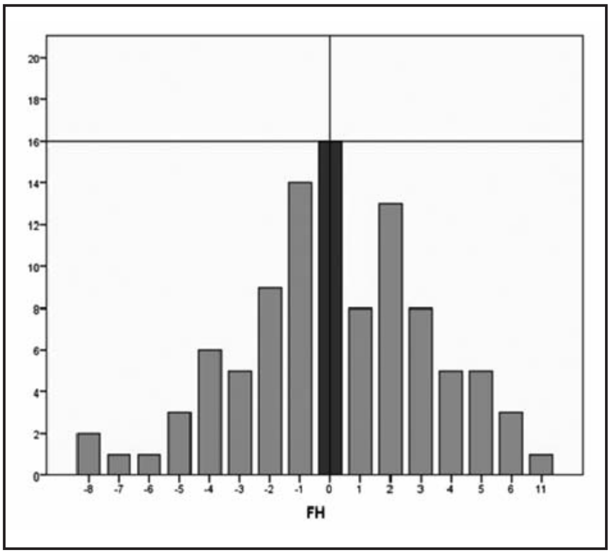


*Fig. 2: The Frankfort plane matches the Horizontal True correlation.*

**Table 1.**

| | NB | FMIA | IMPA | APo | PVV | IMPACOM | MH |
|---|---|---|---|---|---|---|---|
| NB | 1 | .880** | .783** | .797** | .861** | .879** | .841** |
| FMIA | .880** | 1 | .683** | .587** | .889** | 1.000** | .839** |
| IMPA | .783** | .683** | 1 | .644** | .635** | .684** | .800** |
| APo | .797** | .587** | .644** | 1 | .609** | .587** | .633** |
| PVV | .861** | .889** | .635** | .609** | 1 | .889** | .777** |
| IMPACOM | .879** | 1.000** | .684** | .587** | .889** | 1 | .839** |
| MH | .841** | .839** | .800* | .633** | .777** | .839** | 1 |

** The correlation is significant at the 0.01 level (bilateral). n = 100, FMIA: Frankfort horizontal plane – inferior incisive, NB: Nasion line to point B, APo: Point A- Pogonion, IMPA: mandibular plane – inferior incisive, true vertical plane (TVP), IMPACOM: IMPA compensated and MH: McHorris plane.

**Table 2.**

| | Sum of squares | Gl | Mean square | F-ratio | P-value |
|---|---|---|---|---|---|
| Between groups | 5545.98 | 6 | 924.33 | 20.56 | 0.0001* |
| Within groups | 31158.5 | 693 | 44.9617 | | |
| Total (Corr.) | 36704.4 | 699 | | | |

* Statistically significant differences (p< 0.05). n = 100.

comparing IMPA to IMPACOM found statistically significant differences with p<0.0001 (Table 5). The Frankfort plane matches the Horizontal True only in 16% of the cases, and the measurements obtained vary between -8 and 11 (Fig 2).

## DISCUSSION

With the development of orthodontic science, diagnostic accuracy and treatment plan detail have both increased, resulting in the need to evaluate the methods used. In order to apply cephalometric measurements properly, it is important to determine their specificity and sensitivity with regard to the position of teeth and their bony bases, and the relationship between them, the jaws and other cranial structures. If cephalometric measurements are interpreted inappropriately, they can lead in the wrong direction.[44]

Similarly, the literature has emphasized the importance of timely, accurate diagnosis of the lower

**Table 3.**

| Contrast | Sig. | Differences |
|---|---|---|
| NB - FMIA | * | -4.76 |
| NB - IMPA | | 1.65 |
| NB - APo | | -0.88 |
| NB – IMPACOM | * | -4.75 |
| NB - MH | * | 2.01 |
| NB - PVV | * | -4.58 |
| FMIA - IMPA | * | 6.41 |
| FMIA - APo | * | 3.88 |
| FMIA – IMPACOM | | 0.01 |
| FMIA - MH | * | 6.77 |
| FMIA - PVV | | 0.18 |
| IMPA – Apo | * | -2.53 |
| IMPA – IMPACOM | * | -6.4 |
| IMPA – MH | | 0.36 |
| IMPA – PVV | * | -6.23 |
| APo - IMPACOM | * | -3.87 |
| APo - MH | * | 2.89 |
| APo - PVV | * | -3.7 |
| IMPACOM - MH | * | 6.76 |
| IMPACOM - PVV | | 0.17 |
| MH - PVV | * | -6.59 |

* Statistically significant differences, (p< 0.05). FMIA: Frankfort Horizontal Plane – inferior incisive, NB: Nasion line to point B, APo: point line A-Pogonion, IMPA: mandibular plane – inferior incisive, True Vertical Plane (TVP), IMPACOM: IMPA compensated and MH: McHorris plane.

**Table 4.**

| | t | Gl. | Sig. (bilateral) | Mean differences | Lower | Upper |
|---|---|---|---|---|---|---|
| NB | 12.826 | 99 | .001* | 4.58000 | 3.8715 | 5.2885 |
| FMIA | -.555 | 99 | .580 | -.18000 | -.8240 | .4640 |
| IMPA | 10.375 | 99 | .001* | 6.23000 | 5.0385 | 7.4215 |
| MH | 14.127 | 99 | .001* | 6.59000 | 5.6644 | 7.5156 |
| APo | 6.499 | 99 | .001* | 3.70000 | 2.5703 | 4.8297 |
| IMPACOM | -.524 | 99 | .601 | 1.17000 | -.8138 | .4738 |

* Statistically significant differences, (p< 0.05). n = 100, FMIA: Frankfort Horizontal Plane– Inferior incisive, NB: Nasion line to point B, APo: Point line A- Pogonion, IMPA: Mandibular Plane – Inferior incisive, MH: McHorris plane and IMPACOM: IMPA compensated.

**Table 5.**

| | t | Gl. | Sig. (bilateral) | Mean differences | Lower | Upper |
|---|---|---|---|---|---|---|
| IMPA-IMPACOM | -11.549 | 99 | 0.001* | -6.40000 | -7.4995 | -5.3005 |

* Statistically significant differences, (p< 0.05). n = 100, FMIA IMPA: Mandibular plane – Inferior incisive, IMPACOM: IMPA compensated.

incisor by analyzing its position and inclination, as well as of other structures that influence and affect its measurement.[24, 27- 29, 34-38] This information influences decisions for the treatment plan, such as whether extractions or stripping will be performed and what anchorage type and biomechanics will be used, among other aspects. [1-3]

It is also relevant in pre-surgical orthodontics because the measurements used are often based on surgically repositioned structures. Cephalometric analysis should therefore be objective and consider each factor that may change or influence the measurement result.[44]

Based on the reference planes used in the present study, the lower incisor had an average proclination of 4.8°, lower than reported by Gütermann[16] (6.6°) and Asad[45] (7.7°), in similar samples. However, the variations among the means obtained for each plane were considerable, with the McHorris and FMIA angles indicating the lowest and highest proclination, respectively (1.2° ± 7 y 8°± 6). This shows how altered incisor measurement can depend on the reference plane used.[44]

There was correlation among all the reference planes analyzed. Aldreeset al.[46] reported similar results in terms of correlation between the mandibular plane and NB line, contrary to what was reported by Linder and Cornelius.[47]

The weakest correlation was observed for the APo line with regard to other planes, including FMIA (.587), IMPA (.644) and TVP (.609). Another low correlation was for IMPA with regard to FMIA (.683) and TVP (.644). This is due to the great influence of the horizontal and vertical position of the jaw on the APo line[38,44] and IMPA angle, highlighting the need for appropriate compensation in the latter in order to eliminate this variability factor.[34, 35]

The FMIA angle derives from the Frankfort plane, and is therefore strongly influenced by it. Ideally, the position of the Frankfort plane should be as close as possible to a true horizontal, however, in the present study this only occurred in 16% of the samples, with a minimum value of -8°, maximum of 11° and a broad range of 19°, similar to values reported by Madsen[20] and Shetty[21], at 23° and 16°, respectively.

The low correlation between FMIA and the other planes and its large difference with measurements of other groups is due to the fact that it is also affected by the position and rotation of the mandible, as stated by Williams[48,49] (1969, 1985).

The IMPA measurement was compensated following Tweed (1952, 1964), to remove the variation of the vertical and horizontal position of the jaw. The following formula is proposed:

$$FMApx - 25 + IMPApx = compensated\ IMPA$$

i.e., for each degree by which the FMA angle exceeds the norm of 25°, the mandibular incisors must be positioned a lower number of the IMPA's norm of 90°, and vice versa, in case of a decrease in FMA, the mandibular incisors must be positioned a higher number of the IMPA's norm of 90°.[34, 35]

The true vertical plane has not been used previously to measure lower incisor inclination. However, in the sample analyzed in our study, the true vertical plane showed correlation with the other reference planes, and measurements of incisal inclination did not differ significantly from the FMIA angle and IMPACOM.

The planes with greatest differences in the proclination or retroclination degrees were the McHorris plane with regard to TVP (-6.59) and FMIA (-6.77), and the IMPA angle with regard to TVP (-6.23), FMIA (-6.41) and IMPACOM (-6.4). It is thus feasible to state that the most consistent measurements are derived from the true vertical planes, FMIA and IMPA with its compensation, since without it the differences are heightened considerably.

## CONCLUSION

There was correlation among all reference planes. The differences were statistically significant except for FMIA, IMPACOM and TVP. There were statistically significant differences between the IMPA angle and the compensated angle. No significant difference was found between the IMPACOM and the incisive measurement derived from the TVP. The Frankfort horizontal plane and the true horizontal plane are mismatched in 84% of the sample population, with a range of 19°.

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## REFERENCES


1. Richmond S, Klufas ML, Sywanyk M. Assessing incisor inclination: a non-invasive technique. Eur J Orthod 1998; 20: 721-726.
2. Allais D, Melsen B. Does labial movement of lower incisors influence the level of the gingival margin? A case–control study of adult orthodontic patients. Eur J Orthod 2003; 25: 343-352.
3. Hernández-Sayago E, Espinar-Escalona E, Barrera-Mora JM, Ruiz-Navarro MB, Llamas-Carreras JM, Solano-Reina E. Lower incisor position in different malocclusions and facial patterns. Medicina Oral S. L 2013; 18: 343-50.
4. Andrews LF. The diagnostic system: Occlusal analysis. Dent. Clin. North Am 1976; 20: 671-690.
5. Profit WR. Equilibrium theory revisited: factors influencing position of the teeth. Angle Orthod 1978; 48: 175-186.
6. Klontz HA. Facial balance and harmony: An attainable objective for the patient with a high mandibular plane angle. Am J Orthod Dentofacial Orthop 1998; 114: 176-188.
7. Schullhof RJ, Allen RW, Walters RD, Dreskin M. The mandibular dental arch: Part I, lower incisor position. Angle Orthod 1977; 47: 280-287.
8. Margolis HI. Axial inclination of mandibular incisors. Am J Orthod 1943; 29: 571-594.
9. Wylie WL. The mandibular incisor—its role in facial esthetics. Angle Orthod 1955; 25: 32-41.
10. Hezberg. BL. Facial esthetics in relation to Orthodontic treatment. Angle Orthod 1952; 22: 3-22 URL:http://www.angle.org/doi/pdf/10.1043/0003-3219(1952)022%3C0003:FEIRTO%3E2.0.CO%3B2
11. Tweed, CH. The application of the principles of the edgewise arch in the treatment of malocclusions: II. Angle Orthod 1941; 11: 12-67.
12. Williams P. Lower incisor position in treatment planning. Br J Orthod 1986; 13: 33-41.
13. Metzdorf, DW. A cephalometric study of cranial, mandibular and lower incisor morphology in the adult face. Angle Orthod 1977; 47: 288-292.
14. Ceylan I, Baydas B, Bölükbasi B. Longitudinal cephalometric changes in incisor position, overjet, and overbite between 10 and 14 years of age. Angle Orthod 2002; 72: 246-250.
15. Hasund A, Boë O. Floating norms as guidance for the position of the lower incisors. Angle Orthod 1980; 50:165-168.
16. Gütermann C, Peltomäki T, Markic G, Hänggi M, Schätzle M, Signorelli L, Patcas R. The inclination of mandibular incisors revisited. Angle Orthod 2014; 84:109-119.
17. Yared KF, Zenobio EG, Pacheco W. Periodontal status of mandibular central incisors after orthodontic proclination in adults. Am J Orthod Dentofacial Orthop 2006; 130: 1-8.
18. Dorfman HS. Mucogingival changes resulting from mandibular incisor tooth movement. Am J Orthod 1978; 74: 286-297.
19. Kuramae M, Borges M, Nouer D, Bovi G, Inoue R. Analysis of Tweed's facial triangle in black Brazilian youngsters with normal occlusion. Braz J Oral Sci 2004; 3:401-403.
20. Madsen D, Sampson W, Townsend G. Craniofacial reference plane variation and natural head position. Eur J Orthod 2008; 30: 532-540.
21. Shetty D, Bagga DK, Goyal S, Sharma P. A cephalometric study of various horizontal reference planes in natural head position. J Ind Orthod Soc 2013; 47: 143-147.
22. El-Hayeck E, Bou Assi S, Bou-Serhal J. Clinical study of orientation and reference planes in cephalometry. Orthod Fr 2005; 76: 217-227.
23. Foster TD, Howat AP, Naish PJ. Variation in cephalometric reference lines. Br J Orthod 1981; 8: 183-187.
24. Tweed CH. The Frankfort-madibular plane Angle in orthodontic diagnosis, Classification, Treatment Planning and Prognosis. Am J Orthod and Oral Surg 1946; 32: 175-221.
25. Riedel RA. Esthetics and its relation to orthodontic therapy. Angle Orthod 1950; 20: 168-178.
26. Ellis E 3rd, McNamara J Jr. Cephalometric reference planes--sella nasion vs Frankfort. Int J Adult Orthod Orthognath Surg 1988; 3: 81-87.
27. Downs WB. Variations in facial relationships: Their significance in treatment and prognosis. Am J Orthod 1948; 34: 812-840.
28. Downs WB. The role of cephalometrics in orthodontic case analysis and diagnosis. Am J Orthod 1952; 38: 162-182.
29. Downs WB. Analysis of the Dentofacial Profile. Angle Orthod 1956; 26: 191-212.
30. Schudy F. Cant of the occlusal Plane and Axial Inclinations of Teeth. Angle Orthod 1963; 33: 69-82.
31. Knösel M, Engelke W, Attin R, Kubein-Meesenburg D, Sadat-Khonsari R, Gripp-Rudolph L. A method for defining targets in contemporary incisor inclination correction. Eur J Orthod 2008; 30: 374-380.
32. Knösel M, Attin R, Kubein-Meesenburg D, Sadat-Khonsari R. Cephalometric assessment of the axial inclination of upper and lower incisors in relation to the third-order angle. J Orofac Orthop 2007; 68: 199-209.
33. Steiner CC. Cephalometrics for you and me. Am J Orthod 1953; 39: 729-755.
34. Tweed CH. The Frankfort-Mandibular Incisor Angle (FMIA) In Orthodontic Diagnosis, Treatment Planning and Prognosis. Angle Orthod 1954; 24: 121-169. URL:http://www.angle.org/doi/pdf/10.1043/0003-3219%281954%29024%3C0121%3ATFIAFI%3E2.0.CO%3B2
35. Tweed CH. Was the development of the diagnostic facial triangle as an accurate analysis based on fact or fancy? Am J Orthod 1962; 48: 823-840.
36. Ricketts RM. Cephalometric Analysis and Synthesis. Angle Orthod 1961; 31: 141-156.
37. Ricketts RM. Planning treatment on the basis of the facial pattern and an estimate of its growth. Angle Orthod 1957; 27: 14-37.
38. Ricketts RM. Perspectives in the clinical application of cephalometrics. Angle Orthod 1981; 51: 115-150.
39. McHorris WH. Occlusion with particular emphasis on the functional and parafunctional role of anterior teeth- part 1. J Clin Orthod 1979; 13: 606-620.
40. McHorris WH. Occlusion with particular emphasis on the functional and parafunctional role of anterior teeth- part 2. J Clin Orthod 1979; 13: 684-701.
41. Moorees CF. Natural head position – a revival. Am J Orthod Dentofacial Orthop 1994; 105: 512-513.

42. Lundstrom F, Lundstrom. A Natural head posture as a basis for cephalometric analyses. Am J Orthod Dentofacial Orthop 1992; 101: 244-247.
43. Houston WJB, Edler R. Long-term stability of the lower labial segment relative to the A-Pog line. Eur J Orthod 1990; 12: 302-310.
44. McNamara JA, Ellis EE. Cephalometric evaluation of incisor position. Angle Orthod 1986; 56: 324-344.
45. Asad S, Naeem S, Ul-Hamid W, Rcsed M. Relationship between lower incisor inclination, lower lip prominence& vertical pattern of the patient. Pakistan Oral & Dental J 2009; 29: 55-58. URL: http://www.podj.com.pk/Jul_2009/12-PODJ.pdf
46. Aldress M. Evaluation of incisors inclination in five cephalometric analysis methods. Pakistan Oral & Dental J 2010; 30: 419-424. URL:http://hdl.handle.net/123456789/ 14547
47. Cornelius M, Linder-Aronson S. The relationship between lower incisor inclination and various reference lines. Angle Orthod, 1976; 46: 111-117.
48. Williams R. Eliminating lower retention. J Clin Orthod 1985; 19: 342-349.
49. Williams R. The diagnostic line. Am J Orthod 1969; 55: 458-475.

# EFFECTS OF NEW IMPLANT-RETAINED OVERDENTURES ON MASTICATORY FUNCTION, SATISFACTION AND QUALITY OF LIFE

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***ABSTRACT***

*The aim of this study is to evaluate the effects of replacing poorly fitting dentures on patient's masticatory function, satisfaction and oral health-related quality of life. Fourteen patients with conventional maxillary complete dentures and mandibular overdentures retained by two implants - bar clip system had their dentures replaced. The laboratory tests for the analysis of masticatory performance were conducted using an "Optocal" food simulator test. Questionnaires were used to evaluate patient satisfaction with dentures and impact of oral health on quality of life. Tests were conducted and questionnaires were administered before and 1, 3 and 6 months after the patient had adapted to the new dentures. Masticatory performance data and satisfaction with dentures (Visual Analogic Scale) were statistically analyzed by ANOVA and Tukey b test, satisfaction with dentures (SATs P) and impact of oral health on quality of life were evaluated using the Wilcoxon and Friedman tests (a=.05). There was no statistically significant improvement in masticatory function after denture replacement, although better outcomes were observed. Satisfaction with dentures and total score obtained using the OHIP-edent showed significant improvement. It can be suggested that the potential for grinding food, patient satisfaction and aspects of quality of life improved immediately after denture replacement.*

***Key words:*** *edentulous, quality of life, overdenture, patient satisfaction, mastication.*

## EFEITO DE NOVAS OVERDENTURES IMPLANTO RETIDAS NA FUNÇÃO MASTIGATÓRIA, SATISFAÇÃO E QUALIDADE DE VIDA

***RESUMO***

*Avaliar os efeitos da substituição de próteses mal adaptadas na função mastigatória, satisfação e qualidade de vida relacionada à saúde bucal do paciente. Quatorze pacientes com próteses totais convencionais maxilarese overdentures retidas por dois implantes mandibulares- sistem abarra clip etiveram suas prótes essubstituídas .Os testes laboratoriais para análise da performance mastigatória foram realizados utilizando um simulador de alimento "Optocal". Foram utilizados questionários para avaliar a satisfação com a prótese eo impacto da saúde bucal na qualidade de vida. Os testes foram realizados e os questionários foram aplicados antes, 1, 3 e 6 mes esapós o paciente ter adaptado às novas próteses. Os dados da performance mastigatória e satisfação com a prótese (EscalaAnalógica Visual) foram analisados estatisticamente por análise de variância e teste de Tukey b, a satisfação com próteses (SATs P) e o impacto da saúde bucal na qualidade de vida foram avaliados pormeio dos testes de Wilcoxon e Friedman (a = 0,05). Não houvem elhora estatisticamente significativa na função mastigatória após a troca daspróteses, em boram elhores resultados foram observados após a substituição. Satisfação com a prótese e a somatória total obtida utilizando o OHIP-Edent mostraram melhora significativa. Pode-se sugerir que o potencial de trituração dos alimentos, a satisfação do paciente com as nova spróteses e aspectos da qualidade de vida melhorar amimediatamente após a substituição da prótese.*

***Palavras chave:*** *desdentado, overdentures, mastigação, satisfação com as próteses.*

## INTRODUCTION

Despite the declining prevalence and low incidence of edentulism in countries where reliable epidemiological data exist, from a global perspective, large numbers of edentulous individuals, particularly among the elderly, are in need of rehabilitation. Complete dentures are and will remain the mainstay of treatment for the vast majority of edentulous patients. Most patients report satisfaction with denture usage, however, a smaller number are unable to adapt[1]. As it is especially difficult to adjust to a lower denture, because the support and retention areas are compromised, overdentures are recommended.

However, over time, implant-retained overdentures present some complications such as loss of attachment, retention, denture fracture, tooth wear, denture base misfit due to bone resorption, color change and tooth shape[2-5].The fact that the overdenture support system is similar to that of complete dentures, both being mucosa-supported, suggests that overdentures should be replaced in shorter or similar periods to conventional complete dentures.

As with complete dentures, the decision to replace overdentures should follow criteria based on a combination of the examiners' subjective opinion regarding the denture aspect and the patients' satisfaction[6]. However, many patients with clinically unacceptable dentures present greater than expected prosthetic tolerance and level of satisfaction, suggesting that such acceptance depends on how each individual adapts[7]. Dentists should tell patients when to replace dentures and explain the benefits to masticatory function and quality of life.

Many studies have evaluated masticatory function, satisfaction with the new denture and quality of life in individuals who received new complete dentures[6,8-14]. However, there is no published retrospective study comparing the masticatory function, satisfaction and quality of life in individuals who have worn two mandibular implant- supported overdentures before and after replacing new dentures. Considering the increasing use of mandibular implant-retained overdentures, it is relevant to investigate the null hypothesis of this study, which was "denture replacement may improve *masticatory function*, *patient satisfaction and quality of life"*.

## MATERIALS AND METHODS:

This study was approved by the Institutional Review Board of the Federal University of Uberlândia for the use of human subjects in research and has therefore been performed in accordance with the ethical standards set forth in the 1964 Declaration of Helsinki and its later amendments. Written informed consent was obtained from each subject after a full explanation of the research project. In this study, participants are part of a longitudinal follow-up of patients who had their complete mandibular dentures converted to implant-retained overdentures[15,16] The evaluation of denture quality was performed by a single calibrated examiner. Laboratory tests were performed to assess masticatory performance and questionnaires were administered to assess patient satisfaction with the dentures and oral health-related quality before and 1, 3 and 6 months after the patient's adaptation to the new dentures.

### Evaluation of Denture Quality

Dentures were evaluated to determine the need for replacement according to the following criteria: vertical dimension, aesthetics, occlusion, extension of dentures, time of use, retention, stability, and incidence of fracture of dentures and clips. Furthermore, a subjective assessment focused on satisfaction was taken into consideration. All prosthetic procedures and evaluations were performed by the same prosthodontists according to a technique recommended by the Dental School of the Federal University of Uberlândia.

### Objective Analysis of Masticatory Performance

Masticatory performance was determined by chewing 17 cubes (approximately 3 $cm^3$) of Optocal[17], for 40 continuous sequential strokes. Chewed particles were immediately sieved through a stack of eight sieves (Bertel Indústria Metalúrgica), with square apertures between 5.6 mm and 0.5 mm. Based on the weight of particles obtained from each sieve, the geometric mean diameter (GMD) of the particles was calculated using the weighted geometric mean. The GMD was calculated using Excel spreadsheets (Microsoft Corp, One Microsoft Way, Redmond, WA, 98052, USA). If all of the masticated content was retained in the 0.56mm sieve, (6660μm is the maximum GMD value) the patient would have 6660μm, which is considered the initial particle size. Masticatory Performance was calculated as the initial particle size minus GMD converted to a percentage by cross-multiplication. This method has been fully described elsewhere[15,17,18].

### Analysis of satisfaction with the prostheses

Two questionnaires were used to evaluate patient satisfaction: (1) Satisfaction with current prostheses and (2) Visual Analogue Scale (VAS)[16].

*1. Satisfaction with current prostheses*

The questionnaire consisted of six questions related to satisfaction with the appearance, retention and comfort of the prostheses [16,19]: (1) Are you comfortable smiling in proximity to other people; (2) Are you satisfied with your appearance?; (3) Are you able to laugh outright with other people (a) The maxillary prosthesis moves (b) The mandibular prosthesis moves; (4) Do you talk freely to other people (a) The maxillary prosthesis moves (b) The mandibular prosthesis moves; (5) Do you eat in proximity to other people; (6) Do you have pain or any discomfort in your mouth. Patient perception in relation to each question was recorded by the researcher in terms of Yes or No.

*2. Visual Analogue Scale (VAS)*

To complement the questionnaire with regards to satisfaction with current prostheses, four questions were asked to evaluate patient's perception with regard to maxillary, mandibular prostheses, stability/ retention and appearance, which were verified using the Visual Analogue Scale – VAS[16,20-22]. The VAS has a scale of 0-10, with the extremes corresponding to "completely dissatisfied" and "totally satisfied", respectively.

**Analysis of oral health-related quality of life (OHIP- edent)**

The quality of life analysis was carried out by administering the OHIP-edent[16,23], a validated specific questionnaire for edentulous patients, which detects changes in oral health-related quality before and after the placement of new dentures. It is a 19-item questionnaire with the following answering options: A = never, B = sometimes and C = almost always, which are divided into 4 domains: (a) masticatory-related complaints, (b) psychological discomfort and disability, (c) social disability and (d) oral pain and discomfort. Three possible responses with their scores in parentheses were provided as options: never (0), sometimes (1) or almost always (2). The scores for the questionnaire were calculated for the four domains at baseline, and after 1, 3 and 6 months.

## STATISTICAL ANALYSIS

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS), version 17.0 (SPSS Inc.; Chicago, IL, USA). Masticatory performance potential was analyzed by One-Way Anova and satisfaction with prostheses (VAS) by One-Way Anova and Tukey b to compare the values obtained at baseline and at 1, 3 and 6 months. For the data obtained on the nominal scale, a satisfaction questionnaire and OHIP-edent, Wilcoxon's and Friedman's tests were applied. Statistical significance was set at 5%.

## RESULTS

**Evaluation of Denture Quality**

Among the 16 patients whose prostheses had been converted a year and a half before, 14 (10 female and 4 male) , between ages 32 and 74 years (mean age 59.2 years) had an appointment to re-evaluate their prosthesis, which showed that their dentures needed to be replaced: 100% of patients presented unsatisfactory occlusion with poorly distributed contacts; 7 (50%) showed decreased vertical dimension; 11 (78%) showed extension of unsatisfactory dentures; 9 (64%) had over five years of denture use; 5 (35%) showed an incidence of denture base fracture in the clip region; and six (42%) were dissatisfied with the aesthetic appearance of the denture.

**Masticatory Performance**

There was no statistically significant difference in masticatory performance after denture replacement (ANOVA), although higher values were observed mainly after 3 and 6 months (Table 1).

**Analysis of satisfaction with the prostheses**

*1. Satisfaction with current prostheses*

No significant difference was found between satisfaction with the new mandibular overdentures and the old mandibular overdentures. For the complete maxillary denture, there was improvement in denture movement when laughing (question 3a) and talking freely with others (question 4a) in the first month after denture replacement. Greater satisfaction was also observed in terms of pain/discomfort in the mouth (question 6) (Table 2).

*2. Visual Analogue Scale - VAS*

There were statistically significant differences for all satisfaction aspects with the prostheses (VAS), showing improvement in both the complete maxillary dentures and the mandibular overdentures in the first evaluation after prosthesis replacement.

Greater satisfaction with complete maxillary dentures was observed at the 1-month evaluation after replacement. No statistically significant difference was found between baseline, 3 and 6 months only for this aspect (Table 3).

**Table 1. Mean, standard deviation, confidence interval and level of significance using ANOVA test (p< 0.05) for masticatory performance with 40 strokes before and 1, 3 and 6 months after denture replacement.**

| Stages | Mean, SD | Confidence Interval | p-value |
|---|---|---|---|
| Baseline | 25.66 ± 14.83 | 17.09 – 34.22 | 0.140 |
| 1 month | 28.00 ± 15.13 | 19.26 – 36.74 | |
| 3 months | 37.22 ± 15.05 | 28.53 – 45.91 | |
| 6 months | 36.79 ± 19.13 | 25.74 – 47.83 | |

ANOVA p< 0.05

### Analysis of oral health-related quality of life (OHIP- edent)

There was a statistically significant improvement in relation to four questions, namely, questions 2 on food retention, 3 on incorrect fitting, 6 on painful points in the mouth and 10 on avoiding eating due to painful points in the mouth.

There were statistically significant differences for the four domains: masticatory-related complaints, psychological discomfort and disability and social disability. For oral pain and discomfort, no statistically significant difference was observed (Table 4).

## DISCUSSION

After denture replacement, there was no statistically significant improvement for the masticatory function, so the null hypothesis was rejected. For satisfaction with dentures and quality of life, the null hypothesis was partially rejected. The

**Table 2. Level of significance (p) and statistical category (SC)** of questions 1, 2, 3a, 3b, 4a, 4b, 5 and 6 comparing each question for the 4 stages.**

| | Satisfaction with prostheses Statistical Categories** | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3a | 3b | 4a | 4b | 5 | 6 |
| Baseline | - | A | A | - | A | - | - | A |
| 1 month | - | B | B | - | B | - | - | B |
| 3 months | - | B | B | - | B | - | - | B |
| 6 months | - | B | B | - | AB | - | - | B |
| *p-value* | >0.999 | 0.029* | 0.000* | 0.392 | 0.035* | >0.999 | >0.999 | 0.001* |

* Statistically significant difference using Friedman test (p< 0.05)

** Statistically significant difference (Wilcoxon test - p< 0.05) represents different letters, where B represents better results than A.

**Table 3. Mean, standard deviation (SD) and statistical categories at baseline, 1, 3 and 6 months for the evaluation of satisfaction with prostheses using VAS.**

| | Satisfaction with prostheses (VAS) Mean ± SD (Statistical Categories**) | | | |
|---|---|---|---|---|
| | Maxillary prostheses | Mandibular prostheses | Stability and Retention | Appearance of prostheses |
| Baseline | 8.1 ± 1.0 (A) | 8.8 ± 1.1 (A) | 8.9 ± 0.7 (A) | 8.1 ± 1.4 (A) |
| 1 month | 9.4 ± 0.8 (B) | 9.6 ± 0.6 (B) | 9.9 ± 0.4 (B) | 9.9 ± 0.4 (B) |
| 3 months | 8.9 ± 1.1 (AB) | 9.6 ± 0.6 (B) | 9.9 ± 0.4 (B) | 9.4 ± 0.6 (B) |
| 6 months | 9.0 ± 1.0 (AB) | 9.7 ± 0.4 (B) | 9.7 ± 0,7 (B) | 9.4 ± 0.8 (B) |
| p-value | 0.015* | 0.001* | 0.000* | 0.000* |

* Statistically significant difference by Anova One way test (p< 0.05)

** * Statistically significant difference (Tukey b test- p< 0.05) represents different letters, where B represents better results than A.

**Table 4. Statistical Category (SC)** and level of significance * (*p*) of questions, das s, subscales and questionnaire total score- OHIP-edent-19.**

| OHIP-edent | | Stages | | | | |
|---|---|---|---|---|---|---|
| | | Baseline | 1 Month | 3 Months | 4 Months | Level of Significance (*p*) |
| QUESTIONS | **1** | - | - | - | - | 0.340 |
| | **2** | A | B | B | AB | 0.017* |
| | **3** | AB | A | B | B | 0.011* |
| | **4** | - | - | - | - | 0.066 |
| | **5** | - | - | - | - | 0.112 |
| | **6** | A | B | B | B | 0.007* |
| | **7** | - | - | - | - | 0.191 |
| | 8 | - | - | - | - | 0.101 |
| | 9 | - | - | - | - | 0.572 |
| | 10 | A | B | B | AB | 0.001* |
| | 11 | - | - | - | - | 0.392 |
| | 12 | - | - | - | - | 0.223 |
| | 13 | - | - | - | - | 0.572 |
| | 14 | - | - | - | - | 0.392 |
| | 15 | - | - | - | - | 1.000 |
| | 16 | - | - | - | - | 0.392 |
| | 17 | - | - | - | - | 0.300 |
| | 18 | - | - | - | - | 1.000 |
| | 19 | - | - | - | - | 0.300 |
| DOMAINS | 1 | A | A | AB | AB | 0.011* |
| | 2 | A | B | B | AB | 0.016* |
| | 3 | A | B | AB | AB | 0.002* |
| | 4 | - | - | - | - | 0.112 |
| **TOTAL** | | A | B | B | B | 0.007* |

* Statistically significant difference– Friedman test ($p<0.05$).
**Different letters show statistically significant difference –Wilcoxon test ($p<0.05$), where A<B being the worst values for category A.

relevance of this study should be emphasized considering that no previous study has evaluated the effect of mandibular overdenture replacement on masticatory function, patient satisfaction and quality of life. The studies conducted on the subject referred to mandibular and maxillary conventional complete dentures.

Andreiotelli et al. reported the following complications associated with implant overdentures: loss of retention or adjustment, clip or attachment fracture, overdenture fracture and acrylic resin base fracture. In this study, the main problems related to the prostheses were incidence of denture fracture (35%), loss of retention or loose clips (50%), unfavorable occlusion (100%), unsatisfactory prosthesis extension (78%), decreased vertical dimension (50%) and dissatisfaction with the aesthetic appearance (42%) [2]. The incidence of prosthesis fracture may have been due to the conversion of conventional overdenture to immediate prosthesis, which was not initially made for overdenture, causing fragility in the clip region and greater predisposition to fracture. In addition, the conversion time of prostheses of more than one year explains the problems with the clips: lack or loosening of retention. This was observed in the study by Bressan et al., in which few patients experienced loosening of retention (2.8%), but it was a cross-sectional study and patients were recalled once a year for follow-up visits, and if necessary, activation of retentive clips[3].

The prostheses of all patients presented unsatisfactory occlusion, mainly when there was contact with anterior teeth and poor distribution of posterior contacts upon closing. The maxillary prostheses of 9 patients presented unsatisfactory retention and stability, probably suggesting a relationship between unsatisfactory occlusion and prosthesis instability. After treatment, all patients presented bilateral balanced occlusion, which aims to stabilize dentures and minimize the resorption of alveolar bone and abrasion of denture teeth[24].

After replacement, when asked about the buildup of food under the mandibular prosthesis (question 2 OHIP-edent), patients reported that this no longer happened, and that they felt comfortable during mastication. Bressan et al. concluded that more than 50% of the subjects reported food impaction under the prosthesis and that this problem began about a year after treatment[3]. Complaints of food impaction may be related either to the time of denture use, as observed by Bressan, or to denture subextension, in which 78% of individuals had subextended mandibular prosthesis[3].

Other problems related to overdentures, such as decreased vertical dimension and dissatisfaction with the aesthetics, may be due to time of use (over 5 years), according to which they are in need of replacement.

The results of this study showed that although 64% of patients have improved masticatory performance (9 out of 14 patients), there was no statistically significant improvement after 1, 3 and 6 months, even though the prostheses were in satisfactory condition. In this study, patients were wearing complete dentures and after conversion to mandibular overdenture, their masticatory function improved significantly[15,16]. However, the potential for grinding food did not improve immediately after replacing the unsatisfactory dentures, even though there was an improvement for all patients. This may a result of the time it takes to adapt to the new dentures, considering that implant-retained overdentures require 6 months more than CD.

Garret et al.[8] analyzed mucosa-supported prostheses and reported that after denture replacement, there is period of 3 weeks to 18 months, depending on the consistency of the food to be chewed, for prosthesis adjustment and patient adaptation, after which improvement is constant. The adaptation of lower mucosa-support area to the new prosthesis seems to play a key role in masticatory performance. Retention, which is an important aspect to the patient, appears to play a secondary role, similar to the support area. Although it presents better retention, the prosthesis has a support area similar to complete mucosa-supported prosthesis.

Patients' first complaints referred to stability and retention of the upper prosthesis. In this study, patients who previously wore lower CD and converted to overdentures had 100% satisfied with prosthesis retention and stability[16]. This may explain why there were no complaints about the lower denture. Patient satisfaction is related mainly to stability, comfort, ability to chew and to speak, ease of prosthesis cleaning and aesthetics[25,26]. Not all patients were satisfied with aesthetics prior to replacement. Satisfaction was 100% after one month, remaining so after 3 and 6 months.

Patients in our study already enjoyed good quality of life before treatment, according to the OHIP-edent questionnaire, even though they were not satisfied with their prostheses. According to Forgie et al. and Scott et al. [27,28], quality of life involves economic and cultural factors, which were not addressed in this study. The greatest impact on patient quality of life involved retention of food particles under the prostheses (question 2), unsatisfactory adjustment of prostheses (question 3), presence of painful points in the mouth (question 6) and avoiding eating certain foods due to problems with the denture (question 10). After replacement, the answers were more favorable.

Avoiding eating certain foods is typical of conventional complete denture wearers, especially when the quality of dentures is unsatisfactory[19,29]. The retention of food particles under the prostheses can lead people to avoiding certain types of foods whenever possible, although they are able to chew some foods.

The aim of implant-retained overdentures is to improve quality of life for individuals considered functionally and psychologically mutilated, as in completely edentulous patients. Both technical quality in rehabilitation and detailed follow-up after rehabilitation can improve quality of life, satisfaction and masticatory function. It is thus extremely important for the dentist to know about these factors in order to be able to identify the best time to change the prosthesis.

Within the limits of this study, it is suggested that patient satisfaction and aspects of quality of life improved immediately after denture replacement, while the potential for grinding food improved after 3 months.

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## REFERENCES

1. Carlsson GE and Omar R. The future of complete dentures in oral rehabilitation. A critical review. J Oral Rehabil 2010; 37: 143-156.
2. Andreiotelli M, Att W and Strub JR. Prosthodontic Complications with Implant Overdentures: A Systematic Literature Review. Int J Prosthodont 2010; 23(3): 195-203.
3. Bressan E, Tomasi C, Stellini E, Sivolella S, Favero G, Berglundh T. Implant-supported mandibularoverdentures: a cross-sectional study. Clinical Oral Implants Res 2012; 23; 814-819.
4. Bryant SR, MacDonald-Jankowski D, Kim K. Does the type of implant prosthesis affect outcomes for the completely edentulous arch? Int J Oral Maxillofac Implants 2007; 22: 117-139.
5. Dudic A, Mericske-Stern R. Retention mechanisms and prosthetic complications of implant-supported mandibular overdentures: long-term results. Clin Implant Relat Res Dent 2002; 4: 212-219.
6. Nevalainen MJ, Rantanen T, Narhi T, Ainamo A. Complete dentures in the prosthetic rehabilitation of elderly persons: five different criteria to evaluate the need for replacement. J Oral Rehabil 1997; 24:251-258.
7. Bergman B, Carlsson GE. Clinical long-term study of complete denture wearers. J Prosthet Dent 1985; 53:56-61.
8. Garrett NR, Perez P, Elbert C, Kapur KK. Effects of improvements of poorly fitting dentures and new dentures on masticatory performance. J Prosthet Dent 1996; 75:269-275.
9. Fenlon MR, Sherriff M. Investigation of new complete denture quality and patients' satisfaction with and use of dentures after two years. J Dent 2004; 32: 327-333.
10. Allen PF. Association between diet, social resources and oral health related quality of life in edentulous patients. J Oral Rehabil 2005; 32: 623-628.
11. Veyrune JL, Tubert-Jeannin S, Dutheil C, Riordan PJ. Impact of new prostheses on the oral health related quality of life of edentulous patients. Gerodontology 2005; 22: 3-9.
12. Fenlon MR, Sherriff M, Newton JT. The influence of personality on patients' satisfaction with existing and new complete dentures. J Denti 2007; 35: 744-748.
13. Shigli K. Hebbal M. Assessment of changes in oral health-related quality of life among patients with complete denture before and 1 month post-insertion using Geriatric Oral Health Assessment Index. Gerodontology 2010; 27: 167-173.
14. Goiato MC, Garcia AR, Santos DM, Zuim PR. Analysis of masticatory cycle efficiency in complete denture wearers. J Prosthod 2010; 19: 10-13.
15. Borges TF, Mendes FA, Oliveira TRC, Prado CJ, Neves FD. Overdenture with immediate load: mastication and nutrition. Br J Nutr 2011; 105: 990-994.
16. Borges TF, Mendes FA, Oliveira TRC, Gomes VL, Prado CJ, Neves FD. Mandibular Overdentures with Immediate Loading: Satisfaction and Quality of Life. Int J Prosthodont 2011; 24:534-539.
17. Slagter AP, Olthoff LW, Steen WH, Bosman F. Comminution of food by complete denture wearers. J Dent Res 1992; 71:380-386
18. Mendonça DB, Prado MM, Mendes FA, Borges TF, Mendonça G, Prado CJ, Neves FD. Comparison of masticatory function between subjects with three types of dentition. Int J Prosthodont 2009; 22:399-404.
19. Oliveira TR, Frigerio ML. Association between nutrition and the prosthetic condition in edentulous elderly. Gerodontology 2004; 21:205-208.
20. Feine JS, de Grandmont P, Boudrias P, Brien N, LaMarche C, Taché R, Lund JP. Within-subject comparisons of implant-supported mandibular prostheses: Choice of prosthesis. J Dent Res 1994; 73:1105-1111.
21. Awad MA, Lund JP, Shapiro SH, Locker D, Klemetti E, Chehade A, Savard A, Feine JS. Oral health status and treatment satisfaction with mandibular implant overdentures and conventional dentures: A randomized clinical trial in a sênior population. Int J Prosthodont 2003;16: 390-396.
22. Mendonça DB, Prado MM, Mendes FA, Borges TF, Mendonça G, Prado CJ, Neves FD. Comparison of masticatory function between subjects with three diferente types of dentition. Int J Prosthodont 2009; 22: 399-404.
23. Souza RF, Patrocínio L, Pero AC,Marra J, Compagnoni MA. Reliability and validation of a Brazilian version of the oral health impact profile for assessing edentulous subjects. J Oral Rehabil 2007; 34:821-826.
24. Badel T, Panduric J, Kraljevic S, Dulcic N. Checking the occlusal relationships of complete dentures via a remount procedure. Int J Periodontics Restorative Dent 2007; 27: 181-192.
25. Cibirka RM, Razzoog M, Lang BR. Critical evaluation of patient responses to dental implant therapy. J Prosthet Dent 1997; 78:574-581.
26. Awad MA, Feine JS. Measuring patient satisfaction with mandibular prostheses. Community Dent Oral Epidemiol 1998; 26: 400-405.
27. Forgie AH, Scott BJ, Davis DM. A study to compare the oral health impact profile and satisfaction before and after having replacement complete dentures in England and Scotland. Gerodontology 2005; 22: 137-142.
28. Scott BJ, Forgie, AH, Davis DM. A study to compare the oral health impact profile and satisfaction before and after having replacement complete dentures constructed by either the copy or the conventional technique. Gerodontology 2006; 23:79-86.
29. Oliveira TRC, Borges TF, Mendes FA, Neves FD. Malnutrition in the 21st Century.Risk of malnutrition in completely edentulous individuals (Chapter XIV). United States: Nova Science Publishers, 2007; 265-280.

# COMPARISON OF FRICTIONAL RESISTANCE AMONG CONVENTIONAL, ACTIVE AND PASSIVE SELF-LIGATING BRACKETS WITH DIFFERENT COMBINATIONS OF ARCH WIRES: A FINITE ELEMENTS STUDY

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***ABSTRACT***
*The aim of this study was to compare frictional resistance among conventional, passive and active self-ligating brackets using Finite Elements Analysis (FEA). Seventy-nine (79) slide tests were performed by combining an upper first bicuspid conventional bracket, 0.018" stainless steel wires and 0.010" ligature by means of an INSTRON 3345 load system to obtain average maximum static frictional resistance (MSFR). This value was compared to the FR (frictional resistance) obtained by simulation of a slide of the same combination by FEA following conventional bracket modeling by means of Computer Aided Design (CAD). Once the FEA was validated, bracket CADs were designed (upper right first bicuspid conventional, active and passive self-ligating bracket) and bracket properties calculated. MSFR was compared among conventional, active and passive self-ligating brackets with different alloys and archwire cross sections such as 0.018", 0.019" x 0.025"and 0.020" x 0.020". Passive self-ligating brackets had the lowest MSFR, followed by conventional brackets and active self-ligating brackets. In conventional brackets, a 0.018" archwire produced a linear pattern of stress with maximum concentration at the center. Conversely, stress in 0.020 x 0.020" and 0.019 x 0.025" archwires was distributed across the width of the slot. The highest normal forces were 1.53 N for the 0.018" archwire, 4.85 N for the 0.020 x 0.020" archwire and 8.18 N for the 0.019 x 0.025" archwire. Passive self-ligating brackets presented less frictional resistance than conventional and active self-ligating brackets. Regardless of bracket type, greater contact area between the slot and the archwire and the spring clip increased frictional resistance.*

***Key words:*** *Friction, orthodontic brackets, finite element analysis.*

## COMPARACION DE LA RESISTENCIA FRICCIONAL ENTRE BRACKETS CONVENCIONALES, DE AUTOLIGADO ACTIVO Y PASIVO CON DIFERENTES COMBINACIONES DE ARCOS: UN ESTUDIO DE ELEMENTOS FINITOS

***RESUMEN***
*El objetivo de este estudio fue comparar la resistencia friccional entre brackets convencionales, de autoligado pasivo y activo por medio del método de elementos finitos (MEF). Se realizaron setenta y nueve (79) deslizamientos combinando brackets convencionales de primer bicúspide superior con arcos de acero de 0,018" y ligadura metálica de 0,010" en una máquina INSTRON 3345, obteniendo el promedio de la resistencia estática máxima (REM). Este valor fue comparado con la resistencia friccional obtenida por simulación de un deslizamiento de la misma combinación por medio de MEF previo diseño asistido por computador (CAD) del bracket convencional. Una vez se validó MEF, se realizaron diseños CAD de los brackets (convencional, autoligado activo y pasivo de primer bicúspide superior derecho) y cálculos de sus propiedades. Se realizó una comparación entre brackets convencionales, brackets de autoligado activo y pasivo con diferentes aleaciones y secciones cruzadas de alambre 0.018", 0.019" x 0.025" y 0.020" x 0.020". Los brackets de autoligado pasivo mostraron la menor REM, seguidos de los brackets convencionales y finalmente los brackets de autoligado activo. En los brackets convencionales, el arco de 0,018" produjo un patrón lineal de stress en el fondo de la ranura, con su máxima concentración en el centro. Por el contrario, los arcos de 0.020" x 0.020" y 0.019 x 0.025" tuvieron una distribución de esfuerzos a través del ancho de la ranura. La mayor fuerza normal en los brackets convencionales fue para el arco 0.019"x 0.025" (8.18N), seguido por el arco 0.020 x 0.020" (4.85N) y finalmente el arco 0.018" (1.53N). Los brackets de autoligado pasivo presentaron menos resistencia friccional que los brackets convencionales y autoligado activo respectivamente. Independiente del tipo de bracket, una mayor área de contacto entre la ranura del bracket y el arco, y el spring clip aumentaron la resistencia friccional.*

***Palabras clave:*** *fricción, brackets, análisis de elementos finitos.*

## INTRODUCTION

Frictional force is defined as the tangential force produced at the interface between two bodies, which opposes the sliding of one on the other. Friction in orthodontics occurs between bracket, wire and ligature. The resulting frictional resistance reduces the efficiency of orthodontic treatment due to loss in the total force applied (21% to60%)[1].

Greater force is therefore required to move teeth. However, forces of high magnitude can cause biological damage such as root resorption and pulp necrosis[1].

Archwire size and type of alloy, bracket size and material, the slot, ligating forces, inter-bracket distance, saliva and other factors can have direct or indirect influence on frictional resistance[2,3]. Self-ligating brackets emerged in the mid 1930s, becoming popular in the 1980s[4], and are presented as an alternative to conventional brackets for providing greater efficiency in the orthodontic treatment. Greater efficiency is based on reduced friction between bracket and archwire, shorter dental appointments and increased patient comfort, acceptance and cooperation[3].

Several *in vitro* studies agree that self-ligating brackets generate lower frictional resistance than conventional brackets when used with a small, round arch wires[5-11]. Further differences are reported between active and passive self-ligating brackets[12-14]. However, these studies showed that the reduction in frictional force of self-ligating brackets compared to conventional brackets is limited to certain archwire sizes in a 0.022” slot, after which frictional resistance is similar[3, 5] or greater [10]. The study of frictional force and stress distribution in self-ligating brackets will provide additional evidence for their rational use.

Finite element analysis (FEA) is a general numerical method for approximating solutions from partial differential equations. It has been applied in dental research, facilitating the solution of complex biomechanical interactions. However, its use in orthodontic research is scarce.

The aim of this study was to compare frictional resistance between conventional and self-ligating brackets using different alloys and archwire cross sections by FEA.

## MATERIALS AND METHODS

This study was conducted in three phases: experimental, validation and FEA modeling.

### Experimental phase

We used upper right first bicuspid pre-adjusted conventional stainless steel brackets (0.022” x0.028” - Mini Diamond Ormco, Glendora CA, USA) combined with stainless steel round arch wire 0.018” (Stainless Steel, SDS, Ormco, Glendora CA, USA) and stainless steel ligatures 0.010” (SDS Ormco, Glendora CA, USA).

A tensile and compressive test was performed on the wire and on the bracket using a 3345 INSTRON machine with a 5000N±1N cell load at a speed of 5mm/min to obtain the elastic modulus (Young modulus) of each material. Poisson ratio was set to a constant of 0.3 (Table 1).

An acrylic structure adapted to the machine fixed one upper right first bicuspid bracket with vertical arrangement of the slot (Fig 1). Each arch wire was ligated to the bracket with stainless steel ligatures and then slid along the slot at a speed of 5mm/min for 2minutes with a cell load of 10±0.01N. Maximum static frictional resistance (MSFR) was measured. The experiment was repeated 79 times and average frictional resistance was calculated. Each sliding test was conducted with the same bracket, and different 0.018” stainless steel arch wire segments and 0.010” ligatures.

### Validation of FEA

Upper right first bicuspid pre-adjusted conventional stainless steel brackets, round stainless steel arch

**Table 1: Properties of brackets and wires.**

| Element | Elastic modulus (GPa) |
|---|---|
| Wire | 190 |
| Bracket | 205 |

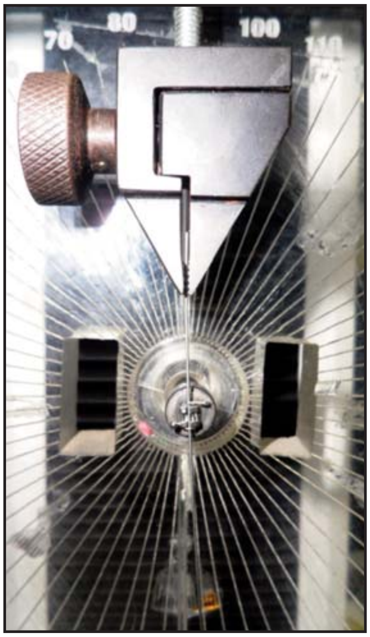


*Fig. 1: An acrylic structure adapted to the INSTRON machine fixed the Upper right first bicuspid bracket, arch wire and ligature combination for the sliding test.*

wires and stainless steel ligatures were measured using a stereoscope(Nikon) with 8X and 80X magnification and a tolerance of ±5 mm, assisted by image analysis software (NIS 3.1 Element, Nikon). A computer-aided design (CAD) model of the bracket was constructed with microscopic measurements using the software Solid Edge 18 (Table 1, Fig. 2). Surface roughness of the slot and the arch wire was measured with a microscope (Eclipse X20 LV100) and average roughness between the observed peaks and valleys on the surface of the materials was calculated.

Frictional resistance was studied by FEA (ALGOR 16) in a simulation that applied the mean MSFR obtained in the experimental phase to the base of the conventional bracket slot with a 0.018” arch wire. The force distribution applied on the surface of the slot of the bracket was calculated as the weighted force on the area of influence (Fig. 3).

The finite element analysis was validated by comparing the frictional resistance from the experimental method and the FEA with 95% reliability by Z test.

**Modeling and FEA simulation**

CAD models of the following upper right first bicuspid brackets were constructed using Solid Edge V18 software, which used 4352, 4521 and 4535 tetragonal elements for (Fig.2 A, B and C): conventional Mini diamond (0.022” x 0.028”), 3MX Damon (0.022” x 0.027”) and In Ovation “R” (0.022” x 0.028”), respectively.

In addition, CAD models of stainless steel, Nickel-Titanium (NiTi), beta-titanium (TMA) and Sentalloy arch wires in 0.018”, 0.019” x 0.025”and 0.020” x 0.020” were constructed.

Surface roughness of the base of the slot was measured using a microscope (Nikon Eclipse100LV), assisted by software (NIS3.1ELEMENT3D reconstruction). Average roughness was 0.03 mm for three different types of brackets and it was incorporated into the CAD model of each bracket.

Sliding simulations using different combinations of brackets and arch wires were performed using the ALGOR16 finite element analysis software. A 3N force was applied to the base of the slot in all simulations. A vertical force represented the ligation force.

The normal force applied in each simulation was calculated by the following equations[15]:

For conventional Mini diamond brackets:

$$FN = 48 \frac{EI}{l^3} \delta$$

where FN is the normal force applied by the ligature to the archwire (and also the base of the slot), i.e. the force produced by the ligature against the slot; $E$ is the modulus of elasticity of the ligature; $I$ is the moment of inertia of the cross section of the ligature; $l$ is the length between the free ends of the ligature, and $\delta$ is the archwire displacement exerted on the ligature.

For In-Ovation R brackets the relationship between force and displacement of the binding was[15]:

$$FN = 3 \frac{EI}{l^3} \delta$$

For 3MX Damon brackets, the normal force was not calculated since there is no metal spring clip or ligature, so that normal force was not taken into account during sliding with this type of bracket.

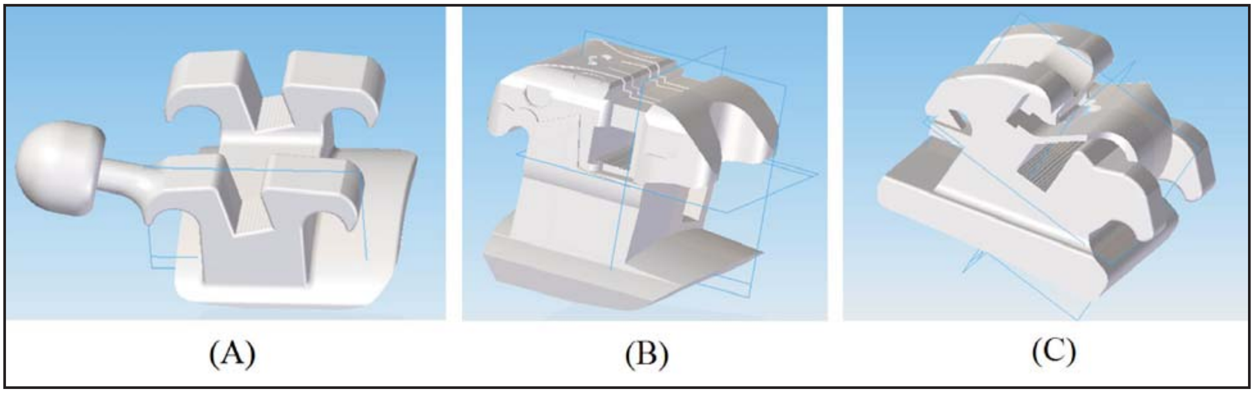


*Fig. 2: Upper right first bicuspid bracket CAD designs. (A) Conventional bracket, (B) Passive self-ligating (C) Active self-ligating.*

To simulate bracket adhesion to the tooth surface, displacement restriction of the bracket base was applied.

Frictional resistance was expressed by stress force ($N/mm^2$) in each simulation by the Von Mises criteria.

## RESULTS

### Experimental phase

The *in vitro* average frictional resistance of the 79 sliding repetitions was 2.41 ± 0.26 N ($p = 0.05$) with95% CI (2.35 - 2.47 N). The results showed high consistency during the experiments.

### Validation of FEA

The highest concentration of frictional force was located in the central area of the bottom of the conventional bracket slot, whereas at the edges of the slot, the forces were almost non-existent (Fig. 3). The weighted sum of the forces according to their area of influence resulted in a force of 2.43 N. This FEA simulation was conducted once, in order to validate the approach with respect to experimental frictional sliding stage.

Comparison of results obtained from the in vitro experiments and the FEA simulation showed that there is no statistical difference ($p = 0.7493 > 0.05$) between the two methods. Hence, the FEA method is validated to study frictional forces in different combinations of brackets and archwires.

### Modeling and FEA simulation

Each combination of brackets and archwires produced characteristic patterns of stress distribution at the bottom of the slot. Fig. 4 shows stress distribution for the three brackets in combination with the three different types of steel archwire. In a conventional bracket, a 0.018” archwire the stress was produced with an arrangement in the same line with a maximum concentration in the center of the line (Fig 4A). Conversely, 0.020 x 0.020“ and 0.019 x 0.025” wires produced stress distribution across the width of the slot. In this case, the contact surfaces are on two planes, and stresses are thus distributed more evenly.

Table 2 shows consolidated maximum stress for each bracket-archwire combination. The DAMON 3 MX bracket was found to produce the least stress in all combinations, followed by the conventional bracket and the In-Ovation R bracket, which produced the greatest stress. With respect to the size of the archwires, least stress occurred with the 0.018” archwires, followed in quantity by the 0.019 x 0.025” archwires, while maximum stress occurred

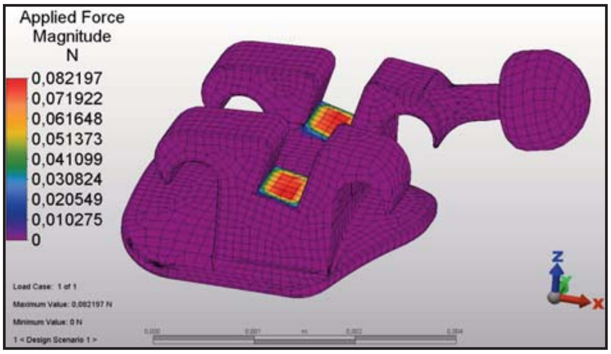


*Fig. 3: Frictional force distribution in conventional bracket slot FEA approach.*

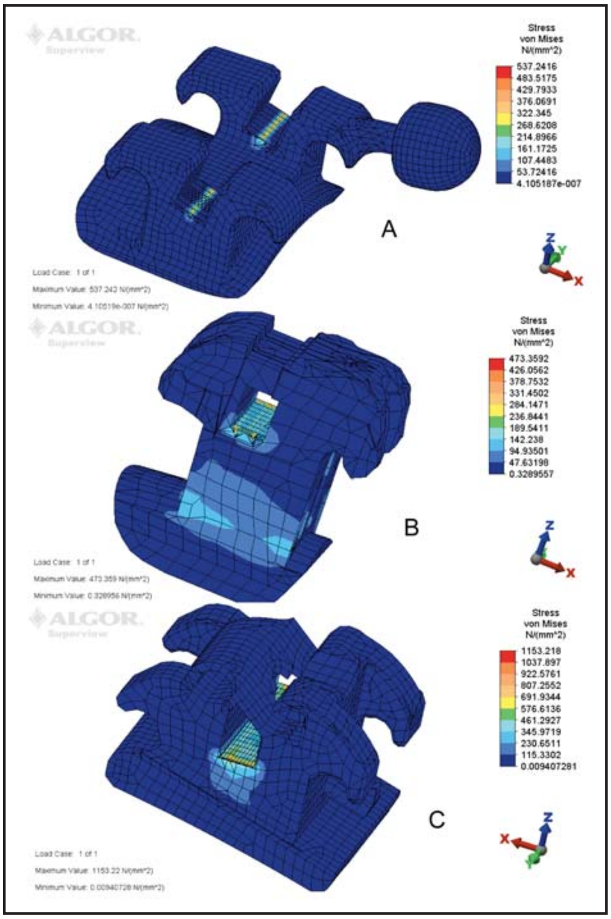


*Fig. 4: Stress distribution for the three types of brackets in combination with the three different types of stainless steel archwire by FEA approach. 4A. Conventional bracket / 0.018” archwire. 4B. Passive self-ligating/ 0.019” x 0.025” archwire. 4C. Active self-ligating/ 0.020” x 0.020” archwire.*

**Table 2: Results in N/mm² of the FEA sliding simulations.**

| Material | Steel | | | NiTi | | TMA | | Sentalloy | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Type of bracket | 0.018” | 0.019 x 0.025” | 0.020” x 0.020” | 0.018” | 0.019 x 0.025” | 0.018” | 0.019 x 0.025 | 0.018” | 0.019 x 0.025” | 0.020” x 0.020” |
| Passive self ligating bracket | 342.6 | 473.36 | 522.5 | 342.6 | 473.36 | 342.6 | 473.36 | 342.6 | 473.36 | 522.5 |
| Conventional 0.022” X 0.028” | 537.24 | 868.22 | 1043.86 | 537.24 | 868.22 | 537.24 | 868.22 | 537.24 | 868.22 | 1043.86 |
| Active self ligating bracket | 986.31 | 1153.2 | 1119.12 | 986.31 | 1153.2 | 986.31 | 1153.2 | 986.31 | 1153.2 | 1119.12 |

with the 0.020” x 0.020” archwires. However, the In-Ovation R bracket with 0020” x 0020” wires showed less stress than with the 0019” x 0025” archwires (Fig.4B, 4C).

Normal forces were only calculated for conventional and In-Ovation R brackets. The highest normal force was 1.53 N for the 0.018” archwire, 4.85 N for the 0.020” x 0.020” archwire and 8.18 N for the 0.019” x 0.025” archwire. For the In-Ovation R bracket,the only combination that activated the spring clip was with 0.019” x 0.025” archwire, in which the normal force was 0.14 N.

## DISCUSSION

Testing frictional resistance in orthodontic systems in *in vitro* studies depends directly on different factors such as the alloy, surface texture, bracket type, size and geometry of the archwire[4,14]. Other factors, including the type of bracket ligating system and the critical angle between the archwire and the slot, have also been discussed[16-18]. However, in these studies it is difficult to control such variables under the same conditions for each experiment. In addition, specific characteristics of the biological environment of the mouth are difficult to replicate[1,3,19,20]. In contrast, finite elements analysis (FEA) allows some of the variables related to frictional resistance (surface, force, angulation, bracket bonding strength) to be controlled more precisely in a simulation model, enabling inferences to be drawn regarding how this phenomenon occurs.

In this study, the use of FEA was validated for the analysis of frictional resistance. The results of the 79 preliminary sliding experiments performed in an INSTRON machine showed that mean maximum static frictional resistance (MSFR) was comparable to the same results in the FEA analysis. FEA was thus used as a valid alternative to study frictional resistance with different types of bracket and archwires.

Kojima et al. conducted a study in 2006 [21]with the aim of assessing the combined effect of friction and stiffness of an archwire applied in the movement of a canine during sliding mechanics. In this study, a 5 mm retraction of canines was simulated by FEA. The authors used conventional brackets and square stainless steel archwires (0.012” to 0.020”), assuming that the archwire and the slot had the same size and there was no clearance between them. They report that there is a reduction in the net force applied to the canine with increasing coefficients of friction. Nonetheless, the simulation showed a distribution of stress, rather than forces, and it is thus difficult to estimate the net force acting on each part of the bracket-archwire system[21]. Other than the fact that the authors did not validate the FEA, the results provide significant insights of the frictional events occuring during orthodontic movement. In this study, the normal force, mechanical properties of the materials and average surface roughness of the archwire and bracket were calculated for stainless steel, because all of the materials have the same composition. Although there is not a friction coefficient, the calculation and use of these properties allowed deductions to be made regarding their effects in each simulation, according to the stress undergone by the surfaces.

The results of this study show that in conventional brackets there is increased frictional resistance in the rectangular archwire compared to the square archwire. It may be attributable to the larger contact area between the bottom of the slot and the 0.020 x

0.020” archwire compared to the 0.019” x 0.025” archwire. This indicates that there is greater normal force on the 0.019 x 0.025”archwire compared to the 0.020” x 0.020”archwire, which means that for simulations with FEA, the bracket-archwire contact surface is more important to MSFR than the normal force itself. The normal force applied by the ligature is a subjective variable that depends on each clinician and is therefore uncontrollable[21]. Clearly, the value of the stress was not affected by the mechanical properties introduced to the model (Young modulus, shear modulus and Poisson modulus), but rather by the contact surface geometry and the applied force. The interpretation of this behavior is that the stress is the intensive form of the applied force.

The passive self-ligating bracket showed the same behavior as conventional brackets with respect to the dimensions of the archwire. Greater bracket-archwire surface contact produced higher MSFR values. Due to the fact that in the simulations with passive self-ligating bracket there was no normal force to press the archwire against the bottom of the bracket, roughness was a constant and the properties of the materials did not influence this behavior. Hence, MSFR was the result of the contact surface between the bottom of the slot and the archwire.

Active self-ligating brackets had the lowest MSFR with the 0.018” archwire; from the stand point of shear stress, frictional force is only a function of the applied force as the same as the roughness and geometry of the slot and wire. In contrast, MSFR produced with the 0.019” x 0.025” archwire was greater than that produced with the 0.020” x 0.020” archwire. This difference was due to the normal force exerted by the spring clip. Spring clips in active self-ligating brackets are activated with archwire sizes≥ 0.025”. Thus, when a 0.020” x 0.020” archwire is placed into the slot, it does not activate the spring clip and consequently there is no normal force present. On the other hand, the 0.019” x 0.025” archwire activates the spring clip and the normal force exerted is similar to that produced by the ligature, resulting in an increase in frictional resistance. From the simulations performed in this study, it can be inferred that the increase in MSFR was determined more by the normal force expressed by the spring clip than by the contact area between the archwire and the slot.

Several *in vitro* studies [5,7,8] are consistent with this study, in which the passive self-ligating brackets generated lower frictional resistance than conventional brackets used with small, round archwires, and even for larger archwires.[18,22,23]

With respect to active self-ligating brackets, this study found that they have a higher frictional resistance than conventional brackets in all combinations, because the active self-ligating bracket has greater contact area than the conventional bracket. Several studies [1,9,10], however, report lower frictional resistance of active self-ligating brackets than conventional brackets, which may be because these studies included other variables such as roughness[24], which was a constant in our study. In this study, the mechanical properties of the materials did not affect frictional resistance.

The result of the FEA simulations, according to several *in vitro* studies[23-28], showed active ligating brackets have higher frictional resistance than passive self-ligating brackets due to the absence of normal force. Nonetheless, studies by Shivapuja and Berger[5] compared the same types of brackets and reported no difference.

Several studies have analyzed how second-order angulation and torque affect frictional resistance. Thorstenson and Kusy[29] found that shear strength at any angle is lower for self-ligating brackets than for conventional brackets. Another study[13], however, noted that when second-order angles were incorporated, active ligating brackets did not reduce frictional resistance when compared to conventional brackets. Unlike these studies, ours did not include angulation or torque as a variables to be analyzed, but future studies may include them, using FEA and evaluate their influence on frictional resistance.

## CONCLUSIONS

According to the FEA, passive self-ligating brackets have less frictional resistance than conventional and active self-ligating brackets. Active self-ligating brackets have the highest frictional resistance, determined by the greater contact area and the spring clip. However, regardless of bracket type, a greater contact area between slot and archwire increases frictional resistance. Passive self-ligating brackets could be used to improve efficiency in initial phases of orthodontic treatment.

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The authors report no conflict of interests.

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## REFERENCES

1. Hain M, Dhopatkar A, Rock P. A comparison of different ligation methods on friction. Am J Orthod Dentofacial Orthop 2006;130:666-670.
2. Gandini P, Orsi L, Bertoncini C, Massironi S, Franchi L. *In vitro* frictional forces generated by three different ligation methods**.** Angle Orthod 2008;78:917-921.
3. Rinchuse DJ, Miles PG. Self-ligating brackets: Present and future. Am J Orthod Dentofacial Orthop 2007;132:216-222.
4. Ehsani S, Mandich MA, El-Bialy TH, Flores-Mir C. Frictional resistance in self-ligating orthodontic brackets and conventionally ligated brackets. Angle Orthod 2009; 79:592-601.
5. Shivapuja PK, Berger J. A comparative study of conventional ligation and self-ligation bracket systems. Am J Orthod Dentofacial Orthop 1994;106:472-480.
6. Cacciafesta V, Sfondrini MF, Ricciardi A, Scribante A, Klersy C, Auricchio F. Evaluation of friction of stainless steel and esthetic self-ligating brackets in various bracket-archwire combinations. Am J Orthod Dentofacial Orthop 2003; 124:395-402.
7. Griffiths HS, Sherriff M, Ireland AJ. Resistance to sliding with 3 types of elastomeric modules. Am J Orthod Dentofacial Orthop 2005;127:670-675.
8. Kim TK, Kim KD, Baek SH. Comparison of friction0al forces during the initial leveling stage in various combinations of self-ligating brackets and archwires with a custom-designed typodont system. Am J Orthod Dentofacial Orthop 2008;133:187-e15-24.
9. Voudouris JC. Interactive edgewise mechanisms: form and function comparison with conventional edgewise brackets. Am J Orthod Dentofacial Orthop 1997;111:119-140.
10. Tecco S, Festa F, Caputi S, Traini T, Di Iorio D, D'Attilio M. Friction of conventional and self-ligating brackets using a 10 bracket model. Angle Orthod 2005;75:1041-1045.
11. Franchi L, Baccetti T, Camporesi M, Barbato E. Forces released during sliding mechanics with passive self-ligating brackets or nonconventional elastomeric ligatures. Am J Orthod Dentofacial Orthop 2008;133:87-90.
12. Kahlon S, Rinchuse D, Robison J, Close JM. *In-vitro* evaluation of frictional resistance with 5 ligation methods and Gianelly-type working wires. Am J Orthod Dentofacial Orthop 2010;138:67-71.
13. Redlich M, Mayer Y, Harari D, Lewinstein I. *In vitro* study of frictional forces during sliding mechanics of ''reduced-friction'' brackets. Am J Orthod Dentofacial Orthop 2003; 124:69-73.
14. Krishnan V. Mechanical properties and surface characteristics of three archwire alloys. Angle Orthod 2004;74: 825-831.
15. Hibbeler R. Mechanics of materials, 4th Ed. Boston USA: Pearson 1998; 607-608.
16. Kusy RP, Whitley JQ. Frictional resistances of metal-lined ceramic brackets vs Conventional stainless steel brackets and development of 3-D friction maps. Angle Orthod 2001; 71: 364-374.
17. Kapur R, Sihna PK, Nanda RS. Comparison of frictional resistance in titanium and stainless steel brackets. Am J Orthod 1999; 116: 271-274.
18. Kusy R.P, Whitley J. Influence of archwire and bracket dimensions on sliding mechanics: derivations and determinations of the critical contact angles for binding. Eur J Orthod 1999;21:199-208.
19. Frank CA, Nikolai RJ. A comparative study of frictional resistance between orthodontic bracket and archwire. Am J Orthod 1980;78:593-609.
20. Scott P, Di Biase A, Sheriff M, Coboume MT. Alignment efficiency of Damon 3 self-ligating and conventional orthodontic bracket systems: A randomized clinical trial. Am J Orthod Dentofacial Orthop 2008;134:470. E1-8.
21. Kojima Y, Fukui H, Miyajima K. The effects of friction and flexural rigidity of the archwire on canine movement in sliding mechanics: A numerical simulation with a 3-dimensional finite element method. Am J Orthod Dentofacial Orthop 2006;130:275.e1-275.e10.
22. Kusy RP, Whitley JQ, de Araújo G. Comparisons of surface roughness and sliding resistances of 6 titanium-based or TMA -type archwires. Am J Orthod Dentofacial Orthop 2004;126:589-603.
23. Sims AP, Waters NE, Birnie DJ. A comparison of the forces required to produce tooth movement ex vivo through three types of preadjusted brackets when subjected to determined tip or torque values. Am J Orthod 1994;21:367-373.
24. Kusy RP, Whitley J Q. Effects of surface roughness on the coefficients of friction in model orthodontic systems. J Biomech 1990;23:913-925.
25. Angolkar PV, Kapila S, Duncanson MG, Nanda RS. Evaluation of friction between ceramic brackets and orthodontic wires of four alloys. Am J Orthod Dentofacial Orthop1990;98:499-506.
26. Drescher D, Bourauel C, Schumacher HA. Frictional forces between bracket and arch wire. Am J Orthod Dentofacial Orthop 1989;96:397-404.
27. Saunders CR, Kusy RP. Surface topography and frictional characteristics of ceramic brackets Am J Orthod Dentofacial Orthop 1994;106:76-87.
28. Oliver C, Daskalogiannakis J, Tompson B. Archwire depth is a significant parameter in the frictional resistance of active and interactive, but not passive, self-ligating brackets. Angle Orthod 2011;81:1036-1044.
29. Thorstenson GA, Kusy RP. Resistance to sliding of self-ligating brackets versus conventional stainless steel twin brackets with second-order angulation in the dry and wet (saliva) states. Am J Orthod Dentofacial Orthop 2001;120: 361-370.


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# IMMUNOLOCALIZATION OF THE TGFB1 SYSTEM IN SUBMANDIBULAR GLAND FIBROSIS AFTER EXPERIMENTAL PERIODONTITIS IN RATS

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***ABSTRACT***

*Saliva is the first barrier to entry of bacteria and viruses into the body. The submandibular glands (SMG) contribute to the maintenance of oral health and regulation of immune/inflammatory responses. Previous studies suggest that transforming growth factor beta 1 (TGFB1) may contribute to salivary gland fibrosis but the expression of the TGFB1 system in the SMG has not been elucidated. Thus, the aim of this study was to analyze in rat SMG the immunolocalization of TGFB1 and its specific receptors ALK5 (pro-fibrotic) and ALK1 (pro-proliferative) and the co-receptor endoglin (EDG) in a bilateral experimental periodontitis (EP) model (cotton thread ligature around the neck of the first lower molars) for 1 and 6 weeks. Fixed SMG were embedded in paraffin and serially cut for routine hematoxylin–eosin staining for histological analysis or immunohistochemical techniques by diaminobenzidine detection. SMG histology from animals with EP showed time-dependent structural changes involving marked reduction in the height of the contoured ducts, cell destruction, loss of secretory granules, periductal congestion and excess connective tissue surrounding these ducts indicative of a fibrotic process, compared to control SMG. TGFB1, ALK5 and ALK1 receptors and the co-receptor EDG were mainly immunolocalized in ductal cells and in the fibrotic areas in EP groups. The expression of the pro-fibrotic ALK5 receptor was increased in areas of fibrosis in SMG of animals with EP. In SMG of rats with EP, the localization of the TGFB1 specific receptors in the ducts and cells from fibrotic areas, due to the expression of TGFB1 in the surrounding areas, might indicate paracrine and autocrine actions exerted by TGFB1 via its specific receptors. The results of this study suggest that TGFB1 promotes fibrosis, inducing cell proliferation via ALK1 and EDG receptors and stimulates fibrosis related-processes via ALK5 receptor, which could lead to abnormal secretor activity of the SMG during periodontal disease.*

***Key words:*** *Submandibular gland, Transforming Growth Factor beta1, Activin Receptors, Fibrosis, Periodontitis.*

## INMUNOLOCALIZACIÓN DEL SISTEMA TGFB1 EN FIBROSIS DE LA GLÁNDULA SUBMANDIBULAR BAJO PERIODONTITIS EXPERIMENTAL EN RATAS

***RESUMEN***

*La saliva es la primera barrera para la entrada de bacterias y virus en el cuerpo. Las glándulas submandibulares (GSM) contribuyen al mantenimiento de la salud oral y a la regulación de las respuestas inmuno-inflamatorias. Estudios previos sugieren que el factor de crecimiento transformante beta 1 (TGFB1) puede contribuir a la fibrosis de las glándulas salivales, pero la expresión y localización del sistema TGFB1 en las GSM no ha sido dilucidada. El objetivo del presente trabajo fue analizar por inmuno-histoquímica en las GSM de ratas la expresión de TGFB1 y sus receptores específicos ALK5 (pro-fibrótico) y ALK1 (pro-proliferativo) y el co-receptor endoglina (EDG) en un modelo de periodontitis bilateral experimental (PE) (hilo de algodón alrededor del cuello de los primeros molares inferiores) durante 1 y 6 semanas. Las GSM fueron fijadas y embebidas en parafina para realizar cortes seriados los cuales se tiñeron con hematoxilina-eosina para analizar la histología o se procesaron para realizar la técnica de inmunohistoquímica mediante detección con diaminobenzidine. La histología de las GSM de animales con PE reveló cambios estructurales tiempo dependientes, con una marcada reducción de la altura de los conductos, destrucción celular, pérdida de gránulos secretores, congestión periductal y exceso de tejido conectivo que rodea los conductos, indicando un proceso de fibrosis respecto de las GSM de animales control. TGFB1, ALK5 y ALK1 y el co-receptor EDG fueron principalmente inmunolocalizados en las células que forman los ductos y en las áreas de fibrosis en los grupos con PE. La expresión del receptor pro-fibrótico ALK5 se incrementó en las áreas de fibrosis en GSM de animales con PE. En GSM de ratas con PE, la localización de los receptores específicos de TGFB1 en las células de los conductos y áreas de fibrosis, junto con la expresión de TGFB1 en las áreas circundantes, podría indicar acciones paracrinas y autocrinas ejercidas por TGFB1 a través de sus receptores específicos. Los resultados de este estudio sugieren que TGFB1 podría inducir un proceso de fibrosis promoviendo la proliferación celular a través de los receptores ALK1 y EDG, y favoreciendo procesos relacionados con la fibrosis a través de su receptor ALK5, lo que conduciría a una actividad secretora anormal de la GSM durante la enfermedad periodontal.*

***Palabras clave:*** *Glándula submandibular, factor de crecimiento transformante beta1, receptores de activina, fibrosis, periodontitis.*

## INTRODUCTION

Periodontal diseases have been well described and characterized according to different degrees of inflammation, eventually leading to the destruction of teeth-supporting tissue. Periodontitis is characterized by chronic activation of the immune response and synthesis of several cytokines and proteases. Cytokines induce alterations in the cellular metabolism of the connective tissue concomitant with the destruction of the periodontal tissue and tooth loss[1].

The pair of submandibular glands (SMG) is involved in the maintenance of the oral health and regulation of immune/inflammatory responses[2]. The SMG has a ductal structure that opens into the oral cavity with secretory end pieces, the mucous and serous acini, producing saliva[3]. A time-dependent link has been observed between experimental periodontitis (EP) and SMG activity, mediated by systemic and neural mechanisms. Initially, the EP stimulates glandular secretion, while the progression of the disease affects the normal function of the gland, leading to histological and biochemical changes, including an inflammatory response and reduced secretion[4,5].

There is growing evidence showing the relation between cytokines and the pathological fibrotic process of different tissues, characterized by the development of excess fibrous connective tissue, as a consequence of a tissue repair process that triggers an increase in the production and deposition of extracellular matrix. Among these cytokines, the transforming growth factor beta 1 (TGFB1) plays an important role. The TGFB1 inhibitory and stimulatory properties have been described in the regulation of cellular homeostasis in both physiological and pathological stages[6-9]. The effects of TGFB1 are exerted via specific type I and type II serine/threonine kinase receptors. Type II receptor (TGFBRII) transphosphorylates and activates type I receptor (TGFBRI). Two TGFBRI have been described: activin receptor-like kinase 1 (ALK1), which signals via phosphorylation of Smads 1/5, and activin receptor-like kinase 5 (ALK5), which signals via phosphorylation of Smads 2/3[10]. In line with this, endoglin (EDG) appears to be a key co-receptor that contributes to an efficient transduction pathway of ALK-1[11]. In this context, it has been observed that deregulation mechanisms of TGFB1 appear to be involved in the progression of diseases such as cancer and autoimmune fibrosis[6]. However, the expression of the TGFB1 system in the SMG affected by periodontal disease is unknown. The aim of this study was to analyze the localization of TGFB1 and its specific receptors ALK5 and ALK1 and the co-receptor EDG in rat SMG after 1 and 6 weeks of EP induction.

## MATERIALS AND METHODS

### Animals

Adult male Wistar rats from our own colony with initial body weight 220-250 g were randomly divided into 4 groups (n=8 animals per treatment): (1) 1- week control, (2) 1- week bilateral EP, (3) 6-week control, (4) 6- week bilateral EP. They were kept in group cages in an animal room with a photoperiod of 12 h light (07.00–09.00 h), room temperature 22–25° C, humidity: 52–56% and fed standard Purina chow pellets and tap water *ad libitum*. All experiments were performed following the National Institute of Health guidelines for the care and use of laboratory animals (NIH 85-23, revised in 1985) and protocols were approved by the Ethical Commission of the School of Dentistry, University of Buenos Aires.

### Induction of experimental periodontitis

EP was performed as previously reported[5]. EP was induced under general anesthesia with a mixture of 2% xylazine hydrochloride (5 mg/kg; i.p.) and 5% ketamine hydrochloride (50 mg/kg; i.p.). A cotton thread ligature was placed around the neck of both first lower molars (bilateral EP) and served as a retention device for oral microorganisms. The ligature was pushed into the gingival sulcus and left in place until sacrifice (1- and 6 weeks post surgery). In the 6 week EP treatment, the thread was renewed every 2 weeks in order to prevent the ligature from coming out of the sulcus, thereby not meeting the desired objective. The SMG and submandibular lymph nodes were removed and weighed (mg), after which they were fixed in 4% PFA for histological and immunohistochemical studies.

### SMG histology and immunohistochemistry

The tissues mounted in paraffin were cut at 5-µm sections, dewaxed in xylene and rehydrated in graded alcohols. For each specimen, at least 3 to 5 slides were stained with hematoxylin/eosin for general histological inspection. Two specialists reviewed all the SMG samples. For immunohistochemical analysis, endogenous peroxidase activity was inhibited in tissue sections using 0.5% v/v $H_2O_2$/methanol. Sections were blocked for 1 h with 15% normal goat serum in phosphate-buffered saline (PBS) and then incubated overnight at 4°C with primary antibody (1:100 diluted rabbit anti-TGFB1, sc-146; 1:100 diluted rabbit anti TGFB RI [V-22], sc-402; 1:100 diluted rabbit anti TGFB RI [T-19], sc-398; 1:100 diluted rabbit anti-endoglin, sc-20632, Santa Cruz Biotechnology, Inc., USA). After 3 rinses in PBS, sections were incubated for 1h at room temperature with the appropriate 1:200 diluted biotinylated secondary antibody (Vector Labs, UK). After further washing in PBS, sections were incubated with 1:100 diluted streptavidin–peroxidase complexes (ABC kit, Vector Labs, UK). Development of peroxidase activity was achieved with 0.05% w/v 3,3-diaminobenzidine and 0.1% v/v $H_2O_2$ in Tris–HCl. Negative controls were processed simultaneously by omitting the primary antibody or pre-absorbing the primary antibody with specific synthetic peptides.

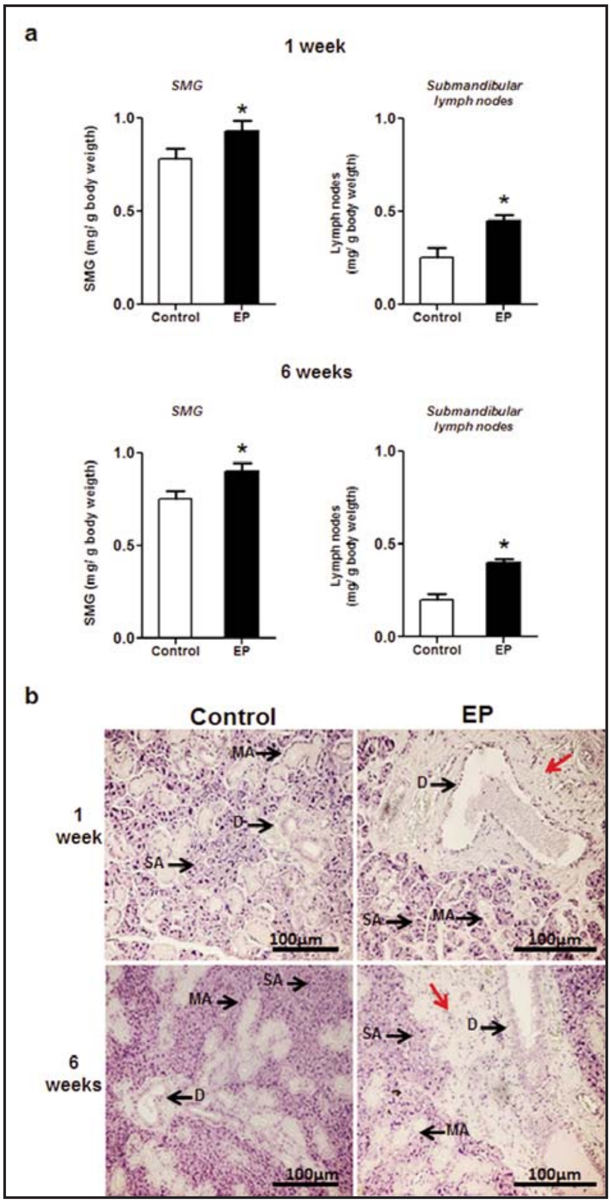


*Fig. 1: Weight of SMG and submandibular lymph nodes (A) and general histology of SMG (B) in animals with 1- and 6-week EP. (B) Note the SMG of periodontitis groups with fibrotic areas surrounding ducts (red arrows). Data are plotted as mean ± SEM (n=6). *p<0.05 different from control group. EP: experimental periodontitis; D: duct; MA: mucous acini; SA: serous acini.*

### Statistical Analysis

Mean and standard error (SEM) were calculated and the InfoStat Software (version 2011, developed by Statistics Department, National University of Córdoba) was used for calculating differences between 2 groups using Student´s t test. A *p*-value of less than 0.05 was considered significant.

## RESULTS

### Histology of the SMG in rats with bilateral EP

The weight of the SMG and submandibular lymph nodes in the EP groups was significantly higher than control SMG in both 1- and 6- week treatments ($p<0.05$) (Fig. 1A). The histology of the SMG of animals with 1- and 6- week EP showed alterations of the epithelial architecture and ducts, partial loss of material granular secretion and periductal edema, as well as an excess in the connective tissue surrounding the ducts, indicative of a fibrotic process compared to the control SMG (Fig. 1B).

### Detection of TGFB1, ALK5, ALK1 and co- receptor EDG in SMG from rats with EP

TGFB1 and its specific receptors ALK1 and AKL5 and co-receptor EDG were immunolocalyzed in the cell cytoplasm of the ducts of control SMG and SMG from animals with 1- and 6 week EP (Fig. 2). TGFB1, ALK1 and EDG were localized in the apical zone and nearest to the lumen while ALK5 showed diffuse localization within cell cytoplasm (Fig. 2).

The expression of TGFB1 and its specific receptors was also predominantly detected in the cytoplasm of cells from the fibrosis areas surrounding the ducts in the affected SMG from animals with 1- and 6- week EP (Fig. 2). TGFB1 was also detected in mucous acinus (MA) and predominantly observed only in SMG of EP groups. Concerning the expression of ALK5, most of the cells from fibrosis areas were immunopositive for this receptor in animals with 6- week EP while the localization of ALK5 was detected in cell clusters in animals with 1-week EP (Fig. 2). Serous acinus (SA) showed no immunostaining for ALK5 and only a few immunopositive MA were observed in SMG from all treatment groups. The expression of ALK1 and its co-receptor EDG was mainly observed in cells of fibrosis areas with intense immunostaining in the affected SMG from animals with 1- and 6-week EP (Fig. 2). Some immunopositive MA for ALK1 and EDG was observed in the SMG from EP treatment groups.

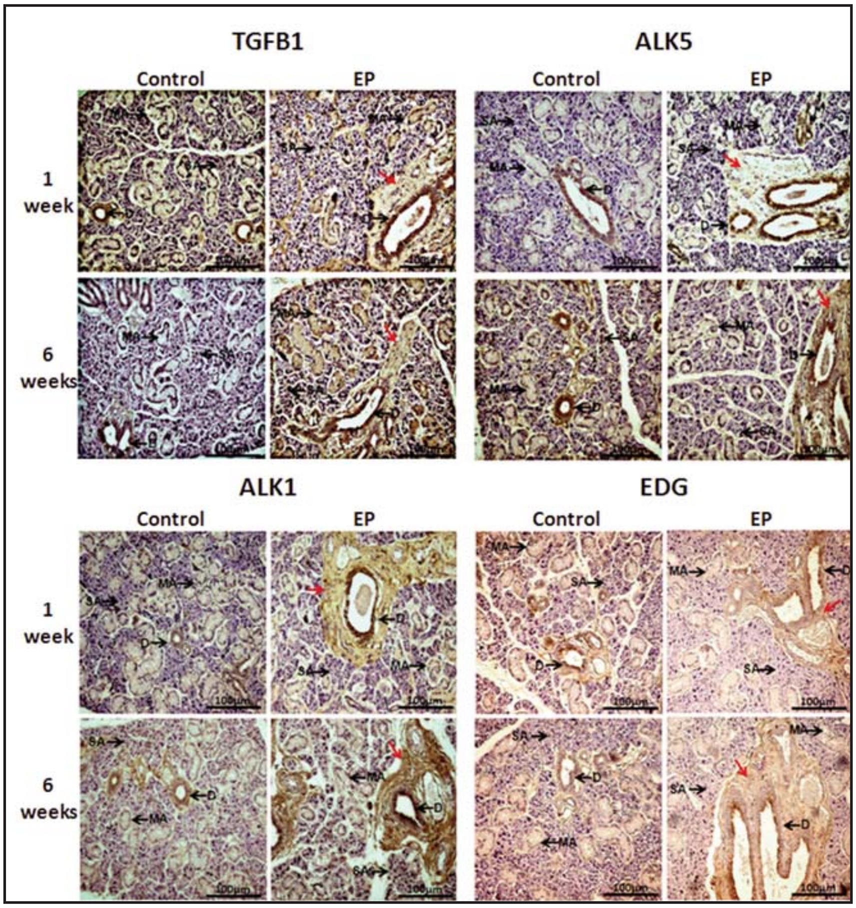


*Fig. 2: Immunolocalization of the TGFB1 system in SMG from animals with 1- and 6-week EP. Note that fibrotic areas (red arrows) of the SMG of periodontitis groups are intensively immunostaned with TGFB1. These areas were also immunopositive for ALK5 and ALK1 receptor and co-receptor EDG, particularly, in 6-week EP. EP: experimental periodontitis; D: duct; MA: mucous acini; SA: serous acini; EDG: endoglin.*

## DISCUSSION

TGFB1, a pro-fibrotic cytokine, participates in diverse biological processes including inflammation, fibrosis, tissue regeneration and epithelial-mesenchymal transition[6,12-15]In this study, we followed the expression of TGFB1 and its specific receptors ALK5 and ALK1 and the co-receptor EDG in normal SMG and in SMG after 1 and 6 weeks of induction of EP in adult rats. To our knowledge, this is the first report of a global analysis of the expression of the TGFB1 system in SMG in EP conditions.

Normal SMG secretor activity is essential because saliva is the first barrier to entry of bacteria into the body, so changes in secretion are important in the onset and progression of oral infectious processes[16].

Using a 1- and 6- week EP model, we observed histological changes in the SMG, mainly including the appearance of large fibrotic areas that could affect the normal secretor function of the gland. We detected alterations of the epithelial architecture as well as of the ducts, partial loss of material granular secretion and excess in the connective tissue surrounding the ducts indicative of a fibrotic process in SMG after induction of EP. Moreover, SMG and lymph nodes weighed more in animals with EP than in control animals regardless of the duration of the treatment. This may be explained by the fact that EP produced severe structural changes involving a marked reduction in the height of the granular convoluted ducts, cell destruction, loss of secretory granules, increased fibrosis and periductal congestion.

Under these experimental treatments, there is an excess of immunopositive cells for TGFB1 in the connective tissue surrounding the ducts, indicative of a fibrotic process. In line with this, Hall *et al.*[17] have reported an increase in TGFB1 expression in salivary glands leading to hypofunction of the tissue due to the replacement of normal glandular parenchyma with interstitial fibrous tissue. We recently reported altered function of the SMG, including partial loss of secretor granular material with reduced salivary secretion and periductal oedema in EP treatments[5].

Concerning the type of the TGFB1 receptor, it has been shown that the presence of the co-receptor EDG promotes TGFB1 signaling via ALK1 receptor leading to cell proliferation and attenuates the ALK5 signaling that leads to cell cycle arrest [9,18]. There is also growing evidence indicating that in pathological conditions, TGFB1 stimulates fibrosis through deposition of extracellular matrix and epithelial-mesenchymal transition via ALK5 receptor and activation of Smad2/3 [19-21]. In the present study, the expression pattern of ALK1 and its co-receptor EDG was similar and remained constant in the fibrotic areas of SMG in all EP treatments. This could indicate that TGFB1 induces a proliferative response in the connective tissue surrounding the ducts, stimulating the fibrotic process. In line with this, the quantity of cells in fibrotic areas expressing ALK5 increased in SMG concomitant with the days of the EP treatment, which may reflect a fibrotic process that becomes more aggressive with the duration of the periodontitis.

In conclusion, to our knowledge, this is the first report of the expression and localization of TGFB1 and its receptors ALK5 and ALK1 and co-receptor EDG in the SMG. We postulate that TGFB1 would be a key factor in SMG during periodontitis conditions, probably stimulating tissue damage, fibrosis and morphological alterations associated to abnormal secretor activity. The SMG of animals with EP appears to be an interesting model for studying diverse mechanisms that promote gland fibrosis exerted by TGFB1. Further insights into TGFB1 signaling in SMG could identify novel therapeutic targets for salivary gland fibrosis, whether or not associated to periodontal disease.

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## REFERENCES

1. Niculescu EA, Crăiţoiu MM, Baniţă MI, Drăghici E, Crăiţoiu Ş. The involvement of TGF-β1 and CTGF in regional gingival overgrowth. Rom J Morphol Embryol 2012; 53:143-150.
2. Sabadini E, Berczi I. The submandibular gland: a key organ in the neuro-immuno-regulattory network? Neuroimmunomodulation 1995; 2:184-202.
3. Holmberg KV, Hoffman MP. Anatomy, biogenesis and regeneration of salivary glands. Monogr Oral Sci 2014; 24:1-13.
4. Vacas MI, Amer M, Chiarenza AP, Luchelli MA, Mandalunis PM, Elverdin, JC. Influence of submandibulectomy on alveolar bone loss in rats. J Periodontol 2008; 79:1075-1080.
5. Amer M, Elverdin JC, Fernández-Solari J, Medina V, Chiarenza AP, Vacas MI. Reduced methacholine-induced submandibular salivary secretion in rats with experimental periodontitis. Arch Oral Biol 2011; 56:421-427.
6. Ingman WV, Robertson SA. Defining the actions of transforming growth factor beta in reproduction. Bioessays 2002; 24:904-914.
7. Ingman WV, Robertson SA. The essential roles of TGFB1 in reproduction. Cytokine Growth Factor Rev 2009; 20:233-239.
8. Gonzalez CR, Matzkin ME, Frungieri MB, Terradas C, Ponzio R, Puigdomenech E, Levalle O, Calandra RS et al. Expression of the TGF-beta1 system in human testicular pathologies. Reprod Biol Endocrinol 2010; 8:148-159.
9. Gonzalez CR, Vallcaneras SS, Calandra RS, Gonzalez-Calvar SI. Involvement of KLF14 and egr-1 in the TGF-beta1 action on Leydig cell proliferation. Cytokine 2013; 61:670-675.
10. Feng XH, Derynck R. Specificity and versatility in TGF-beta signaling through Smads. Annu Rev Cell Dev Biol 2005; 21:659-693.
11. Lebrin F, Goumans MJ, Jonker L, Carvalho RL, Valdimarsdottir G, Thorikay M, Mummery C, Arthur HM,et al. . Endoglin promotes endothelial cell proliferation and TGF-beta/ALK1 signal transduction. EMBO J 2004; 23:4018-4028.
12. Thiery JP. Epithelial-mesenchymal transitions in tumour progression. Nat Rev Cancer 2002; 2:442-454.
13. Kalluri R, Neilson EG. Epithelial-mesenchymal transition and its implications for fibrosis. J Clin Invest 2003; 112: 1776-1784.
14. Xu J, Lamouille S, Derynck R. TGF-β-induced epithelial to mesenchymal transition. Cell Res 2009; 19:156-172.

15. Woods LT, Camden JM, El-Sayed FG, Khalafalla MG, Petris MJ, Erb L, Weisman GA. Increased Expression of TGF-β Signaling Components in a Mouse Model of Fibrosis Induced by Submandibular Gland Duct Ligation. PLoS One 2015; 10:1-24.
16. Dodds MW, Johnson DA, Yeh CK. Health benefits of saliva: a review. J Dent 2005; 33: 223-233.
17. Hall BE, Zheng C, Swaim WD, Cho A, Nagineni CN, Eckhaus MA, Kathleen CF, Ambudkar IS, et al.Conditional overexpression of TGF-β1 disrupts mouse salivary gland development and function. Lab Invest 2010; 9:543-555.
18. Lebrin F, Mummery CL. Endoglin-mediated vascular remodeling: mechanisms underlying hereditary hemorrhagic telangiectasia. Trends Cardiovasc Med 2008; 18:25-32.
19. Kemaladewi DU, Pasteuning S, van der Meulen JW, van Heiningen SH, van Ommen GJ, Ten Dijke P, Aartsma-Rus A, ‘t Hoen PA et al. Targeting TGF-β Signaling by Antisense Oligonucleotide-mediated Knockdown of TGF-β Type I Receptor. Mol Ther Nucleic Acids 2014; 3:e156.
20. Park JH, Ryu SH, Choi EK, Ahn SD, Park E, Choi KC, Lee SW SKI2162, an inhibitor of the TGF-β type I receptor (ALK5), inhibits radiation-inducedfibrosis in mice. Oncotarget 2015; 6:4171-4179.
21. Smoktunowicz N, Alexander RE, Franklin L, Williams AE, Holman B, Mercer PF, Jarai G, Scotton CJ, et al. The anti-fibrotic effect of TGFβ/ALK5 inhibition in experimental pulmonary fibrosis is attenuated in the presence of concurrent γherpesvirus infection. Dis Model Mech 2015; 9:1129-1139.

# CHARACTERIZATION OF THIRD MOLAR MORPHOMETRIC VARIABLES

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***ABSTRACT***

*The third molar is a tooth of anatomical, surgical, prosthetic and forensic dental interest. However, there is currently a lack of updated data regarding its morphology. The aim of this study was to determine the morphometric features of third molars and their predictive capability as regards dental arch and side. Two calibrated operators (ƙ = 0.83) determined the cervical-occlusal-vestibular (COV), cervical-occlusal-palatal (COP) and occlusal-apical (OA) distances, mesio-distal (MD), and vestibular-palatal (VP) diameters, number of roots (R) and number of cusps (C) of 961 cadaveric third molars, both upper (n = 462) and lower (n = 499), using a CONCOR 0-50 thin mandible caliper (resolution 0.01 mm). Median and range for each variable were calculated and compared using Mann Whitney non-parametric test ($p < 0.05$). Multivariate cluster analysis was used to determine the predictive capability of each variable for dental arch and side. For upper molars (UM), 50.6% were from the right side (RS) and 49.4% from the left side (LS), while for lower molars (LM), 60.9% were from the RS and 39.1% from the LS. No significant difference was found in the study variables in LM according to side. For UM, MD diameter (10.90 mm), COP(7.42 mm) distance and number of R (3) were significantly higher ($p < 0.05$) forRS, and number of C (3) was higher ($p < 0.0001$) for LS. They were also significant predictive grouping factors for side. For dental arch, OA (17.84 mm) and COV (7.60 mm) distances, MD (11.26 mm) diameter and the number of C (5) were significantly higher ($p < 0.0001$) for LM, while VP (10.84 mm) and COP (7.34 mm) distances, and the number of R (3) were significantly higher ($p < 0.0001$) for UM. These variables were significant predictive factors for dental arch. Despite the morphometric heterogeneity of third molars, there are intrinsic parameters with predictive capability for dental arch and side, but it would be advisable to supplement this study with data from topographic occlusal variables in order to validate their predictive capability.*

**Key words:** *Tooth, morphology, third molar.*

# CARACTERIZACIÓN DE VARIABLES MORFOMÉTRICAS DEL TERCER MOLAR

***RESUMEN***

*El tercer molar es una pieza dentaria de interés odontológico anatómico, quirúrgico, endodóntico, protético y forense. Sin embargo, no hay disponibles hoy en día datos morfométricos actualizados del molar. El objetivo del trabajo fue determinar las características morfométricas de terceros molares y establecer el carácter predictivo de las mismas en cuanto a arco dental y lado. Dos operadores calibrados (ƙ = 0.83) determinaron la longitud oclusocervical vestibular (OCV) y palatina (OCP), oclusoapical (OA), diámetro mesiodistal (MD), diámetro vestíbulopalatino (VP), número de raíces (R) y número de cúspides (C), de 961 terceros molares cadavéricos, superiores (n = 462) e inferiores (n = 499), mediante el uso de un calibre de mandíbula fina CONCOR 0-50 (resolución 0.01 mm). Calculamos mediana y rango para cada variable y las comparamos haciendo uso de la prueba de Mann Whitney ($p < 0.05$). Utilizamos el análisis de cluster para determinar el valor predictivo de cada variable en cuanto a arco y lado. De los molares superiores (MS), 50.6% correspondió al lado derecho (LD) y 49.4% al lado izquierdo (LI). De los inferiores (MI), 60.9% correspondió al LD y 39.1% al LI. No hallamos diferencias significativas para las variables en estudio para los MI según su lado. Para los MS, el MD (10.90 mm), la OCP (7.42 mm) y el R (3) resultaron significativamente mayores ($p < 0.05$) para el LD, y el C (3), mayor ($p < 0.0001$) para el LI; y, además evidenciaron significancia como factores predictivos de agrupamiento para la predicción del lado. En relación al arco, la OA (17.84 mm), la OCV (7.60 mm), el MD (11.26 mm) y el C (5), resultaron significativamente mayores ($p < 0.0001$) en los MI, mientras que el VP (10.84 mm), la OCP (7.34 mm) y el R (3) fueron significativamente mayores ($p < 0.0001$) en MS. Dichas variables evidenciaron significancia como factores predictivos para el arco. Pese a la heterogeneidad morfométrica del tercer molar, existen parámetros característicos con valor predictivo para el arco y lado, aunque sería recomendable complementar el estudio con variables topográficas oclusales a fin de validar la capacidad predictiva de los mismos.*

**Palabras clave:** *Diente, morfología, tercer molar.*

## INTRODUCTION

The different types of teeth perform different functions, such as mastication, speech, esthetics and preservation (maintenance of the integrity of periodontal tissues). Teeth are important in preserving the balance and harmony of the dental arch and the dental and masticatory systems[1]. The third molar fulfills all the abovementioned functions; however, it has specific features. Its calcification begins at 9 years of age, and it usually erupts between 18 and 25 years of age. After its eruption, the calcification and conformation of the third molar is completed over a period of 1 to 1.5 years[2]. Given the omnivorous diet of humans, like the rest of the molars, it performs cutting, friction and grinding functions during mastication[3]. The third molar shows more morphological variations than any other tooth[4-6]. The occlusal surface is rough due to the presence of short, shallow grooves; roots are usually short, fused and curved distally at the apical third[7].

Ontogenic and phylogenetic studies suggest that, in modern man, the molar series is decreasing in size distally as a consequence of a reduction in the masticatory function, with a decrease in the number and size of teeth, number of molar cusps and size of jaws, over the ages[8]. However, the opposite is observed in monkeys and primitive races, the consequence of which is that the third molar is smaller than the first and second molars and is usually retained or absent[9].

In dentistry, not only is the third molar a tooth of anatomical interest, but also of surgical, endodontic, prosthetic and forensic interest. The morphometric study of this particular tooth is of vital importance for said specialties. The study of the retained third molar, indication for its removal and potential complications associated with it require precise morphometric assessment of the tooth in question[10]. This is emphasized by the fact that some morphometric characteristics of the lower third molar have been associated with a higher incidence of mandibular angle and condylar fractures[11]. The morphometric characteristics of this tooth have also been recently included as a measure of interest in predictive models regarding the duration of third molar surgery[12]. Moreover, the morphometric study of this particular tooth is of interest in the determination and validation of parameters concerning the measurements and architecture of the dental arches[13]. In the field of forensic dentistry, the morphometric study of the third molar is vital in dental recognition of fossil remains[14]. From the prosthetic point of view, the need for preservation of this tooth is not infrequent, prior to which the relevant endodontic therapy should be performed, which requires accurate knowledge of the tooth and root morphology[15].

Despite the importance of studying dental morphometry and topography[14, 16], there is no consistent update of morphometric data of the third molar. The available national literature only refers to data published almost four decades ago[17]. The advent of new techniques for morphometric and topographical study of teeth[7,16]provides favorable prospects for updating such data. The incorporation of mathematical tools for creating computational models predictive of the occlusal morphology and topography of teeth and the jaw bone constitutes a broad field where updated morphometric data for the third molar are to be specifically applied[18]. The pressing need for updating the morphometric data of the third molar and its subsequent statistical analysis is the main reason for undertaking this research, and the aim of this study was to identify the morphometric characteristics of the upper and lower third molar and their predictive capability as regards dental arch and side.

## MATERIALS AND METHODS

This research was conducted as a descriptive study. Nevertheless, analytic comparison and predictive capability of the observed dental parameters have been included herein. The study population consisted of cadaveric teeth, including bilateral upper and lower third molars from the ossuary of the Department of Anatomy of the School of Dentistry, University of Buenos Aires, Argentina. The molars were prepared for handling according to the requirements set by the Biosafety Commission of the School of Dentistry.

Nine hundred and sixty-one third molars (462 upper and 499 lower) were studied. Molars with abnormal crown or roots, or any that were partially destroyed or filled were not included in the study population. The morphometric determinations comprised the occlusal-apical (OA), cervical-occlusal-vestibular (COV) and cervical-occlusal-palatal (COP) lengths (Fig. 1), and mesio-distal (MD) and vestibular-palatal (VP) diameters (Fig. 2). Measurement data corresponding to the different tooth morphometric parameters were determined in mm by means of a CONCOR 0-50 mm thin mandible digital caliper (resolution: 0.01 mm) and a TESA 25/50 (0-25 mm) digital micrometer. The measurement data corresponding to the number of roots (R) and cusps (C), of the occlusal surface of the third molars were determined macroscopically by dental recognition.

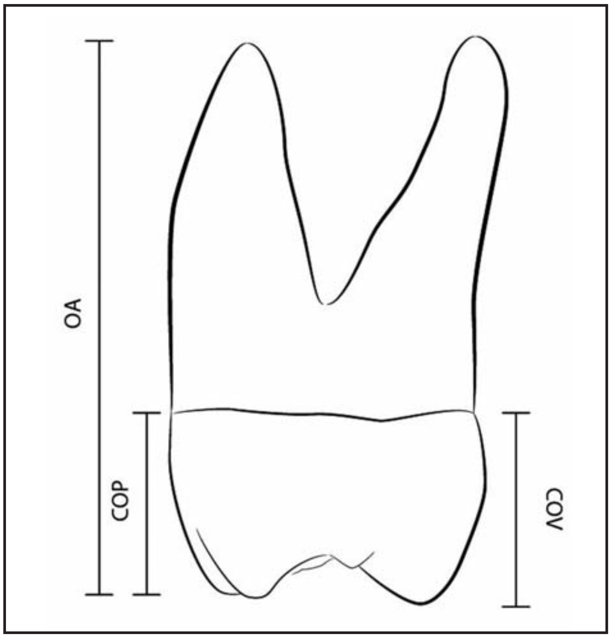


*Fig. 1: Proximal view of a generic third molar. The drawing illustrates the occlusal-apical (OA), cervical-occlusal-vestibular (COV) and cervical-occlusal-palatal (COP) lengths.*

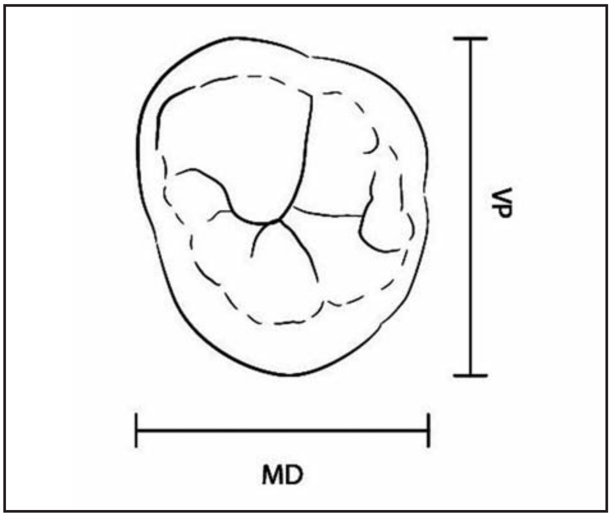


*Fig. 2: Occlusal view of a generic third molar.The drawing illustrates the mesio-distal (MD) and vestibular-palatal (VP) diameters.*

Data were collected by two calibrated operators (Cohen's kappa coefficient of 0.83).

The descriptive statistical analysis included the calculation of median and range for each studied variable. Differences in the morphometric parameters were tested for significance by Mann Whitney test using GRAPHPAD Prism version 5.03 for Windows (GraphPadSoftware, San Diego, CA, USA). Multivariate cluster analysis of the abundance levels of dental measurements was used to determine the hierarchical dissimilarity and predictive capability of each variable as for dental arch and side. The level of significance used was $p < 0.05$.

**RESULTS**

For upper molars, 50.6% were from the right side and 49.4% from the left side, while for lower molars, 60.9% were from the right side and 39.1% from the left side.

The boxplot in Fig. 3 shows that the upper third molars have a statistically significantly higher number of cusps (Mann Whitney U = 22230, p = 0.0003) on the left side (median, range: 3, 3-6) than on the right side (3, 3-5), whereas the number of roots was statistically significantly higher (Mann Whitney U = 24650, p = 0.028) in molars on the right side (3, 1-5) than on the left (3, 1-4).The OA length (Fig. 4 A) and the VP and COV distances did not differ significantly between sides (Fig. 4 B);

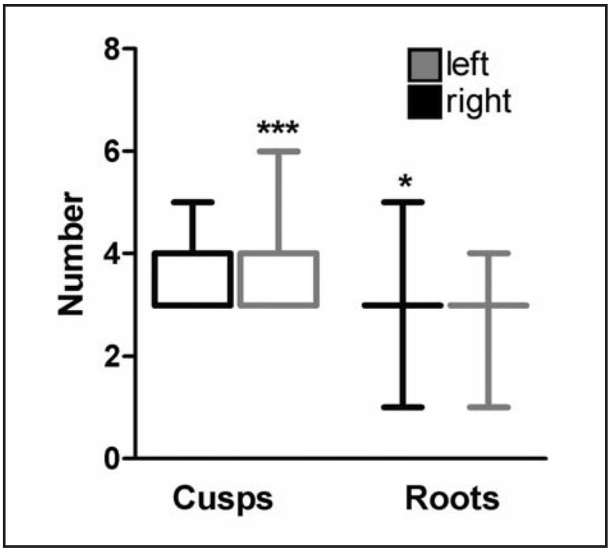


*Fig. 3: Distribution of the number of cusps and roots of upper third molars according to side. We determined the number of cusps and roots of right side (n=234) and left side (n = 228) molars. *p < 0.05; ***p < 0.0001.*

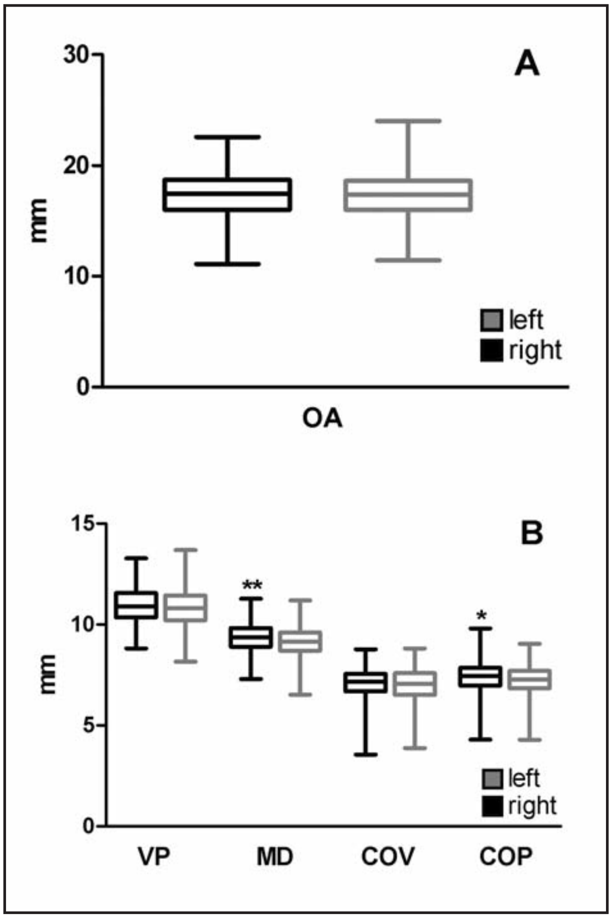


*Fig. 4: Distribution of the measurements of upper third molars according to side. A. Total length. We determined the occlusal-apical (OA) length;B. Coronal measurements.We determined the vestibular-palatal (VP), mesio-distal (MD), cervical-occlusal-vestibular (COV), and cervical-occlusal-palatal (COP) diameters of right side (n = 234) and left side (n = 228) molars. *p <0.05; **p<0.001.*

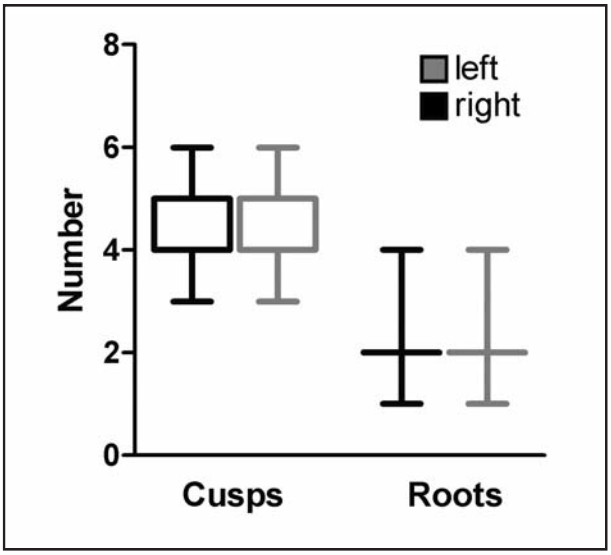


*Fig. 5: Distribution of the number of cusps and roots of lower third molars according to side. We determined the number of cusps and roots of right side (n = 304) and left side (n = 195) molars.*

however, the median of the MD and COP measurements of right molars were significantly higher (Mann Whitney U = 23540, p = 0.0287 for MD measurements and Mann Whitney U = 22820, p = 0.0089 for COP measurements, respectively, than those of left molars (Fig. 4 B). For right molars, measurements (median, range) were: OA:17.45, 11.12-22.56 mm; VP: 10.90, 8.81-13.27 mm; MD: 9.37, 7.29-11.27 mm; COV: 7.15, 3.55-8.77 mm; COP: 7.42, 4.29-9.79 mm. For left molars, they were: OA: 17.35, 11.45-24.00 mm; VP: 10.80, 8.16-13.68 mm; MD: 9.16, 6.52-11.18 mm; COV: 7.06, 3.87-8.81 mm; COP: 7.26, 4.28-9.03 mm. The lower left and right third molars showed no significant differences in the number of cusps (4, 3-6) or roots (2, 1-4), as shown in Fig. 5,or in the different measurements taken (Fig. 6 A and 6 B). Fig. 6 A shows the boxplot of OA measurements obtained for lower right (17.83, 12.01-22.52 mm) and lower left molars (17.88, 13.21-22.10 mm). Fig. 6 B shows that vestibular-lingual (VL) measurements were 10.04, 4.62-12.25 mm for right molars and 10.10, 8.14-11.36 mm for left molars; MD diameters were 11.25, 6.05-14.03 mm for right molars and 11.28, 9.08-13.38 mm for left molars; COV was 7.57, 3.81-9.63 mm for right molars and 7.64, 5.73-9.44 mm for left molars; cervical-occlusal-lingual (COL) values were 6.66, 3.30-8.69 mm for right molars and 6.72, 5.54-8.03 mm for left molars.

Upon comparison of the molars from both jaws regardless side, it was observed that the medians for cusps (5) and the COV (7.60 mm), MD (11.26 mm) and OA (17.89 mm) measurements were significantly higher (p< 0.0001) in the lower molars, whereas the number of roots (3) and the COP (7.34 mm) and VP measurements were significantly higher (p < 0.0001) in the upper molars.

The dissimilarity and the correlation analysis were performed on arcsinh-normalised dental measurements abundance levels. Dental measurements could then be clustered according to dental arch and side to determine how closely correlated they were. The dendrogram in Fig. 7 shows the dissimilarity profile of dental measurements grouped according

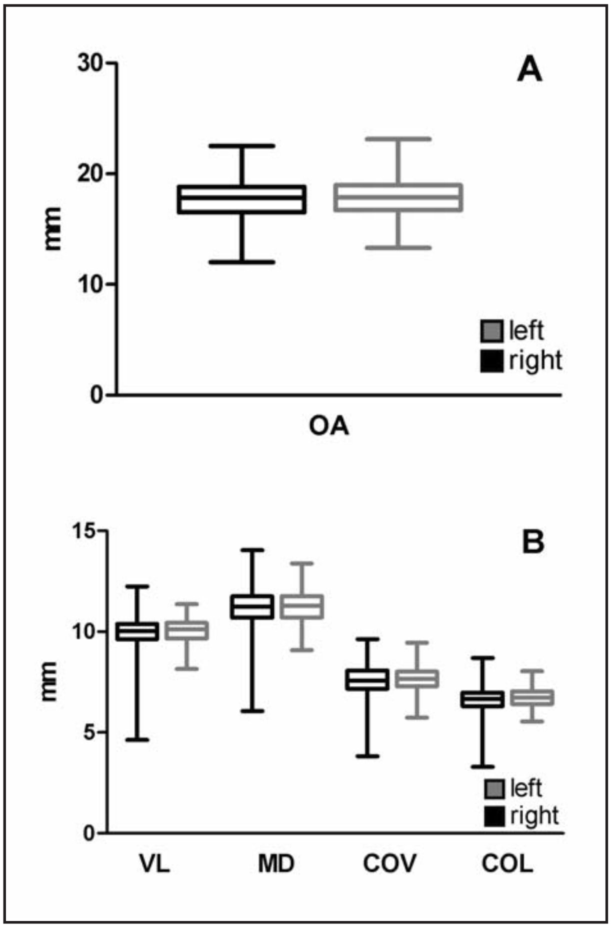


*Fig. 6: Distribution of the measurements of lower third molars according to side. A. Total length. We determined the occlusal-apical (OA) length; B. Coronal measurements. We determined the vestibular-lingual (VL), mesio-distal (MD), cervical-occlusal-vestibular (COV), and cervical-occlusal-palatal (COP) diameters of right side (n = 304) and left side (n = 195) molars.*

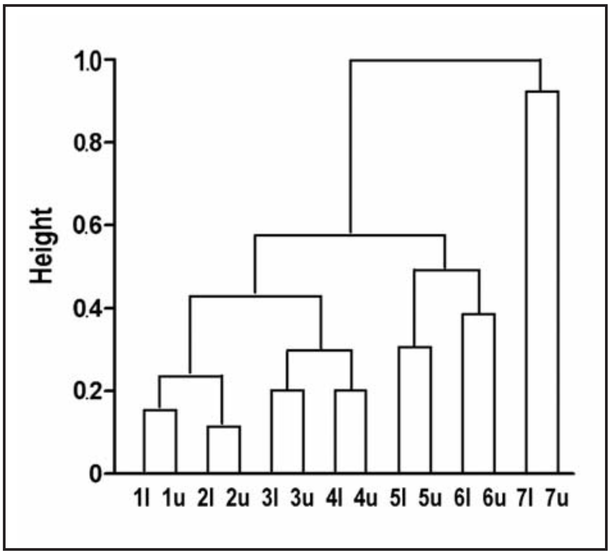


*Fig. 7: Dendrogram of third molar measurements, roots and cusps.Dissimilarity of the molar morphometric parameters according to dental arch.1 = OA: occlusal-apical length; 2 =COV: cervical-occlusal-vestibular diameter; 3 = COP: cervical-occlusal-palatal diameter; 4 = MD: mesio-distal diameter; 5 = VP: vestibular-palatal diameter; 6 = R: number of roots;7 = C: number of cusps; u: upper; l: lower.*

to dental arch. The height of each leaf shows dissimilarity of the dental parameter regarding dental arch, i.e., the longer the line of the leaf, the greater the difference. All the resulting clusters were *bifolious* and each leaf represented the upper and lower arch for each studied dental parameter. There was only one *bifolious* outlier (leaves 7l and 7u), evidencing the main distinctive feature of this parameter (number of cusps) as regards dental arch. The analysis of the dissimilarity of each clade (dendrogram branch) and its correlation to each dental arch, as merged from the cluster analysis, enabled the assessment of the predictive capability of each dental parameter as regards dental arch. The dental parameters C, MD, OA and COV resulted predictive for lower arch whereas R, VP and COP were predictive for upper arch. The same analysis was performed to assess the predictive capability of the studied parameters for dental side (data not shown). They showed no predictive capability for side in lower molars, although they did in upper molars. In the latter case, C was predictive for left side and R, COP and MD for right side.

## DISCUSSION

This is the first study updating morphometric data of third molars in recent decades in our country. It is worth noting that the size of the study population of third molars used in this research is the largest ever used. Sample sizes used in previous studies[17, 19-21] have always been smaller than one half of the size we used here. In general, most previous studies[19-21] have reported morphometric data from molar measurements taken by means of Vernier calipers. In contrast, we report data obtained using digital calipers rather than Verniers, ensuring accuracy and precision of the measurements, as proposed for anthropometric measurements of skeleton remains or cadaveric bones and teeth[22]. Another interesting feature of our research is the complete descriptive statistical report of the molar measurements, including median, 95% confidence interval and range. In contrast, previous investigations reported only average measurements of some dental parameters of the molar and no dispersion statistics. However, those averages, although different, are comparable to ours. As a rule, average third molar measurements reported previously[17, 19-21] are within the 95% confidence interval of our data reported in this paper. This includes COV, COP, OAdistances, MD and VP, R and C, of both upper and lower third molars. One original contribution of this study is the analysis of the capability of dental morphometric parameters to predict the side or dental arch the molar belongs to. This kind of analysis had not been performed to date. In our research, the study population analyzed is appropriately large to perform such analysis. At present, virtual modeling of bones and teeth is of dental, forensic and anthropological interest[7, 16, 18, 23, 24]. These specialties require the most accurate and precise data possible. The accuracy and precision of models usually increases when input data are weighted according to their predictive capability[18]. In this regard, the data reported in this paper will contribute to the development of more accurate mathematical models. However, one limitation of this study to that aim is the number of dental parameters considered. We only studied morphometric parameters, though occlusal topographical parameters such as the perimeter of the molar occlusal view, perimeter of the occlusal surface, number of pits and fissures, cusps height and angles, should also be considered, some of which might reveal higher predictive capability for side or dental arch.

We conclude that despite the morphometric heterogeneity of third molar, there are intrinsic parameters with predictive capability for dental arch and side, but it would be advisable to supplement this study with data from topographic occlusal variables in order to validate the accuracy of their predictive capability.

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## REFERENCES

1. Jokstad A. Methodological challenges in the study of dental occlusion. J Oral Rehabil 2012; 39: 480-488.
2. Thevissen PW, Fieuws S, Willems G. Third molar development: measurements versus scores as age predictor. Arch Oral Biol 2011; 56:1035-1040.
3. Koolstra JH. Dynamics of the human masticatory system. Crit Rev Oral Biol Med 2002; 4:366-376.
4. Sert S, Sahinkesen G, Topçu FT, Eroğlu SE, Oktay EA. Root canal configurations of third molar teeth. A comparison with first and second molars in the Turkish population. Aust Endod J 2011; 37: 109-117.
5. Johan NA, Khamis MF, Abdul Jamal NS, Ahmad B, Mahanani ES. The variability of lower third molar development in Northeast Malaysian population with application to age estimation. J Forensic Odontostomatol 2012; 1: 45-54.
6. Kuzekanani M, Haghani J, Nosrati H. Root and canal morphology of mandibular third molars in an Iranian population. J Dent Res Dent Clin Dent Prospect 2012; 6:85-88.
7. Gaspersic D. Morphometry, scanning electron microscopy and X-ray spectral microanalysis of protostylid pits on human lower third molars. AnatEmbryol (Berl) 1996; 193: 407-412.
8. Peterkova R, Lesot H, Peterka M. Phylogenetic memory of developing mammalian dentition. J Exp Zool B Mol Dev Evol 2006; 306: 234-250.
9. Esposito M, Coulthard P. Impacted wisdom teeth BMJ Clin Evid. 2008; pii: 1302.
10. Marciani RD. Third molar removal: an overview of indications, imaging, evaluation, and assessment of risk. Oral Maxillofac Surg Clin North Am 2007; 19:1-13
11. Choi BJ, Park S, Lee DW, Ohe JY, Kwon YD. Effect of lower third molars on the incidence of mandibular angle and condylar fractures. J Craniofac Surg 2011; 22: 1521-1525.
12. Susarla SM, Dodson TB. Predicting third molar surgery operative time: avalidated model. J Oral Maxillofac Surg 2013; 71:5-13.
13. Al-Khatib AR, Rajion ZA, Masudi SM, Hassan R, Townsend GC.Validity and reliability of tooth size and dental arch measurements: a stereo photogrammetric study. Aust Orthod J 2012; 28:22-29.
14. Ganguli A, Bhattacharyya B. Role of morphometry in the identification of teeth: Maxillary permanent molars from an eastern Indian population. Forensic Sci Int 1986; 32: 103-107.
15. Sidow SJ, West LA, Liewehr FR, Loushine RJ.Root canal morphology of human maxillary and mandibular third molars. J Endod 2000; 26:675-678.
16. Klukkert ZS, Dennis JC, M'kirera F, Ungar PS. Dental topographic analysis of the molar teeth of primates. Methods Mol Biol 2012; 915: 145-152.

17. Figún ME, Garino R. Anatomía odontológica funcional y aplicada. Buenos Aires, Argentina. El Ateneo 1978; 244-245, 247-249, 266.
18. Szucs A, Bujtár P, Sándor GK, Barabás J. Finite element analysis of the human mandible to assess the effect of removing an impacted third molar. J Can Dent Assoc 2010; 76:a72.
19. Scott JH, Symons NBB. Introducción a la anatomía dentaria. Buenos Aires, Argentina. Mundi 1980; Cap.1: 22-28.
20. Riojas Garza, MT. Anatomía dental. México DF, México: El Manual Moderno 2006; 97-98, 108-109.
21. Scheid R, Weiss G. Woefel. Anatomía dental. Barcelona, Wolters Kluwer/Lippincott Williams & Wilkins 2012; 153-156.
22. Burns K. Forensic anthropology training manual. New York, NY, USA: Routledge 2012; 153-180.
23. Barone S, Paoli A, Razionale AV. Geometrical modeling of complete dental shapes by using panoramic X-ray, digital mouth data and anatomical templates. Comput Med Imaging Graph 2015; 43:112-121.
24. Teshima TL, Patel V, Mainprize JG, Edwards G, Antonyshyn OM. A three-dimensional statistical average skull: application of biometric morphing in generating missing anatomy. J Craniofac Surg 2015; 26:1634-1638.

# ORAL HEALTH-RELATED QUALITY OF LIFE IN THE ELDERLY POPULATION RECEIVING HEALTH CARE AT THE PUBLIC HOSPITAL NETWORK IN MEDELLÍN, COLOMBIA, AND ITS RELATED FACTORS

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***ABSTRACT***

*Metrosalud is the largest public hospital network in the city of Medellin and one of the most important in Colombia providing health care to the most vulnerable population. The objective of the study was to determine the Oral Health-Related Quality of Life (OHRQoL) and its related factors in the elderly population receiving health care at the public hospital network in Medellin (Colombia). A cross-sectional design was used. Men and women ≥ 65 years old were considered for this research, selected from first consultation records by the institution's statistical unit for 2011, who accepted to participate after being contacted by telephone. Sampling was performed in two stages: simple random sampling for selecting Hospital Units -HU- and Health Centers -HC- throughout the hospital network in the city, followed by random quota sampling in proportion to the number of elderly population assigned to each HU and HC. A total 342 patients (58.2% women) participated in the study. The project involved the use of a structured questionnaire and complete dental examination with information on sociodemographic data, self-perceived health variables (mental, general and oral), use of oral health services, Oral Health-Related Quality of Life (OHRQoL as measured with GOHAI index), temporomandibular joint test, oral mucosa, soft tissue evaluation, periodontal, dental and prosthetic examination. Descriptive and bivariate analyses were conducted to determine statistically significant differences. Multivariate analysis was performed, using logistic regression, calculating crude and adjusted odds ratios (OR) with their 95% confidence intervals (95% CI). Impacts were found to be generated by education levels, differences in socioeconomic status and urban or rural housing conditions. The results of this research show low OHRQoL levels in the elderly population receiving health care services at the public hospital network in Medellin.*

**Key words:** *Quality of life, oral health, perception, geriatric assessment.*

# CALIDAD DE VIDA RELACIONADA CON LA SALUD BUCAL EN LA POBLACIÓN ADULTA MAYOR ATENDIDA EN LA RED HOSPITALARIA PÚBLICA DE MEDELLÍN (COLOMBIA) Y SUS FACTORES RELACIONADOS

***RESUMEN***

*Metrosalud es la Empresa Social del Estado (E.S.E.) y red hospitalaria pública más grande de la ciudad de Medellín y una de las más importantes del país en materia de atención en salud a la población más vulnerable. El objetivo de este estudio fue determinar la calidad de vida relacionada con salud bucal (CVRSB) en la población adulta mayor atendida en la red hospitalaria pública de Medellín (Colombia) y sus factores relacionados. Se realizó un estudio descriptivo trasversal. La muestra fue constituida por población adulta mayor de la ESE Metrosalud. Hombres y mujeres mayores de 65 años fueron considerados para esta investigación y fueron seleccionados de los registros de consulta de la unidad de estadística de la institución (año 2011) e invitados a participar a través de contacto telefónico. Se realizó un muestreo en dos etapas, en primer lugar se seleccionaron aleatoriamente los centros y unidades hospitalarias y en segundo lugar se realizó un muestreo por cuotas teniendo en cuenta la distribución de adultos mayores, uso del Índice GOHAI, examen de articulación temporo-mandibular, mucosa bucal, evaluación de tejidos blandos, examen periodontal, dental y protésico. Se realizaron análisis de 342 adultos mayores (58,2% mujeres) participaron en el estudio. El proyecto contempló la utilización de encuesta estructurada y examen odontológico completo, con información sobre datos sociodemográficos, variables de salud auto-percibida (mental, general y bucal), utilización de servicios de salud bucal, calidad de vida relacionada con la salud bucal (CVRSB). Análisis descriptivos y bivariados fueron realizados para observar diferencias estadísticamente significativas. Se realizó análisis multivariado por regresión logística, calculando odds ratios (OR) crudas y ajustadas con sus intervalos de confianza al 95% (IC 95%). Los hallazgos indican impactos en la Calidad de Vida Relacionada con la Salud Bucal -CVRSB- generados por variables como los niveles de educación, las diferencias en niveles socioeconómicos y las condiciones de la vivienda urbana o rural. Los resultados de esta investigación muestran bajos niveles de CVRSB en la población adulta mayor vinculados a los servicios de la red de publica hospitalaria en la ciudad de Medellín.*

**Palabras clave:** *Calidad de vida, salud bucal, percepción, evaluación geriátrica.*

## INTRODUCTION

Like many other countries in Latin America, Colombia has undergone a major demographic and epidemiological transition in recent decades,with rapid aging of the population[1]. Data from the 2012 World Health Organization (WHO) projection placed life expectancy at 79 years (76 years for men and 83 years for women)[2]. According to the statistics from the National Demographic and Health Survey (ENDS-2010) and the projections from the National Administrative Department of Statistics (DANE), the population older than 60 years represents 10% of the population, and those over 65 years 7% with indicators for the Department of Antioquia being quite similar to the national average[3,4].

With regard to social and health diagnosis, it is clearly important to analyze certain conditions because of the challenges involved in developing policies for the elderly population. For example, among the social and health conditions reported by the ENDS-2010[3], the following stand out: one third of households with elderly membershave two or more unfavorable conditions, including housing and access to health services; three out of five seniors feel emotionally affected because of their health condition; 45% have high blood pressure, followed by allergies (17%), arthritis (16%), other heart diseases (16%), diabetes (11%) and lung diseases (10%). For the population aged over 65 years, the latest National Survey of Oral Health (ENSAB IV, 2013-2014) reported higher DMFT, partial tooth loss in over 95%, total tooth loss 33%, and periodontal disease in over 70%[5].

These situations mean that the elderly population is an increasingly significant group, but as it is vulnerable to inequalities and social exclusion, the higher life expectancy is not accompanied by better quality of life or optimal conditions of general and oral health[6].

Scientific research has increasingly been evaluating the relationship between quality of life and oral health [7-10]. Oral health-related quality of life is defined as *"a person's perceived level of enjoyment regarding his/her mouth and the service it provides to his daily life, taking into account his/her past and present circumstances, involvement in care, expectations, paradigms, and of course the value system inspired by his/her sociocultural context"*[11]. Tools to evaluate the impact of quality of life in oral health have been available since the early 1970s. Although the review of recent literature shows that there are many studies on the subject [12-16], there has been little research in this line of work in Colombia. There are afew studies evaluating oral health conditions in specific samples [17-20], but they address neither the quality of life component nor this age group.

METROSALUD is the largest public hospital network in the city of Medellin and one of the most important in Colombia providing health care to the most vulnerable population. It thus provides an interesting opportunity for studying the factors related to quality of life and their relationship to oral health in the population that receives healthcare services at the hospital's different facilities. Scientific knowledge of these issues will provide support to decision-making for the implementation of public oral health policies for vulnerable populations.

The purpose of this study is to determine the Oral Health-Related Quality of Life (OHRQoL) and its related factors in the elderly population receiving healthcare at the public hospital network in Medellin, Colombia.

## MATERIALS AND METHODS

### *Design, data collection and setting*

A cross-sectional design was used. The study population was constituted by the elderly population receiving primary and specialized health care at the public hospital network in Medellín (E.S.E METROSALUD). Men and women ≥ 65 years old were considered for this research. They were selected from first consultation records by the institution's statistical unit for 2011 and accepted to participate after being contacted by telephone. The exclusion criterion was motor disabilities preventing the subject from traveling to the healthcare facility.

An initial sample of 352 elderly persons was determined by sampling in two stages. First, simple random sampling was used to select Hospital Units -HU- and Healthcare Centers -HC- throughout the hospital network in the city. Second, random quota sampling was carried out in proportion to the number of elderly persons assigned to each HU and HC. To calculate sample size we considered prevalence of 29.1% of low quality of life, as demonstrated by other studies[21, 22], 95% confidence level and 5% sampling error, plus 10% over sampling for any possible loss of information. However, for this analysis, data from 342 seniors (58.2% female) was used, which constitutes 97% of the total sample after debugging data.

The project used a structured questionnaire and complete dental examination with information on sociodemographic data, self-perceived health variables (mental, general and oral), use of oral health care services, Oral Health-Related Quality of Life, temporomandibular joint test, oral mucosa, soft tissue evaluation, periodontal, dental and prosthetic examination. Questionnaires were administered by a fieldwork group (two examiners and 4 interviewers), who were trained and calibrated to take quality surveys (concordance >80%). A pilot test was conducted on 10 people to revise and correct the language, adapt the questions culturally and check the overall consistency of the instruments for data collection. Fieldwork was conducted between March and December 2013. Oral test standards followed the parameters defined in the Basic Methods according to parameters of the World Health Organization (WHO) for surveys of oral health [23].

### *Variable Definitions*

This study considered as a principal variable the Oral Health-Related Quality of Life (OHRQoL) through the Geriatric Oral Health Assessment Index (GOHAI) [6,8,21], which consists of 12 multiple-choice questions and provides a score on a scale of 0 to 60 (where zero corresponds to the worst assessment of GOHAI and 60 the best). The GOHAI scales where divided into three categories: high (57 to 60), moderate (51 to 56) and low (≤50). However, for multivariate analysis this variable was dichotomized, and the moderate and high score were aggregated as good quality of life and low scores were aggregated as poor quality of life.

Sociodemographic variables considered were: sex, age (65-74, ≥75), level of education (≤Primary, ≥Secondary), socioeconomic level (defined according to the classification provided for housing by the city's public services company and categorized as low -0, 1 and 2- and medium -3 and 4-), marital status (single, married, cohabiting, widowed, separated), and zone of residence (urban/rural). The Duke-UNC-11 questionnaire, validated and adapted for the purpose of this study, was used to measure social support. This self-administered instrument containing 11 items evaluates perceived functional or qualitative social support in two dimensions: confidence (possibility of relying on others to communicate) and affective (manifestations of love, affection and empathy). Each item is scored on a frequency rating (Likert-type scale) from 1:"Much less than I would like" to 5:"As much as I would like". The score was calculated by adding up the responses to each item, with a higher score denoting greater social support. The cut-off point for low levels of social support is the 15th percentile, corresponding to a score of 32 [24, 25].

Several variables were selected to evaluate health and oral health status: medical treatment (Yes/No), medication use (Yes/No), presence of upper/lower denture (Yes/No), satisfaction with upper/lower denture (Satisfied/Dissatisfied), need to change upper/lower denture (Yes/No), oral mucosa (Normal/Affected), need for treatment (Yes/No), temporomandibular joint (Asymptomatic/ Symptomatic), satisfaction with dental status (Satisfied/ Dissatisfied).

### Data Analysis

First, the study variables were described using absolute and relative frequencies. For GOHAI, mean and median, interquartile range (IQR), minimum and maximum ranges were calculated according to sociodemographic variables and general and oral health, and chi-square tests (median, Student's t-test and Mann-Whitney) were performed to determine statistically significant differences between variables. OHRQoL categories were analyzed as high, moderate or low, according to the aforementioned variables, and Chi-square tests for frequencies were performed to determine relationships between variables. Finally, multivariate analysis was performed, using logistic regression, calculating crude and adjusted odds ratios (OR) with their 95% confidence intervals (95% CI). For these analyses, we used a model that includes all the confounders mentioned, and we show the complete adjusted models. All calculations were made using Stata10® y SPSS® 19.0.

### *Ethics*

This research followed international guidelines (Declaration of Helsinki) and the legal regulations of Colombia (Resolution N° 008430, October 4, 1993, Ministry of Health). Confidentiality was guaranteed throughout the research process and all respondents provided informed consent to participate. Since the elderly population is considered highly sensitive, all participants and their relatives received information on the objectives of the research and were informed they could withdraw freely with no retaliation at any time if they wished to do so. In addition, they received information about the treatment they required and where they needed to go. The study protocol was approved by the Ethical Committee of the E.S.E Metrosalud (act 09-2011).

## RESULTS

The sample consisted of 342 elders with mean age 72.6 years (±5.9), and the predominant age group was 65 - 74 years (69%). The percentage of females was slightly higher (58%). Fifty-eight percent reported primary education and a higher frequency of subjects was from socioeconomic levels1 and 2 (86%). Over 40% were married or cohabiting, 90% lived in urban areas and almost a quarter reported low social support (Table 1).

Considering the behavior of the GOHAI index according to sociodemographic variables (Table 1), a median of 46 (IR = 13) was obtained. The survey found higher Oral Health-Related Quality of Life (OHRQoL) scores for men, adults under 74, higher education, middle socioeconomic level and married, although the differences were not statistically significant. Those living in rural areas reported higher quality of life score with statistically significant difference ($p<0.05$)

Regarding the health indicators selected in the study (Table 2), it was found that 86% of adults were in medical treatment and 85% used some type of medication. The variables related to oral health findings indicated that just over three-quarters of

**Table 1: Mean (SD) and median of the GOHAI test score according to sociodemographic characteristics in the target population. Medellin (Colombia), 2013. (n=342).**

| Variables | Sample | | GOHAI Score | | | | *p-value*** |
|---|---|---|---|---|---|---|---|
| | n | % | $\bar{X}$ (SD) | Me (Q3 - Q1)* | Min | Max | |
| *Sex* | | | | | | | |
| Male | 143 | 41.8 | 44.8 (9.6) | 47 (12) | 22 | 60 | 0.534 |
| Female | 199 | 58.2 | 44.3 (9.8) | 46 (14) | 16 | 60 | |
| *Age (years)* | | | | | | | |
| 65-74 | 235 | 68.7 | 44.9 (9.7) | 47 (13) | 16 | 60 | 0.085 |
| ≥ 75 | 107 | 31.3 | 43.6 (9.6) | 44 (15) | 22 | 60 | |
| *Education level* | | | | | | | |
| ≤ Primary | 316 | 92.4 | 44.2 (9.8) | 46 (14) | 16 | 60 | 0.058 |
| ≥ Secondary | 26 | 7.6 | 47.6 (8.0) | 51 (11) | 28 | 58 | |
| *Socioeconomic level* | | | | | | | |
| Low (1-2) | 293 | 85.7 | 44.0 (9.8) | 46 (13) | 16 | 60 | 0.154 |
| Middle (3-4) | 49 | 14.3 | 47.5 (8.3) | 49 (10) | 24 | 60 | |
| *Marital status* | | | | | | | |
| Single | 75 | 21.9 | 44.1 (10.9) | 46 (15) | 19 | 60 | 0.453 |
| Married | 127 | 37.1 | 45.5 (8.8) | 48 (11) | 16 | 60 | |
| Cohabiting | 21 | 6.1 | 45.8 (9.5) | 46 (8) | 23 | 60 | |
| Widowed | 90 | 26.3 | 43.2 (10.0) | 44 (15) | 22 | 60 | |
| Separated | 29 | 8.5 | 44.3 (9.4) | 47 (14) | 22 | 57 | |
| *Zone of residence* | | | | | | | |
| Urban | 306 | 89.5 | 44.0 (9.8) | 46 (14) | 16 | 60 | 0.007 |
| Rural | 36 | 10.5 | 48.5 (7.3) | 50 (6) | 27 | 60 | |
| *Social support (Duke-11)* | | | | | | | |
| Normal | 261 | 76.3 | 45.1 (9.5) | 48 (12) | 16 | 60 | 0.056 |
| Low | 81 | 23.7 | 42.4 (10.0) | 44 (17) | 20 | 60 | |
| ***Total (All )*** | ***342*** | ***100*** | ***44.5 (9.7)*** | ***46 (13.3)*** | ***16*** | ***60*** | **--** |

* Median (Interquartile range (Quartile 3- Quartile 1) ** Chi square median tests for independent samples

the elders were using upper dentures, of whom 63% were dissatisfied and 70% needed change according to clinical criteria; slightly less than half (46%) used lower dentures, of whom 82% were dissatisfied and 58% needed change. Affected oral mucosa was observed in two thirds of the examinees. A little over 40% presented subjective symptoms in Temporomandibular Joint (TMJ). Overall, nearly half the respondents were satisfied with the state of their teeth and structures of the oral cavity.

For clinical variables, highest scores according to GOHAI index were achieved by those not receiving medical treatment, taking medication, with upper and lower dentures, and without need for dental

**Table 2: Mean (SD) and median of the GOHAI test score according to selected general health and oral health indicators in the target population. Medellin (Colombia), 2013. (n=342).**

| Variables | Sample | | GOHAI Score | | | | *p-value*** |
|---|---|---|---|---|---|---|---|
| | n | % | X̄ (SD) | Me(Q3-Q1)* | Min | Max | |
| *Medical treatment* | | | | | | | |
| Yes | 293 | 85.7 | 44.3 (9.6) | 46 (14) | 16 | 60 | 0.217 |
| No | 49 | 14.3 | 45.8 (10.2) | 48 (13) | 20 | 60 | |
| *Medication use* | | | | | | | |
| Yes | 290 | 84.8 | 44.3 (9.6) | 46 (14) | 16 | 60 | 0.344 |
| No | 52 | 15.2 | 45.4 (10.4) | 48 (13.5) | 20 | 60 | |
| *Presence of upper denture* | | | | | | | |
| Yes | 79 | 23.1 | 44.6 (9.8) | 47 (14) | 23 | 60 | 0.604 |
| No | 263 | 76.9 | 44.2 (9.3) | 45 (11) | 16 | 60 | |
| *Presence of lower denture* | | | | | | | |
| Yes | 184 | 53.8 | 44.4 (10.1) | 46.5 (15) | 22 | 60 | 0.878 |
| No | 158 | 46.2 | 44.5 (9.4) | 46 (11) | 16 | 60 | |
| *Satisfaction with upper denture* | | | | | | | |
| Satisfied | 126 | 36.8 | 47.4 (8.9) | 50 (13) | 16 | 60 | <0.001 |
| Dissatisfied | 216 | 63.2 | 42.8 (9.8) | 45 (14) | 19 | 60 | |
| *Satisfaction with lower denture* | | | | | | | |
| Satisfied | 63 | 18.4 | 48.7 (9.1) | 52(9) | 16 | 60 | <0.001 |
| Dissatisfied | 279 | 81.6 | 43.5 (9.6) | 46 (15) | 19 | 60 | |
| *Need to change upper denture* | | | | | | | |
| Yes | 102 | 29.8 | 43.6 (10.0) | 46 (15) | 24 | 60 | 0.017 |
| No | 240 | 70.2 | 46.5 (8.6) | 48 (11) | 16 | 60 | |
| *Need to change lower denture* | | | | | | | |
| Yes | 145 | 42.4 | 43.4 (10.2) | 45 (15) | 22 | 60 | 0.020 |
| No | 197 | 57.6 | 46.0 (8.8) | 48 (11) | 16 | 60 | |
| *Oral mucosa* | | | | | | | |
| Normal | 115 | 33.6 | 46.7 (8.9) | 49 (12) | 22 | 60 | 0.002 |
| Affected | 227 | 66.4 | 43.4 (9.9) | 45 (15) | 16 | 60 | |
| *Need for dental treatment* | | | | | | | |
| No | 10 | 2.9 | 44.0 (11.1) | 48.5 (20) | 26 | 56 | 0.984 |
| Yes | 332 | 97.1 | 44.5 (9.7) | 46 (13) | 16 | 60 | |
| *Temporomandibular joint* | | | | | | | |
| Asymptomatic | 192 | 56.1 | 46.8 (8.9) | 49.5 (12) | 19 | 60 | <0.001 |
| Symptomatic | 150 | 43.9 | 41.6 (9.9) | 43 (15) | 16 | 60 | |
| *Satisfaction with dental status* | | | | | | | |
| Satisfied | 167 | 48.8 | 48.6 (8.1) | 50 (9) | 16 | 60 | <0.001 |
| Dissatisfied | 170 | 49.7 | 40.3 (9.4) | 42 (16) | 19 | 56 | |

** Median -Interquartile range (Quartile 3- Quartile 1)- ** Mann Whitney U tests* treatment (differences between groups were not statistically significant). Higher scores were achieved by the groups satisfied with their prostheses (both lower and upper), those that do not need to change their prostheses, those with normal oral mucosa, those without TMJ symptoms and those who are satisfied with their dental status; these findings being statistically significant (Table 2).

The reclassification of the GOHAI score overall showed that 68% had a low level of OHRQoL (Table 3). No statistically significant difference ($p<0.05$) was found according to sociodemographic characteristics. It is worth highlighting that women, adults over 74 years old, subjects with high school and college education, subjects with low socioeconomic level, the widowed, urban dwellers, and people with low social support all reported low OHRQoL.

The analysis of the GOHAI index categories according to general and oral health variables (Table 4) shows lower OHRQoL in those who are in medical treatment and take medication (69% in both cases), those with upper and lower prosthesis (70% in both cases), those who need to change their prostheses, both upper and lower (60% and 64% respectively), although no statistically significant difference was found ($p<0.05$). In contrast, statistically significant differences ($p<0.001$) were found for those who perceived lower OHRQoL, those dissatisfied with their upper and lower prosthesis (76% and 73% respectively), those with

**Table 3: Test GOHAI categories (Oral health-related quality of life) according to sociodemographic categories in the target population. Medellin (Colombia), 2013. (n=342).**

| Variables | GOHAI categories (Oral-health related quality of life) | | | | | | *p-value*** |
|---|---|---|---|---|---|---|---|
| | High | | Moderate | | Low | | |
| | n | % | n | % | n | % | |
| *Sex* | | | | | | | |
| Male | 11 | 5.5 | 55 | 27.6 | 133 | 66.8 | 0.509 |
| Female | 11 | 7.7 | 33 | 23.1 | 99 | 69.2 | |
| *Age (years)* | | | | | | | |
| 65-74 | 15 | 6.4 | 65 | 27.7 | 155 | 66.0 | 0.477 |
| ≥ 75 | 7 | 6.5 | 23 | 21.5 | 77 | 72.0 | |
| *Education level* | | | | | | | |
| ≤ Primary | 21 | 6.6 | 76 | 24.1 | 219 | 69.6 | 0.046 |
| ≥ Secondary | 1 | 4.5 | 12 | 46.2 | 13 | 50.0 | |
| *Socioeconomic level* | | | | | | | |
| Low (1-2) | 17 | 5.8 | 73 | 24.9 | 203 | 69.3 | 0.297 |
| Middle (3-4) | 5 | 10.2 | 15 | 30.6 | 29 | 59.2 | |
| *Marital status* | | | | | | | |
| Single | 6 | 8.0 | 21 | 28.0 | 48 | 64.0 | 0.946 |
| Married | 6 | 4.7 | 34 | 26.8 | 87 | 68.5 | |
| Cohabiting | 2 | 9.5 | 6 | 28.6 | 13 | 61.9 | |
| Widowed | 6 | 6.7 | 19 | 21.1 | 65 | 72.2 | |
| Separated | 2 | 6.9 | 8 | 27.6 | 19 | 65.5 | |
| *Zone of residence* | | | | | | | |
| Urban | 17 | 5.6 | 78 | 25.5 | 211 | 69.0 | 0.130 |
| Rural | 5 | 13.9 | 10 | 27.8 | 21 | 58.3 | |
| *Social support (Duke-11)* | | | | | | | |
| Normal | 20 | 7.7 | 69 | 26.4 | 172 | 65.9 | 0.181 |
| Low | 2 | 2.5 | 19 | 23.5 | 60 | 74.1 | |
| ***Total (All )*** | 22 | 6.4 | 88 | 25.7 | 232 | 67.8 | --- |

* Chi square tests

Temporomandibular Joint symptoms (81%) and those who said they were dissatisfied with their oral health status (85%).
Table 5 shows the multivariate model for poor quality of life related to oral health adjusted for sociodemographic variables (model 1), clinical conditions (model 2) and all variables together (model 3). Overall, the significant variables that best explained poor quality of oral health were the presence of Temporomandibular Joint symptoms

**Table 4: Mean (SD) and median of the GOHAI test score according to selected general health and oral health indicators in the target population. Medellin (Colombia), 2013. (n=342).**

| Variables | GOHAI categories (Oral-health related quality of life) | | | | | | *p-value*** |
|---|---|---|---|---|---|---|---|
| | High | | Moderate | | Low | | |
| | n | % | n | % | n | % | |
| *Medical treatment* | | | | | | | |
| Yes | 17 | 5.8 | 73 | 24.9 | 203 | 69.3 | 0.297 |
| No | 5 | 10.2 | 15 | 30.6 | 29 | 59.2 | |
| *Medication use* | | | | | | | |
| Yes | 16 | 5.5 | 75 | 25.9 | 199 | 68.6 | 0.263 |
| No | 6 | 11.5 | 13 | 25.0 | 33 | 63.5 | |
| *Presence of upper denture* | | | | | | | |
| Yes | 4 | 5.1 | 20 | 25.3 | 55 | 69.6 | 0.838 |
| No | 18 | 6.8 | 68 | 25.9 | 177 | 67.3 | |
| *Presence of lower denture* | | | | | | | |
| Yes | 10 | 5.4 | 46 | 25.0 | 128 | 69.6 | 0.646 |
| No | 12 | 7.6 | 42 | 26.6 | 104 | 65.8 | |
| *Satisfaction with upper denture* | | | | | | | |
| Satisfied | 13 | 10.3 | 45 | 35.7 | 68 | 54.0 | <0,001 |
| Dissatisfied | 9 | 4.2 | 43 | 19.9 | 164 | 75.9 | |
| *Satisfaction with lower denture* | | | | | | | |
| Satisfied | 8 | 12.7 | 27 | 42.9 | 28 | 44.4 | <0,001 |
| Dissatisfied | 14 | 5.0 | 61 | 21.9 | 204 | 73.1 | |
| *Need to change upper denture* | | | | | | | |
| Yes | 7 | 6.9 | 34 | 33.3 | 61 | 59.8 | 0.096 |
| No | 15 | 6.3 | 54 | 22.5 | 171 | 71.3 | |
| *Need to change lower denture* | | | | | | | |
| Yes | 10 | 6.9 | 42 | 29.0 | 93 | 64.1 | 0.446 |
| No | 12 | 6.1 | 46 | 23.4 | 139 | 70.6 | |
| *Oral mucosa* | | | | | | | |
| Normal | 9 | 7.8 | 38 | 33.0 | 68 | 59.1 | 0.048 |
| Affected | 13 | 5.7 | 50 | 22.0 | 164 | 72.2 | |
| *Need for dental treatment* | | | | | | | |
| No | 0 | 0.0 | 3 | 30.0 | 7 | 70.0 | 0.690 |
| Yes | 22 | 6.6 | 85 | 25.6 | 225 | 67.8 | |
| *Temporomandibular joint* | | | | | | | |
| Asymptomatic | 16 | 8.3 | 65 | 33.9 | 111 | 57.8 | <0,001 |
| Symptomatic | 6 | 4.0 | 23 | 15.3 | 121 | 80.7 | |
| *Satisfaction with dental status* | | | | | | | |
| Satisfied | 21 | 12.6 | 60 | 35.9 | 86 | 51.5 | <0,001 |
| Dissatisfied | 0 | 0.0 | 26 | 15.3 | 144 | 84.7 | |

** Chi square tests*

**Table 5: Multivariate model by logistic regression for low oral health-related quality of life according to selected sociodemographic and oral health indicators in the target population. Medellin (Colombia), 2013. (n=342).**

| Variables | Low quality of life | | | | | |
|---|---|---|---|---|---|---|
| | Model 1 | | Model 2 | | Model 3 | |
| | OR | 95% CI | OR | 95% CI | OR | 95% CI |
| *Age (years)* | | | | | | |
| 65-74 | 1.00 | --- | | | 1.00 | --- |
| ≥ 75 | 1.32 | 0.79- 2.21 | | | 1.37 | 0.76- 2.46 |
| *Education level* | | | | | | |
| ≤ Primary | 2.29 | 0.99- 5.13 | | | 2.48 | 0.98- 6.93 |
| ≥ Secondary | 1.00 | --- | | | 1.00 | --- |
| *Socioeconomic level* | | | | | | |
| Low (1-2) | 1.56 | 0.82- 2.96 | | | 1.72 | 0.83- 3.58 |
| Middle (3-4) | 1.00 | --- | | | 1.00 | --- |
| *Zone of residence* | | | | | | |
| Urban | 1.69 | 0.82- 3.49 | | | 2.11 | 0.87- 5.13 |
| Rural | 1.00 | --- | | | 1.00 | --- |
| *Social support (Duke-11 )* | | | | | | |
| Normal | 1.00 | --- | | | 1.00 | --- |
| Low | 1.50 | 0.84- 2.65 | | | 1.16 | 0.61- 2.22 |
| *Medical treatment* | | | | | | |
| Yes | | | 1.24 | 0.60-2.55 | 1.06 | 0.50- 2.27 |
| No | | | 1.00 | --- | 1.00 | --- |
| *Satisfaction with upper denture* | | | | | | |
| Satisfied | | | 1.00 | --- | 1.00 | --- |
| Dissatisfied | | | 1.48 | 0.77- 2.85 | 1.52 | 0.77- 2.99 |
| *Satisfaction with lower denture* | | | | | | |
| Satisfied | | | 1.00 | --- | 1.00 | --- |
| Dissatisfied | | | 1.45 | 0.66- 3.18 | 1.58 | 0.71- 3.53 |
| *Need to change upper denture* | | | | | | |
| Yes | | | 1.27 | 0.65- 2.50 | 1.26 | 0.64- 2.52 |
| No | | | 1.00 | --- | 1.00 | --- |
| *Need to change lower denture* | | | | | | |
| Yes | | | 1.30 | 0.68- 2.48 | 1.33 | 0.69- 2.57 |
| No | | | 1.00 | --- | 1.00 | --- |
| *Oral mucosa* | | | | | | |
| Normal | | | 1.00 | --- | 1.00 | --- |
| Affected | | | 1.40 | 0.82- 5.37 | 1.36 | 0.77- 2.41 |
| *Temporomandibular joint* | | | | | | |
| Asymptomatic | | | 1.00 | --- | 1.00 | --- |
| Symptomatic | | | 3.09 | 1.77- 5.37 | 3.17 | 1.80- 5.70 |
| *Satisfaction with dental status* | | | | | | |
| Satisfied | | | 1.00 | --- | 1.00 | --- |
| Dissatisfied | | | 4.36 | 2.47- 7.71 | 4.31 | 2.40- 7.77 |

*The reference category for oral health related quality of life according to the GOHAI score is moderate or high (<50).
Model 1: Adjusted for sociodemographic variables, Model 2: Adjusted for health indicators,
Model 3: Adjusted for sociodemographic and health variables.

and satisfaction with dental status. People who reported Temporomandibular Joint symptoms (OR 3.17; 95% CI: 1.80- 5.70) and satisfaction with their dental status (OR 4.31, 95% CI 2.40- 7.77) were more likely to report poor oral health-related quality of life.

## DISCUSSION

This study aims to explain factors related to OHRQoL in the elderly population receiving health care at the public hospital network in Medellín. Overall, men and women perceived a low level of their Oral Quality of Life. Educational level, dissatisfaction with current dental prosthesis and the need to change these prostheses, state of oral mucosa, state of TMJ and self-perception of the status of dental and oral health, appear as factors associated to OHRQoL in the bivariate analysis. The multivariate analysis shows that after adjusting sociodemographic and clinical variables, elders that referred presence of symptoms in Temporomandibular Joint and dissatisfaction with their dental status were more likely to report poor oral health-related quality of life with statistically significant differences. To the best of our knowledge, this is one of few studies in Colombia focusing on elderly population including oral health-related quality of life and its relationship with sociodemographic and clinical conditions.

These findings on the status of low oral health-related quality of life according to the model provided by the GOHAI index reveal aspects that should be taken into consideration, particularly the impacts generated by education levels, socioeconomic differences and urban or rural housing condition. They reveal the situation of vulnerability expressed by these indicators and perceived quality of life, suggesting that efforts should be sustained, particularly when users of the public health care network are from middle and low socioeconomic classes. Likewise, findings related to clinical indicators are relevant, highlighting the positive impact associated with positive clinical conditions and satisfaction of subjects assessed. This information should be taken into account because health care providers should appraise how decisive health careactions have a positive impact on the perceptions of the population over 65 years of age.

The main results of this study are consistent with research conducted in recent years. The first study that provided data on the OHRQoL in the elderly with use of the GOHAI index was performed by Atchison and Dolan[21], who found a GOHAI index value of 52.5 points for a sample of people older than 65 years. The German version of GOHAI index by Alexander Hassel et al.[26] found an average of 56 points for people reporting satisfaction with their oral status and an average of 44 points for those who were not satisfied. In France, Tuber-Jeanninet al.[27] reported an average value of GOHAI index of 46 points, being lower (38 points) for those with worse oral health status and much higher (52 points) for those with better conditions .

The GOHAI index, has also been applied in China, reporting 48.9 points on average, with 55.9 for people with good oral health and 40.4 for people with poor oral health[28]. In Japan, Naito et al.[29] studied 175 participants of average age 70 years, finding a GOHAI index of 57.9 among those who rated their oral health as very good, and 38.7 among those who rated their oral health the lowest. Pinzon and Zunzunegui[30] validated GOHAI index in Spain and published a study on oral health care needs in an institutionalized geriatric population comprising 100 individuals. They found that 68% had scores below 57 points with moderate and low levels; while the rest had higher scores and were defined as having high oral health-related quality of life.

Dias da Silva et al.[31] conducted a study at the University of Campinas, finding an average GOHAI index of 33.61 points. Another study conducted in Taubaté, Brazil on institutionalized and non-institutionalized subjects found an average of GOHAI index of 33.45 points for the former and 32.66 points for the latter[32]. Similar OHRQoL results are reported for France, China, Brazil, Spain, Malaysia and Saudi Arabia. In Colombia, a study conducted on adults treated at dental clinics at the University of Cartagena reported that most of them show high impact onOHRQoL[33].

Results such as those presented by the University of Cartagena show an association between the existence of oral problems and their impact on quality of life in older adults. Likewise, other studies indicate a positive impact of dental treatment and rehabilitation primarily based on findings based on the GOHAI[34]. The study in China reported a GOHAI of 53 points for toothless patients and 49.1 points for partial denture wearers. It concluded that the loss of teeth and use of dentures do not have great impact on quality of life; however, partial denture users experienced a negative impact, perhaps due to lower satisfaction with denture and the presence of teeth with advanced support problems. Wong and MacMillan[35] report a tendency to better oral health-related quality of life in subjects who do not wear dentures. The study conducted at the University of Clermont-Ferrand, France, by Veyrune et al.[36], shows the impact of new dentures through evaluations made when a new prosthesis was first used and after 6 and 12 months, concluding that GOHAI index improved after 12 months of wearing the new prosthesis.

It is important to discuss the strengths and limitations of our study. As far as we know, it is one of the first studies to address the research topic in Medellin city. There was a sufficiently representative sample of older adults from the Hospital Units and Health Care Centers in the public health service provider network in Medellin city to enable visualization of oral health status in a large segment of special social vulnerability. Likewise, the use of previously validated instruments (self-perception/clinical) enabled international comparisons to be made. Quality control performed on the questionnaires increases reliability. Finally, including health variables enables new perspectives for epidemiological analysis. However, the results of this study should be interpreted with caution in view of its limitations. It should be mentioned that the cross-sectional nature of the study does not allow causality to be established among the relationships and associations found. However, the results show the need to establish methods for monitoring and surveillance, in view of the fact that the study population has an institutional allocation responsible for providing health care services.

## CONCLUSION

The results of this research show low levels of OHRQoL in the senior population receiving health care services at the public hospital network in Medellin. They also provide evidence of sociodemographic and general and oral health factors that should be taken into account for formulating strategies and social policies, while working together to ensure that this sensitive and socially vulnerable population segment has more opportunities to receive health care, in order to contribute to improving its quality of life.

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## REFERENCES

1. Chesnais JC. Demographic Transition Patterns and the Impact of Age Structure. Popul Dev Rev 2000; 16: 327-336. URL: http://www.jstor.org/stable/1971593
2. World Health Organization. World Health Statistics 2014. URL: http://apps.who.int/iris/bitstream/10665/112738/1/9789240692671_eng.pdf?ua=1
3. Colombia: Profamilia. Encuesta Nacional de Demografía y Salud: ENDS-2010. URL: http://www.profamilia.org.co/encuestas/Profamilia/Profamilia/index.php
4. Colombia. Departamento Administrativo Nacional de Estadística -DANE-. Estudios Postcensales No. 7. Proyecciones nacionales y departamentales de población 2005-2020. Bogotá: DANE, 2009. URL: http://www.dane.gov.co/files/investigaciones/poblacion/proyepobla06_20/7Proyecciones_poblacion.pdf
5. Colombia. Ministerio de Salud. IV Estudio Nacional de Salud Bucal -ENSAB IV. Situación en salud Bucal. Bogotá: Ministerio de Salud, 2015. URL: https://www.minsalud.gov.co/sites/rid/Lists/BibliotecaDigital/RIDE/VS/PP/ENSAB-IV-Situacion-Bucal-Actual.pdf
6. Ham Chande R. El envejecimiento: una nueva dimensión de la salud. Salud Pública Mex 1996; 38: 409-418. URL:http://bvs.insp.mx/rsp/_files/File/1996/num_6/ENVEJECI386.pdf
7. Locker D. Oral health and quality of life. Oral Health Prev Dent 2004; 2:247-253.
8. Locker D. Concepts of oral health, disease and the quality of life. In: Slade, G.D. (Ed.) Measuring Oral Health and Quality of Life. Chapel Hill: Department of Dental Ecology, School of Dentistry, University of North Carolina, 1997: 11-23.
9. Allen PF. Assessment of oral health related quality of life. Health and Quality of Life Outcomes. 2003; 1:40. DOI: 10.1186/1477-7525-1-40.
10. Sheiham A. Oral health, general health and quality of life. Bull World Health Organ, 2005 83:644.
11. Montero J. Calidad de vida oral en población general. Granada: Universidad de Granada. Facultad de Odontología, 2006. URL: http://0-hera.ugr.es.adrastea.ugr.es/ tesisugr/16093823.pdf
12. Hernández Mejía R, Calderón García R, Fernández López J, Cueto Espinar A. Escala para medir la calidad de vida en

desdentados totales rehabilitados con prótesis completa mucosoportada. RCOE 2006; 11:181-191. URL:http://scielo.isciii.es/pdf/rcoe/v11n2/181_191.pdf
13. Jiménez FR, Esquivel HR, Martínez VN. Percepción de salud bucal, calidad de vida y bienestar subjetivo en ancianos. Revista ADM 2006; 63: 181-184. URL: http://www.medigraphic.com/pdfs/adm/od-2006/od065e.pdf
14. De la Fuente-Hernández J, Sumano-Moreno O, Sifuentes-Valenzuela MC, Zelocuatecatl-Aguilar A. Impacto de la salud bucal en la calidad de vida de adultos mayores demandantes de atención dental. Univ Odontol. 2010 Jul-Dic; 29: 83-92. URL: http://www.redalyc.org/pdf/2312/231216366010.pdf
15. Castrejón-Pérez RC, Borges-Yáñez SA, Irigoyen-Camacho ME. Validación de un instrumento para medir el efecto de la salud bucal en la calidad de vida de adultos mayores mexicanos. Rev Panam Salud Publica. 2010; 27:321-329. URL: http://www.scielosp.org/pdf/rpsp/v27n5/a01v27n5.pdf
16. Montero J, Yarte JM, Bravo M, López-Valverde A. Oral health-related quality of life of a consecutivesample of Spanish dental patients. Med Oral Patol Oral Cir Bucal. 2011; 16:e810-5. DOI:10.4317/medoral.16790
17. Munevar Torres M, Rojas García JK, Marín Zuluaga DJ. Perfil epidemiológico bucal de los pacientes de 55 años y más que asisten a las clínicas del geronte y a la facultad de odontología de la Universidad Nacional de Colombia durante el primer semestre del año 2000 y cuál es la percepción que tienen estos pacientes sobre su propio estado de salud bucal. Revista de Odontología. URL: http://encolombia.com/ odontologia/foc/foc20102-perfil5.htm
18. Bermúdez WF, Concha Sánchez SC, Camargo Lemos DM. Perfil orofacial de las personas mayores institucionalizadas de la ciudad de Bucaramanga y su Área Metropolitana. UstaSalud Odontología (Bucaramanga) 2003; 2: 13- 19. URL: file:///C:/Users/menesesgasca/Downloads/vustabmanga 51279120120524141426%20(1).pdf
19. López Soto OP, Cardona Rivas D, Parra Sánchez H, Montes Rojas DM, Arango Ossa MA. Morbilidad oral y factores de riesgo en adultos mayores. Revista Digital de Salud. Universidad Autónoma de Manizales 2005; 1: 1-13.
20. Mesa GL. Estado de salud bucal de los adultos mayores del Centro Geriátrico Presbítero Rogelio Arango, Municipio de Bello (Antioquia), 2005. Medellín: Universidad de Antioquia, 2005.
21. Atchison KA, Dolan TA. Development of the Geriatric Oral Health Assessment Index. J Dent Educ 1990; 54:680-687.
22. Pinzón Pulido SA, Gil MJ. Validación del Índice de Valoración en Salud Oral en Geriatría en una población Geriátrica Institucionalizada de Granada. Rev Esp Geriatr y Gerontol 1999; 34:273-282. URL: http://www.elsevier.es/es-revista-revista-espanola-geriatria-gerontologia-124-articulo-validacion-del-ndice-valoracion-salud-13006100
23. Organización Mundial de la Salud. Encuestas de salud bucodental: métodos básicos. 4ed. Ginebra: OMS, 1997. URL: http://apps.who.int/iris/bitstream/10665/41997/1/9243544934_spa.pdf
24. Broadhead WE, Gehlbach SH, Degruy FV, Kaplan BH. The Duke-UNC functional social support questionnaire: measurement of social support in family medicine patients. MedCare 1988; 26:709-23. URL: https://www.jstor.org/stable/3765493?seq=1#page_scan_tab_contents
25. Bellón JA, Delgado A, Luna JD, Lardelli P. Validez y fiabilidad del cuestionario de apoyo social funcional Duke-UNC-11. Aten Primaria 1996; 18:153-62. URL: http://www.elsevier.es/es-revista-atencion-primaria-27-articulo-validez-fiabilidad-del-cuestionario-apoyo-14325
26. Hassel AJ, Rolko C, Koke U, Leisen J, Rammelsberg P. A German version of the GOHAI. Community Dent Oral Epidemiol 2008; 36:34-42
27. Tubert-Jeannin S, Riordan PJ, Morel-Papernot A, Porcheray S, Saby-Collet S.Validation of an oral health quality of life index (GOHAI) in France. Community Dent Oral Epidemiol 2003; 31:275-284.
28. Wong MC, LuiJk, Lo Ec. Translation and Validation of Chinese Version of GOHAI. J Public Health Dent 2002; 62:78-83.
29. Naito M, Suzukamo Y, Nakayama T, Hamajima N, Fukuhara S. Linguistic adaptation and validation of the General Oral Health Assessment Index (GOHAI) in an elderly Japanese population. J Public Health Dent 2006; 66:273-275.
30. Pinzon PS, Zunzunegui MA. Detección de necesidades de atención bucodental en ancianos mediante la autopercepción de la salud oral. Rev Mult Gerontol 1999; 9:216-224.URL: https://www.researchgate.net/profile/Maria_Victoria_Zunzunegui/publication/237777456_Deteccion_de_necesidades_de_atencion_bucodental_en_ancianos_mediante_la_autopercepcion_de_la_salud_oral/links/5444e5b60cf2a76a3ccdbf8a.pdf?inViewer=0&pdfJsDownload=0&origin=publication_detail
31. Silva DD, Sousa MLR, Wada RS. Self-perception and oral health conditions in an elderly population. Cad Saúde Pública 2005; 21:1251-1259. URL :http://dx.doi.org/10.1590/S0102-311X2005000400028
32. Abud MC, Fernandes dos Santos JF, Prisco da Cunha V, Marchini L. TMD and GOHAI indices of Brazilian institucionalised and community dwelling elderly. Gerodontology 2009; 26:34-39.
33. Díaz CS, Arrieta VK, Ramos MK. Impacto de la Salud Oral en la Calidad de Vida de Adultos Mayores. Rev Clin Med Fam 2012; 5: 9-16. URL: http://scielo.isciii.es/pdf/ albacete/v5n1/original2.pdf
34. Ilhan B1, Cal E, Dündar N, Güneri P, Dağhan S. Oral health-related quality of life among institutionalized patients after dental rehabilitation. Geriatr Gerontol Int 2015; 15: 1151-1157.
35. Wong MC, Mac Millan AS. Tooth loss, denture wearing and oral health related quality of life in elderly Chinese people. Community Dent Health 2005; 22:156-161.
36. Veyrune JL, Tubert-Jeannin S, Dutheil C, Riordan PJ. Impact of new prostheses on the oral health related quality of life of edentulous patients. Gerodontology 2005; 22:3-9.

# MASKING ABILITY OF OPAQUE THICKNESS ON LAYERED METAL-CERAMIC

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***ABSTRACT***

*This study evaluated the masking ability of two opaques applied in different thicknesses. Eighty Ni-Cr metal discs 16 mm in diameter and 1.0 mm thick were prepared. The disks were divided into 8 groups (n = 10). Ceramic opaque in paste (groups 1 to 4) or powder (groups 5 to 8) presentations were applied. They were machined with aluminum oxide burs to the following thicknesses: G1 and G5 = 0.10 mm; G2 and G6 = 0.15 mm; G3 and G7 = 0.20 mm and G4 and G8 = 0.30 mm. Dentin ceramic 0.7 mm thick was applied over these discs, sintered and glazed according manufacturer's instructions. Color was assessed with a Minolta CR-10 spectrocolorimeter on the CIE-Lab scale. Powder opaque had higher values on (L) and (ΔE) variables, and lower values on (a) and (b) variables compared to paste opaque. For opaque thickness, 0.10 mm had higher ΔE than all other thicknesses. L values were higher for 0.20 mm and 0.30 mm. Lowest and highest a* values were observed for 0.10 mm and 0.30 mm, respectively. No difference was observed for b* values. There were differences between paste and powder opaque types; 0.10 mm thickness behaves differently from the other thicknesses, with higher ΔE, while 0.15 mm does not differ statistically from thicker layers.*

***Key words:*** *Metal ceramic alloys, ceramic, dental porcelain, color.*

## CAPACIDADE DE MASCARAMENTO DE DIFERENTES ESPESSURAS DA CAMADA OPACA EM CERÂMICA ESTRATIFICADA

***RESUMO***

*O objetivo deste estudo foi avaliar a capacidade de mascaramento de duas cerâmicas opacas para metalocerâmicas aplicadas em espessuras diferentes. Foram confeccionados 80 discos metálicos de Ni-Cr (High Bond) com 16 mm de diâmetro e 1,0 mm de espessura. Os discos foram divididos em 8 grupos (n=10) e aplicadas as cerâmicas opacas (Noritake) em pasta (grupos de 1 a 4) e em pó (grupos de 5 a 8). Estas foram usinadas com pontas de óxido de alumínio até atingir as seguintes espessuras: G1 e G5 = 0,10 mm; G2 e G6 = 0,15 mm; G3 e G7 = 0,20 mm e G4 e G8 = 0,30mm de espessura. A cerâmica de dentina opaca foi aplicada (0,7 mm) e realizado o glazeamento. A cor foi avaliada com Espectrocolorímetro (Minolta CR-10) e foi aplicada análise de variância com para p<5%. Resultados: O opaco em pó apresentou valores maiores estaticamente significantes nas variáveis (L*) e (ΔE), ocorrendo o inverso nas outras duas variáveis. Quanto à espessura de opaco, os valores podem ser agrupados da seguinte forma, segundo os testes complementares aplicados: variável (L*): (0,10 = 0,15) < (0,20 = 0,30); variável (a*): (0,10) < (0,15 = 0,20) < (0,30); variável (b*): não houveram diferenças estatisticamente significantes; variável (ΔE): (0,10) < (0,15 = 0,20 = 0,30). Conclui-se que houve diferença entre os tipos de opaco em pasta e em pó e que todas as espessuras testadas, exceto a de 0,10 mm, podem ser usadas sem alteração significativa da cor.*

***Palavras Chave:*** *Ligas metalo-cerâmicas. Cerâmica. Cor.*

## INTRODUCTION

A esthetic dental restorations have always been one of the major challenges in prosthetic and restorative dentistry[1]. Even when dentists request an adequate color for an indirect dental restoration, the result is not always satisfactory and there may be a significant difference in color, the cause of which is difficult to ascertain[2].

Despite the high acceptability and longevity of metal-ceramic, its aesthetics in anterior teeth are still a major challenge as a result of the underlying metal structure [3,4]. To camouflage the metal, a layer of opaque porcelain about 0.3 mm thick is applied[4], which affords masking ability due to its high metal oxide opacifier content.[5]

The most commonly used system for evaluating color change was established by CIE – *Commission Internacionale de E'clairage* in 1976[3]. The CIE color system considers the light transmitted or reflected from an object. Since the CIE 1931 diagram did not have visual uniformity, mathematicians changed the colors in order to facilitate color readings. This improved system, called CIEL*a*b* color space or CIELab[1] color space can be used to study color in dental materials.

Previous studies have established the influence of each metal-ceramic layer on color and determined the influence of the layering pattern on the final color of dental restorations, concluding that the thickness of the layers affects the final color of a dental restoration [5-7]. The opaque ceramic application method had also been studied, evaluating its bond strength and the final metal-ceramic color[8]. No visible differences have been found in the final porcelain color between application methods. Although many studies evaluate the effect of the thickness of different layers of opaque dentin on ceramic color, few studies show which thickness suffices to mask the underlying metal. The study of opaque layer thickness is clinically relevant because it influences the amount of tooth reduction necessary to make aesthetically appropriate metal-ceramic prostheses. The aim of this study is to evaluate whether there is a thickness lower than the recommended 0.3 mm which would have comparable masking ability, using a Ni-Cr (High Bond) metal alloy and Super-Porcelain EX-3 (Noritake) ceramic.

## MATERIALS AND METHODS

Eighty circular wax discs 16 mm in diameter and 1.0 mm thick (Newwax, techNew) were obtained from a circular pattern and molded in phosphate-bonded investment (Heat Shock, Polidental). Ni-Cr discs (High Bond, Leona Indústria e Comércio de Materiais e LigasOdontológicas e Médicas Ltda.) were obtained from these molds. The discs were removed from the molds, cleaned and sandblasted with 200 µm aluminum oxide particles to remove the excess covering and the oxide layer produced by the casting. Discs were manually sanded on both sides using 200-grit water sandpaper until they formed flat, but not polished, surfaces. The discs were left in ultrasonic baths for 10 minutes with distilled water to remove debris and dried with paper towels. Using a diamond drill (1012 KG Sorenson), four marks were made on the side of the discs at 90 degrees from one another. Guided by these marks, a digital caliper (Mitutoyo, Digimatic Caliper) was used to measure disc thickness. The specimens were randomly divided into 8 groups (n=10) to be covered in one of two opaque types: G1, G2, G3 and G4 paste opaque (Noritake paste opaque, Noritake, Kizao CO.) and G5, G6, G7 and G8 powder opaque (Super Porcelain EX-3 Noritake Kizao CO.). Opaques were applied in the following thickness: G1 and G5 = 0.1 mm; G2 and G6 = 0.15 mm; G3 and G7 = 0.2 mm and G4 (control) and G8 = 0.3 mm. The discs were washed with distilled water in an ultrasonic bath and dried. Dentin opaque ceramic (Super Porcelain EX-3 Color A3 or Noritake Kizao CO.) was applied in two layers, totaling 0.7 mm thick altogether. After obtaining the adequate dentin thickness, the discs were glazed. All the sintering steps were done according to the manufacturer's recommendations. The color of the discs was measured with a portable spectro-colorimeter (Minolta CR-10) according to the CIEL*a*b* scale. The mean values of L*a*b* readings for G4 were used as control to calculate ΔE. Statistical analysis was performed on four variables (L*, a*, b* e ΔE), with two variation factors: opaque type (paste or powder) and opaque thickness (0.10 mm; 0.15 mm; 0.20 mm or 0.30 mm). All the variables fit the normal curve and homogeneity of errors. Under these circumstances, 2-way ANOVA was performed, followed by post-hoc Tukey´s test when needed, at 5% significance level.

## RESULTS

Tables 1 to 3 show means and standard deviation (L*, a*, b* and ΔE respectively) and their grouping according to post-hoc Tukey's tests. For opaque type, Tables 1 to 4 show significant statistic differences for all variables. The (L*) and (ΔE) values in groups G5, G6, G7 and G8 were greater than in groups G1, G2, G3 and G4, the opposite being true for the other two variables.

Concerning opaque thickness, the tables show that the values can be grouped as follows, according to the complementary tests applied: variable (L*): (0.10mm = 0.15mm) < (0.20mm = 0.30mm); variable (a*):(0.10mm) < (0.15mm = 0.20mm) < (0.30mm); variable (b*): there was no statistically significant difference, variable (ΔE): (0.10mm) < (0.15mm = 0.20mm = 0.30mm). Since the behavior of the two opaque types was different in all cases, a complementary statistical analysis was performed (ANOVA with a single variation factor – thickness)

to see how the variable (ΔE) behaved for each opaque type separately (Table 5). The means and standard deviation values used in this analysis are the same as those shown in Table 4.
Fig. 1 shows the respective curves for (ΔE) values according to opaque type and thickness.

## DISCUSSION

The CIE-L*a*b* scale was used, which represents color in a three-dimensional space represented by axes L* (black-white axis), a* (green-red axis) and b* (blue-yellow axis). The distance between any two points on this scale, represented by ΔE, can be obtained from the formula $\Delta E = (((L^*1-L^*2)^2+(a^*1-a^*2)^2+(b^*1-b^*2)^2)^{0.5})$ [3,4,9].
The opaque type significantly influenced the color of metal-ceramics, since they presented significant differences for all variables tested (ΔE, L*, a* and b*). Although color perception is closely linked to variations in ΔE [2,3, 5,7,9-11] it is important to evaluate

**Table 1. Means and standard deviation for L* values.**

| | Opaque thickness (mm) | | | | |
|---|---|---|---|---|---|
| | 0.1 | 0.15 | 0.2 | 0.3 | Means |
| Paste | 74.6(0.8) | 75.2(0.6) | 76.3(0.6) | 75.6(1.0) | 75.50 (α) |
| Powder | 75.4(0.6) | 75.8(0.9) | 76.5(0.3) | 76.9(0.4) | 76.12 (β) |
| Means | 75.00 | 75.49 | 76.39 | 76.2 | |
| | (a) | (a) | (b) | (b) | |

Groups with same letters were not statistically different. Lowercase represents grouping among opaque thickness while Greek letters represent grouping among opaque types.

**Table 2. Means and standard deviation for a* values.**

| | Opaque thickness (mm) | | | | |
|---|---|---|---|---|---|
| | 0.1 | 0.15 | 0.2 | 0.3 | Means |
| Paste | 2.5 (0.2) | 3.3 (0.4) | 3.0 (0.3) | 3.2 (0.2) | 3.00 (α) |
| Powder | 0.4 (0.3) | 1.1 (0.3) | 1.4 (0.3) | 2.7 (0.1) | 1.38 (β) |
| Means | 1.45 | 2.16 | 2.21 | 2.93 | |
| | (a) | (b) | (b) | (c) | |

Groups with same letters were not statistically different. Lowercase represents grouping among opaque thickness while Greek letters represent grouping among opaque types.

**Table 3. Means and standard deviation for b* values.**

| | Opaque thickness (mm) | | | | |
|---|---|---|---|---|---|
| | 0.1 | 0.15 | 0.2 | 0.3 | Means |
| Paste | 20.6 (0.6) | 20.8(0.3) | 19.7(0.6) | 20.1(0.6) | 20.26 (α) |
| Powder | 18.4(0.7) | 19.0(0.9) | 19.3(0.8) | 19.5(0.3) | 19.05 (β) |
| Means | 19.49 | 19.90 | 19.41 | 19.81 | |
| | (a) | (a) | (a) | (a) | |

Groups with same letters were not statistically different. Lowercase represents grouping among opaque thickness while Greek letters represent grouping among opaque types.

**Table 4. Means and standard deviation for ΔE values.**

| | Opaque thickness (mm) | | | | |
|---|---|---|---|---|---|
| | 0.1 | 0.15 | 0.2 | 0.3 | Means |
| Paste | 1.6 (0.2) | 1.0(0.4) | 1.0(0.6) | 1.0(0.6) | 1.14 (α) |
| Powder | 3.4(0.5) | 2.7(0.5) | 2.3(0.4) | 1.5(0.3) | 2.48 (β) |
| Means | 2.49 | 1.83 | 1.64 | 1.25 | |
| | (a) | (b) | (b) | (b) | |

Groups with same letters were not statistically different. Lowercase represents grouping among opaque thickness while Greek letters represent grouping among opaque types.

**Table 5. Grouping of ΔE values considering each opaque type separately.**

| | Opaque thickness (mm) | | | | |
|---|---|---|---|---|---|
| | 0.1 | 0.15 | 0.2 | 0.3 | Control |
| Paste | A | B | B | B | B |
| Powder | ∑ | Ω | Ω | ψ | ψ |

Groups with same letters were not statistically different. Capital letters represent grouping of ΔE varying thickness for paste opaque while Greek letters represent grouping of ΔE varying thickness for powder opaque.

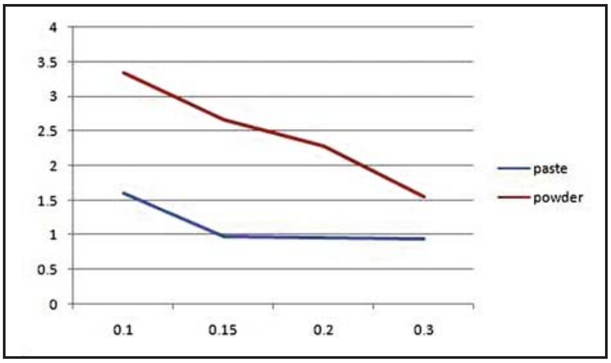


*Fig. 1: ΔE values according opaque thickness.*

the axes separately in order to understand which one contributed most to this color difference. The paste opaque was more uniform and easier to use than the powder opaque. Powder opaque tends to be more difficult to spread uniformly because of the presence of water, its surface tension and the contact angle between its surface and the metal. This may explain why paste is easier to apply, as well the differences found in all variables. Nevertheless, according to the intensity which each axis was affected, it is possible to say that the opaque type affects the "a*" axis more and the "L*" axis less, (Δa*=1.62; Δb*=1.21; ΔL*=0.62). The paste opaque presented greater redness and yellowness and was slightly less brilliant than the powder opaque. The presence of oxides tends to turn ceramic color green, and the lack of uniformity of powder opaque may lead to lower control of oxide formation, explaining this greater variation on the a* axis. Since the red color component is less present in teeth than the yellow component and variations in the red color are less tolerable than the yellow variations[2], the main variation on axis "a*" is a very important factor when choosing the opaque type to be used.

The ΔE values for paste are within the ΔE values considered little or not clinically noticeable (1 to 2), while the values for powder are within the limit of 2 to 3.3 ΔE units, considered perceptible, but clinically acceptable [9, 12-15]. A change in the referential (control group) could lead to a change in the order of this observation. The results of our study differ from Waddell and Swain,[8] who found no color difference according to the type of opaque used. This difference may be due to the opaque/ ceramic system used or to the fact that Waddel and Swain used discs without metal substrate as control. Thicker opaque layers increased the values of "L*", in contrast to other studies that used pure ceramic [16,17] or metal-ceramics [18], which stratified the dentin layer and not the opaque layer. Some studies found no (L*) variation from the opaque dentin layers [5, 7, 11]. The lack of agreement with these studies may be explained considering that the metal used in the substructure turns dark after oxidation, and smaller, or more irregular, opaque layers tend to favor light absorption by this oxidized metal. As the metal becomes adequately covered by the opaque, light absorption is expected to decrease until it is stable at a certain value of L*. On the other hand, the dentin layer can lead to greater light scattering and/or light absorption by the pigment oxide particles present in it [4, 16]. Variations in dentin and enamel thickness lead to variations in the final color of the ceramics and this was the reason why in this study it was chosen to add a simple opaque dentin layer 0.7 mm thick. Future studies can evaluate the effect of the opaque layer stratification in combination with stratification of the dentin and enamel layers to better understand the interference of these factors in the final color of metal-ceramic restorations. The variations in the values of a* represented a single axis where the values of 0.20 thickness differed from the values of 0.30 thickness. Generally the values of 0.30 thickness were more reddish than in 0.20 and 0.15 thicknesses; however, despite being statistically significant, this difference was in the order of 0.7 points, which constitutes color variation which is not noticeable *per se*. Although Douglas and Brewer[2] report major perception capacity of changes in the red color component, they claim that these changes in the red component are noticeable just above 1.1 points, which did not usually happen between the 0.15 and 0.30 thicknesses. The results show that the paste opaque was even more effective in this aspect, because the component (a*) variations were at most 0.3 points between the 0.15 and 0.30 thicknesses. To calculate the values of ΔE, the average values of opaque paste L*a*b* of "0.30" thickness were used, because it is considered an adequate thickness for metal-ceramic dental restorations and because it is easier to cover with this opaque. It is relevant that on all axes, the 0.10 thickness differed from the others and thus presented higher ΔE than the control group. Other authors report the inability of a 0.10 mm layer to mask non-noble metals [19] or metals with high palladium content [20,21], which is in agreement with our study. The observation that 0.20 mm opaque thickness is sufficient to mask the metal is consistent with previous findings [22], but differs from what the same author mentioned in another study, which found similarity only in the color [23]. In our study, however, statistically, even 0.15 mm thickness can be considered the same as 0.30 mm thickness. This statement is particularly true to the paste opaque, where it is possible to graphically observe the stabilization of the ΔE value in all the thickness from 0.15. This graphic observation confirms the statistic result that the color variations from one 0.15 mm paste opaque thickness derive from the inherent variations in the making process of the metal-ceramic dental restoration and not from the thickness variation in the opaque layer, which are all below the 1 point perceptible limit. In a specific analysis on paste opaque, it is noteworthy that although there is a statistically significant difference between the 0.10 thickness and the others, ΔE for this thickness is within the range of not clinically perceptible (ΔE “0.10” =1.6), which is even more significant when we consider the color variation inherent to the ceramic production process found in the other thickness (e.g.: ΔE “0.30” = 0.95). In “0.10” thickness, and for this opaque type, color was affected most by the “L*” and “a*” axes. Future studies can ascertain whether the 0.10 mm thickness can be clinically acceptable when compared to the 0.30 mm thickness and/or if the addition of pigments can improve it at this thickness. For the powder opaque, however, it should be observed that the values of ΔE for “0.30” thickness differ from the ΔE values for “0.20” and “0.15” thicknesses, which are both the same. The data axes analysis, in particular, shows that the axis which contributed the most to this variation was the “a*” axis. Future studies can help elucidate whether the addition of red pigments to the powder opaque would enable its application in layers thinner than 0.30 mm, such as 0.20 mm and 0.1 5 mm. Some studies noted that the kind of metal used may change the color values in the metal-ceramic final restoration [14-16] as well as in the color of the opaque layer [19, 21, 24, 25]. In this regard, our study has the limitation of having used only the Ni-Cr alloy, which was chosen because it is a type of alloy very often used in the Brazilian market due to its low price. The influence of opaque type used combined with the metal variations may be the subject of a future study. Furthermore, and taking into consideration the limitations of our study, only the opaque dentin color was used (A3), without adding enamel. This decision was taken because earlier observations showed that thicker ceramic layers provide less noticeable background color, in this case, from the opaque layer, which is why we did not use enamel ceramic [4, 16]. Another reason for not using more than one color or layering dentin/enamel was to simplify the statistic model. Other studies may be necessary to clarify whether the stratification of these layers allows the use of the thickness mentioned or whether the 0.10 mm thickness is feasible with the stratification of the upper layers.

Based on the experimental procedures performed and taking into consideration the limitations of this study, it is possible to conclude that:

a) There is a difference between the powder and paste opaque; the paste opaque provides more predictable results than the powder opaque;
b) The 0.10 mm thickness behaves differently from the other thickness, showing greater ΔE; the 0.10 mm thickness proved unsatisfactory for masking the metal substructure;
c) The ΔE for 0.15 mm thickness is statistically the same as the others, showing that it is sufficient to mask the metal used in both ceramic systems.

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## REFERENCES

1. Goveia JC. Estudo colorimétrico da translucidez de materiais restauradores odontológicos [Dissertation]. São Paulo: University of São Paulo- USP; 2004.
URL: http://www.teses.usp.br/ teses/disponiveis/23/23134/tde-06062005-163034/publico/GoveiaJC.pdf. Accessed in 2016/07/11
2. Douglas RD, Brewer JD. Variability of porcelain color reproduction by commercial laboratories. J Prosthet Dent 2003; 90:339-346.
3. Douglas RD, Przybylska M. Predicting porcelain thickness required for dental shade matches. J Prosthet Dent 1999; 82:143-149.
4. Goodacre CJ. Designing tooth preparations for optimal success. Dent Clin North Am 2004; 48:359-385.
5. Corciolani G, Vichi A, Louca C, Ferrari M. Influence of layering thickness on the color parameters of a ceramic system. Dent Mater 2010; 26:737-742.
6. Fazi G, Vichi A, Corciolani G, Ferrari M. Spectrophotometric evaluation of color match to VITA classical shade guide of

four different veneering porcelain systems for metal ceramic restorations. Am J Dent 2009; 22:19-22.
7. Corciolani G, Vichi A, Louca C, Ferrari M. Color match of two different ceramic systems to selected shades of one shade guide. J Prosthet Dent 2011;105:171-176.
8. Jorn D, Waddell JN, Swain MV. The influence of opaque application methods on the bond strength and final shade of PFM restorations. J Dent 2010; 38 Suppl 2:e143-149.
9. Xu BT, Zhang B, Kang Y, Wang YN, Li Q. Applicability of CIELAB/CIEDE2000 formula in visual color assessments of metal ceramic restorations. J Dent. 2012; 40 Suppl 1:e3-9.
10. Douglas RD, Steinhauer TJ, Wee AG. Intraoral determination of the tolerance of dentists for perceptibility and acceptability of shade mismatch. J Prosthet Dent. 2007; 97: 200-208.
11. Corciolani G, Vichi A, Goracci C, Ferrari M. Colour correspondence of a ceramic system in two different shade guides. J Dent. 2009; 37: 98-101.
12. Ragain JC Jr, Johnston WM. Minimum color differences for discriminating mismatch between composite and tooth color. J Esthet Restor Dent. 2001; 13: 41-48.
13. Goiato MC, Zuccolotti BC, Moreno A, dos Santos DM, Pesqueira AA, Dekon SF. Colour change of soft denture liners after storage in coffee and coke. Gerodontology. 2011; 28:140-145.
14. Imirzalioglu P, Karacaer O, Yilmaz B, Ozmen Msc I. Color stability of denture acrylic resins and a soft lining material against tea, coffee, and nicotine. J Prosthodont. 2010; 19:118-124.
15. Ergun G, Nagas IC. Color stability of silicone or acrylic denture liners: an in vitro investigation. Eur J Dent. 2007; 1:144-151.
16. Dozic A, Kleverlaan CJ, Meegdes M, van der Zel J, Feilzer AJ. The influence of porcelain layer thickness on the final shade of ceramic restorations. J Prosthet Dent. 2003; 90:563-570.
17. Ozturk O, Uludag B, Usumez A, Sahin V, Celik G. The effect of ceramic thickness and number of firings on the color of two all-ceramic systems. J Prosthet Dent 2008; 100:99-106.
18. Jacobs SH, Goodacre CJ, Moore BK, Dykema RW. Effect of porcelain thickness and type of metal-ceramic alloy on color. J Prosthet Dent 1987; 57:138-145.
19. Ozcelik TB, Yilmaz B, Ozcan I, Kircelli C. Colorimetric analysis of opaque porcelain fired to different base metal alloys used in metal ceramic restorations. J Prosthet Dent 2008; 99:193-202.
20. Stavridakis MM, Papazoglou E, Seghi RR, Johnston WM, Brantley WA. Effect of different high-palladium metal-ceramic alloys on the color of opaque and dentin porcelain. J Prosthet Dent 2004; 92:170-178.
21. Stavridakis MM, Papazoglou E, Seghi RR, Johnston WM, Brantley WA. Effect of different high-palladium metal-ceramic alloys on the color of opaque porcelain. J Prosthodont 2000; 9:71-76.
22. Barghi N, Richardson JT. A study of various factors influencing shade of bonded porcelain. J Prosthet Dent 1978; 39:282-284.
23. Barghi N, Lorenzana RE. Optimum thickness of opaque and body porcelain. J Prosthet Dent 1982; 48:429-431.
24. Yilmaz B, Ozcelik TB, Wee AG. Effect of repeated firings on the color of opaque porcelain applied on different dental alloys. J Prosthet Dent 2009; 101:395-404.
25. Ozcelik TB, Yilmaz B, Ozcan I, Wee AG. Color change during the surface preparation stages of metal ceramic alloys. J Prosthet Dent 2011; 106:38-47.

# SEQUENTIAL ADMINISTRATION OF ALENDRONATE AND STRONTIUM RANELATE: HISTOMORPHOMETRY AND BONE BIOMECHANICS IN OVARIECTOMIZED ANIMALS

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***ABSTRACT***

*Bisphosphonates are the first choice therapy for the pharmacological treatment of osteoporosis. Following reports of cases of bisphosphonate-related osteonecrosis of the jaw and atypical femur fracture, the safety of long-term use of bisphosphonates has been evaluated, resulting in the proposal of strontium as an alternative drug. No experimental study using a sequential administration design has been reported to date. Hence, the aim of this study was to evaluate the effect on bone tissue of ovariectomized rats of administration of alendronate followed by strontium ranelate. Forty-eight female Wistar rats were ovariectomized on day 1 of the experiment. Beginning on day 30, they were administered 0.3 mg/kg/week of alendronate (ALN) or vehicle (VEH) for 8 weeks. Two groups (ALN and corresponding control) were euthanized at this time, and the remaining animals were divided into 4 groups and given 290 mg/kg/day of strontium ranelate (SR) in their drinking water (TW) or only water for 4 months. Experimental groups were: ALN+SR, ALN+TW, VEH+SR, VEH+TW, ALN and VEH. The tibiae and hemimandibles were resected for histomorphometric evaluation, and the right femur was used to perform biomechanical studies. ANOVA and Bonferroni test were applied. Diaphyseal stiffness, maximum elastic load and fracture load increased in animals that received alendronate, regardless of whether or not they received subsequent SR treatment. Fracture load also increased in VEH+ SR versus control (VEH+TW). Subchondral and interradicular bone volumes were significantly higher in animals that received ALN than in those that received vehicle. No difference was observed in cortical area or thickness of the tibia among treatments. The results obtained with the model presented here, evaluating tibial and mandibular interradicular bone, showed that the combination of ALN and SR and administration of ALN alone are equally effective in preventing bone loss associated with ovariectomy-induced estrogen depletion.*

**Key Words:** *Bone tissue, alendronate, strontium ranelate.*

# ADMINISTRACIÓN SECUENCIAL DE ALENDRONATO Y RANELATO DE ESTRONCIO: HISTOMORFOMETRÍA Y BIOMECÁNICA ÓSEA EN ANIMALES OVARIECTOMIZADOS

***RESUMEN***

*Si bien la primera opción terapéutica para el tratamiento farmacológico de la osteoporosis son los bisfosfonatos (BPs), luego de los primeros reportes en 2003 de los casos de osteonecrosis de mandíbula asociada al uso de dichas drogas y las fracturas atípicas de fémur, se ha evaluado su seguridad a largo plazo. Además, en aquellos pacientes que no responden al tratamiento con BPs y mantienen elevado el riesgo de fractura, es necesario suspender su administración y alternar con otras drogas. Una de las que se ha utilizado en la clínica luego del tratamiento con BPs es el ranelato de estroncio (SR). Existen varios trabajos clínicos que reportan los efectos de la administración secuencial de ambas drogas, aunque estudios experimentales con un diseño secuencial aun no se han reportado. Por ello el objetivo de este trabajo ha sido evaluar el efecto de la administración secuencial de alendronato, seguido de ranelato de estroncio sobre el tejido óseo de ratas ovariectomizadas. Se utilizaron 48 ratas Wistar hembras de dos meses de edad divididas en 6 grupos de 8 animales cada uno. El día 1 de experiencia todas fueron ovariectomizadas. El día 30 se comenzó con la administración de alendronato (ALN) en una dosis de 0.3 mg/kg/semana o vehículo (VEH) durante 8 semanas. Luego de este período se sacrificaron dos grupos (uno que recibió ALN y su correspondiente control (sólo vehículo). Los cuatro grupos restantes continuaron con ranelato de estroncio (SR) en el agua de bebida durante 4 meses en una dosis de 290 mg/kg/día o sólo agua corriente( TW) Luego de ese período fueron eutanasiados. Así, los grupos experimentales conformados fueron: ALN+SR, ALN+TW, VEH+SR, VEH+TW, ALN y VEH. Para los estudios histomorfométricos se extrajeron ambas tibias y hemimandíbulas; para el estudio biomecánico se utilizó el fémur derecho. Los resultados fueron analizados mediante el test de ANOVA y el test de Bonferroni. Incrementaron significativamente la rigidez diafisaria, la carga elástica límite y la carga de fractura aquellos grupos que recibieron alendronato versus aquellos que no lo recibieron, independientemente del tratamiento posterior con SR. La carga de fractura además fue mayor en el grupo VEH+SR versus el control (VEH+TW). En cuanto al volumen óseo subcondral e interradicular evaluado histomorfométricamente fue significativamente mayor en aquellos animales que recibieron ALN versus aquellos que recibieron vehículo. No se detectaron diferencias entre aquellos grupos que recibieron SR y sus controles. El área y espesor cortical de la tibia no mostraron diferencias entre grupos. Los resultados obtenidos en el modelo estudiado tanto a nivel del volumen óseo subcondral y cortical de la tibia como a nivel del hueso interradicular del maxilar inferior, mostraron que la combinación de ALN con SR y la administración aislada de ALN son igualmente efectivas para prevenir la pérdida ósea causada por la depleción estrogénica de la ovariectomía.*

**Palabras clave:** *Tejido óseo, alendronato, ranelato de estroncio.*

## INTRODUCTION

Osteoporosis is a systemic skeletal disease characterized by low bone mass and structural deterioration of the bone tissue leading to bone fragility and increased risk of fracture. There are currently various options for pharmacological treatment of this common pathology: use of anti-catabolic agents (bisphosphonates [BPs], selective estrogen receptor modulators and denosumab), anabolics (peptides of the parathyroid hormone family) or an agent with dual action mechanism (strontium ranelate, SR)[1]. For decades, BPs have proven to be safe, effective drugs, and are therefore one of the most often used for pharmacological treatment of osteoporosis[2,3]. However, regarding long-term safety of BPs, adverse effects have been reported which, though rare, are cause for concern: osteonecrosis of the jaw[4] and atypical femur fractures[5]. It should be highlighted that in addition, some patients do not respond to BP treatment and remain at high risk of fracture. Hence, patients who have received BPs for a long time, or who do not respond adequately to the treatment, should stop taking them[6,7] and alternate with other drugs. One of the drugs which has been used clinically after treatment with BPs is strontium ranelate (SR)[8-11]. In general, papers describe better response in bone mineral density (BMD) in BP-naïve patients than in patients previously treated with BPs[8,9,11]. They also report that during the first 6 months' treatment with SR following treatment with BPs, patients have an attenuated response in BMD, which varies according to the site evaluated (spine, hip or ankle)[9]. This period during which patients respond less to densitometrically evaluated treatment agrees with other authors' reports of improvement in the histomorphometric parameters of the iliac crest at 12 months but not at 6 months after suspending BPs[10]. BPs are powerful inhibitors of bone resorption and bone turnover general. They become included in the bone matrix on the mineralization fronts due to their great affinity for hydroxyapatite[12]. Once included, they are not released until subsequent cycles of bone remodeling take place, during the resorption or reversal phase, and can therefore continue to exert their effect for years. SR is a drug composed of ranelic acid and strontium, in which the active component is strontium, which becomes included in the bone matrix at the formation fronts. In cases where BPs and SR are administered sequentially, the inhibitory effect on bone turnover caused by BPs may cause SR to be incorporated less, generating a weaker clinical response in patients.

The literature includes clinical studies such as Rizzoli *et al.*, which used high-resolution peripheral quantitative computed tomography to study the effect of ALN and SR administered separately to women with osteoporosis[13,14]. Other authors studied the effect of sequential administration of these two drugs[15] on formation and resorption markers. Still others analyzed the densitometric response to SR of patients who had previously received BP compared to those who had never received BP (BP-naive)[8-11]. Experimental studies have compared the effect of BPs to the effect of SR[16,17] or evaluated the combined effect of both drugs administered separately or together,[18] but not sequentially.

Even though the most frequent clinical situation is sequential administration of BP and SR, to date, the literature contains no experimental evidence of the effect on bone of BP followed by SR. The aim of this study is thus to evaluate the effect of administering alendronate followed by strontium ranelate on bone tissue in ovariectomized rats.

## MATERIALS AND METHODS

### Experimental design

Forty-eight 2-month-old female Wistar rats weighing 180±10 g were used, which were ovariectomized at the beginning of the experiment. They were housed in groups of six animals per cage, with a 12-hour light, 12-hour darkness cycle, temperature 22±2°C and humidity 52-56 %, fed *ad libitum* (standard balanced feed, Cooperación, Argentina, which contains 23% protein, 1-1.4% calcium and 0.5-0.8% phosphorus) and water. Use, care and treatment of the animals followed National Research Council standards (Guide for the Care and Use of Laboratory Animals, 8th Ed. 2011) and guidelines approved by the Ethics Committee of the School of Dentistry of Buenos Aires University (28/11/2012-38).

The animals were divided into 6 groups (n=8 per group): Alendronate + Strontium Ranelate (ALN+SR), Vehicle + Strontium Ranelate (VEH+SR), Alendronate + Water (ALN+TW), Vehicle + Water (VEH+TW), Alendronate (ALN) and Vehicle (VEH), as shown in Fig 1. They were euthanized using a solution based on sodium pentobarbital and sodium diphenylhydantoin (Euthanyle, Laboratorios Brouwer, Argentina), after which both tibiae, right femur and hemimandibles were extracted from each animal.

**Morphometric analysis**
Right femurs (free from any soft tissues by dissection) were weighed on precision scales (E. Mettler, Zürich, Switzerland) immediately after dissection and prior to fixing. Their length was measured using a Vernier-type caliper and they were kept for biomechanical analysis. They were preserved by freezing at -70°C without fixing.

**Biomechanical analysis**
For the three-point flexural test, the test machine (Instron model 4442, Instron Corporation, Canton, MA, USA) was operated in stroke control at a constant rate of 5mm/min, in order to determine diaphyseal stiffness and maximum elastic load[19-21].

**Histology and histomorphometry**
**Decalcified sections**
Left tibias and hemimandibles were fixed in formalin buffer for 48 hours and then decalcified in EDTA at pH 7 for one month at room temperature.
Left tibias were processed for hematoxylin and eosin staining, and longitudinal sections were made of the proximal epiphysis. An area 1 mm high by 1.5 mm wide beneath the mataphyseal cartilage was analyzed in order to determine subchondral bone volume (BV/TV, %). The area studied included primary and secondary spongy bone (Fig 2).
Hemimandibles were processed to obtain histological sections stained with hematoxylin and eosin oriented mesio-distally at the level of the first lower molar. Interradicular bone volume was measured in a zone comprised between a tangent line at the level of root apexes and the space limited by the roots of the first molar (BV/TV, %) (Fig 3).
For histomorphometric measurement of sections, digital micrographs were taken with a brightfield photomicroscope (Axioskop 2, Carl Zeiss Jena, Germany) and measured using Image Pro Plus 6.1 software (Media Cybernetics).

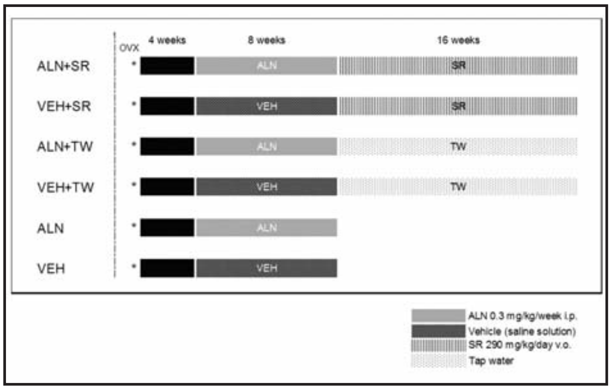


*Fig. 1: Experimental design. One month after ovariectomy (OVX), groups ALN, ALN+SR and ALN+TW were given a weekly i.p. injection of alendronate (Gador SA) in a single 0.3 mg/kg dose diluted in sterile saline, for 8 weeks (8 doses) between 11 a.m. and 12 a.m. The dose was adjusted to each animal according to its weight immediately prior to injection. Groups VEH, VEH+SR and VEH+TW were injected with an equivalent volume of saline over the same period. After two months of weekly alendronate or vehicle injections, groups ALN and VEH were sacrificed. Alendronate or vehicle was suspended in the four remaining groups. Groups ALN+SR and VEH+SR were then administered SR in their drinking water at a dose of 290 mg/kg/day p.o., while groups ALN+TW and VEH+TW were only given tap water. Four months later, all animals were euthanized.*

**Non-decalcified sections**
Right tibias were embedded in methyl methacrylate. In order to determine cortical thickness (Ct.Th, μm) and cortical area (Ct. Ar, $\mu m^2$) histomorphometrically, the area to be evaluated was first standardized by means of two transversal cuts at the level of the diaphysis: one at 15 and another at 17 mm from the proximal epiphysis, providing a section 2 mm thick. This section was manually worn down with decreasing grits to provide thin sections which were mounted with Canada balsam. Digital micrographs were taken with epifluorescent microscopy. The full cross section was reconstructed

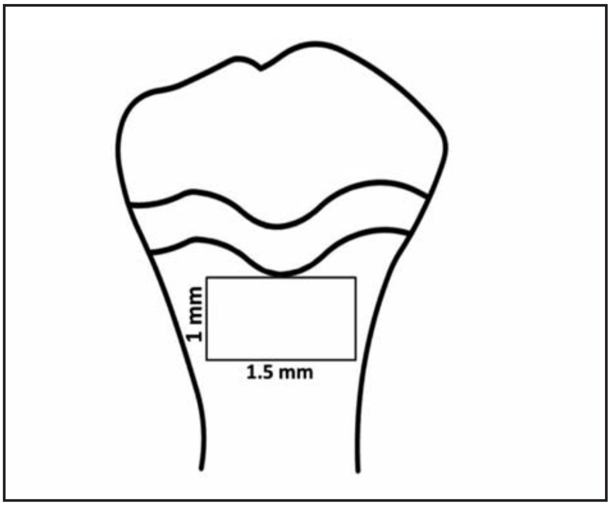


*Fig. 2: Area evaluated in tibia. An area 1mm high by 1.5 mm wide was marked in the subchondral trabeculae zone of the proximal epiphysis, located in the central zone immediately beneath the growth cartilage.*

by means of photomontage. Measurements were taken using Image Pro Plus on these photomontages. To measure cortical thickness, 4 parallel lines were drawn perpendicular to the bone surface on each face of the tibia. The 12 values for each section were averaged. To determine cortical area on the same photomontages, the area covered by the medullary canal was subtracted from the total area of the section (Fig. 4).

Names and abbreviations follow the recommendations of the American Society for Bone and Mineral Research[22, 23].

**Statistical analysis**

Results are expressed as arithmetic mean and standard deviation. All results were analyzed using one-way Anova and post-hoc Bonferroni. Data were analyzed using Primer software (Mc Graw-Hill 1992). Values of p lower than 0.05 were considered statistically significant. Some parameters were also tested using the hypothesis test for planned comparisons by means of the orthogonal contrast technique (SSPS Statistics, IBM USA). The three contrasts used were: groups receiving alendronate vs. groups receiving vehicle (whether or not they received strontium ranelate), groups receiving alendronate combined with strontium ranelate vs. groups receiving only alendronate and groups receiving only strontium ranelate vs. those not receiving any treatment.

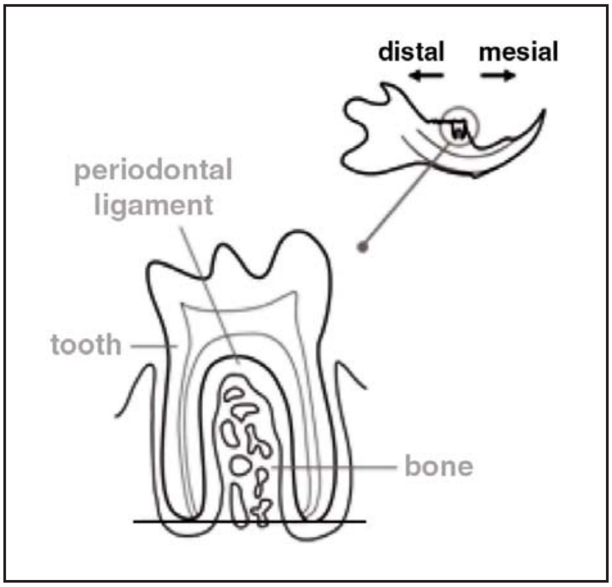


*Fig. 3: Interradicular bone tissue area evaluated. We evaluated the area included between the roots of the first lower molar, apically delimited by a line tangential to both apices. The top right corner shows the position of the molar studied in the mandible.*

**RESULTS**

**Morphometric analysis**

For femur length and weight, it was found that femurs of animals euthanized 7 months after the beginning of the experiment were significantly heavier than those of animals euthanized 3 months after the beginning of the experiment (Table 1).

**Biomechanical analysis**

The following values were recorded for diaphyseal stiffness (N/mm): ALN+SR: 398.8±50.1 (n=6), ALN+TW: 421±36.7 (n=8), VEH+SR: 384.8±47.5 (n=6), VEH+TW: 337.0±85.5 (n=8), ALN: 356.4±60.0 (n=4), VEH: 329.0±32.9 (n=4), Anova

**Table 1. Femur weights and lengths.**

| | Weight (g) | Lenght (mm) |
|---|---|---|
| ALN+SR | 0.987±0.087* | 36.54±0.38 |
| ALN+TW | 1.007±0.098* | 36.63±1.19 |
| VEH+SR | 0.951±0.052* | 36.95±0.88 |
| VEH+TW | 0.936±0.1* | 37.02±0.99 |
| ALN | 0.748±0.110# | 36.96±1.96 |
| VEH | 0.729±0.094# | 37.36±2.60 |

Weight: Anova p=0.001, Bonferroni groups ALN+SR, ALN+ TW, VEH+SR and VEH+TW vs. ALN and VEH; Length: Anova p=0.91. Different symbols (* and #) indicate statistically significant differences.

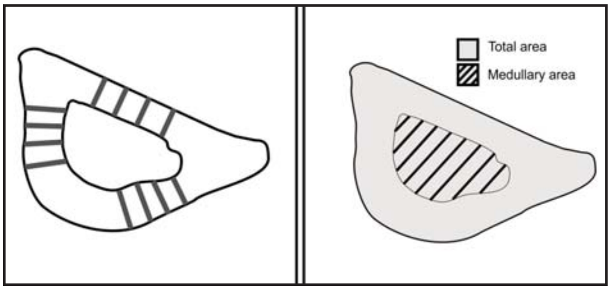


*Fig. 4: Determination of cortical thickness and cortical area. Left: cortical thickness was determined by measuring four lines perpendicular to the endosteal and periosteal surfaces of the three faces of the tibia. Right: cortical volume was determined by measuring total area occupied by the bone tissue and the medullary cavity (area shaded in gray), from which the area occupied by the medullary space (lined area) was subtracted.*

p=0.059. In addition, orthogonal contrasts detected differences between groups that received alendronate vs. those that did not (ALN+SR and ALN+TW vs. VEH+SR and VEH+SR) (Fig 5).
Maximum elastic load (N) showed the same trend: ALN+SR: 111±17.8 (n=6), ALN+TW: 116.1±30.1 (n=8), VEH+SR: 104.8±12.4 (n=6), VEH+TW: 83.4±19.3 (n=8), ALN: 103.0±33.8 (n=4), VEH: 87.7±22.5 (n=4), Anova p=0.043, Bonferroni ALN+TW vs. VEH+TW. Orthogonal contrasts: there are differences between groups that received alendronate vs. groups that did not (ALN+SR and ALN+TW vs. VEH+SR and VEH+SR) (Fig. 5).
Fracture load (N): ALN+SR: 165.8±32.7 (n=6), ALN+TW: 170.2±30.1 (n=8), VEH+SR: 164.1± 18.7 (n=6), VEH+TW: 141.7±25.5 (n=8), ALN: 125.5±35.5 (n=4), VEH: 147.7±13.8 (n=4), Anova p=0.02, Bonferroni ALN+SR vs. VEH+TW, ALN+TW vs. ALN+TW and VEH+SR vs. VEH+ TW. Orthogonal contrasts did not detect any differences (Fig. 5).

**Histology and histomorphometry**

The tibia and maxillary sections were evaluated qualitatively. Tibia sections from animals that received alendronate and strontium ranelate (ALN+ SR) or alendronate followed by tap water (ALN+ TW) showed a zone of high trabecular density corresponding to the period during which they received alendronate. Between this high-density trabecular zone and the growth cartilage, there was a zone with fewer trabeculae, corresponding to the period of time during which the animals received strontium ranelate or only tap water (Fig 6).
In addition, giant osteoclasts were observed in all groups that received ALN (ALN, ALN+SR and ALN+TW) (Fig 7). In groups ALN+TW and ALN+SR these osteoclasts were observed both in the high-density trabecular zone corresponding to the 8 weeks during which they received BP and in recent trabecular zones formed during the BP-free period.

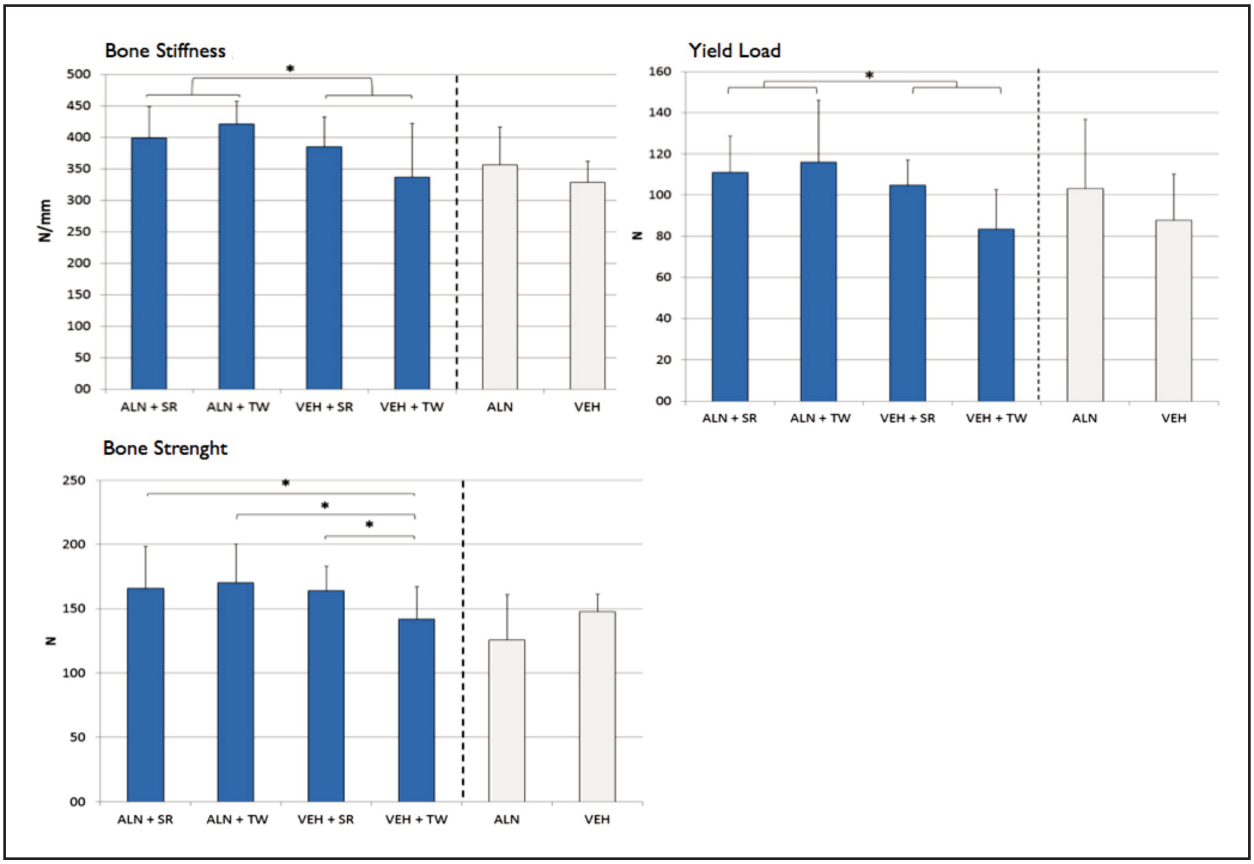


*Fig. 5: Biomechanical results. Diaphyseal stiffness showed differences between groups that received ALN (ALN+SR and ALN+TW) versus those that did not (VEH+SR and VEH+TW). Maximum elastic load showed the same tendency as diaphyseal stiffness, with differences between the groups that received ALN (ALN+SR and ALN+TW) versus those that did not (VEH+SR and VEH+TW). Fracture load showed differences between all groups versus the absolute control: ALN+SR, ALN+TW and VEH+SR vs. VEH+TW.*

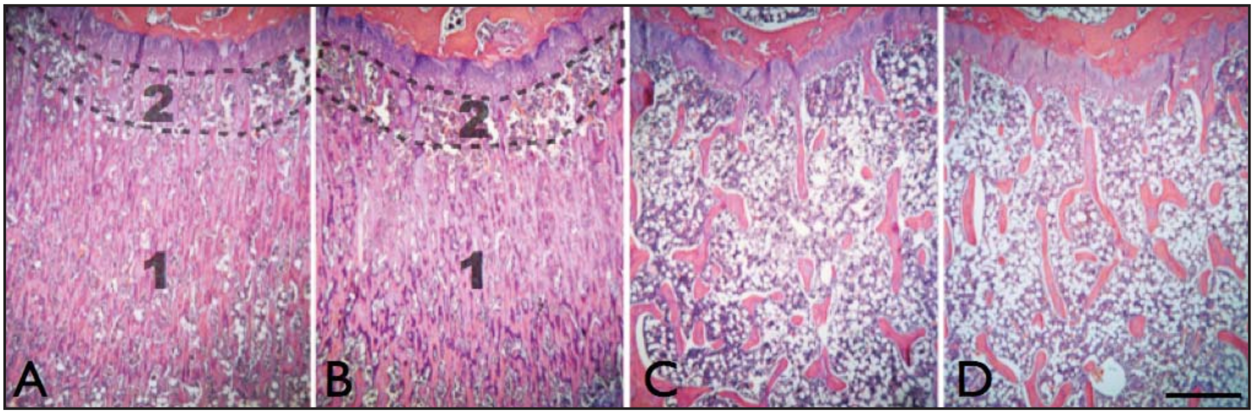


*Fig. 6: Subchondral bone volume in tibia. Animals that received ALN ( A and B) had a zone of high trabecular density (1). During the bisphosphonate-free period, they developed a less dense zone (2) between the growth cartilage and the zone with high trabecular density; whether during that period they received strontium ranelate (A) or tap water (B). The animals that received strontium ranelate only (C) had bone volume similar to the control group (D). Bar, 1000 µm.*

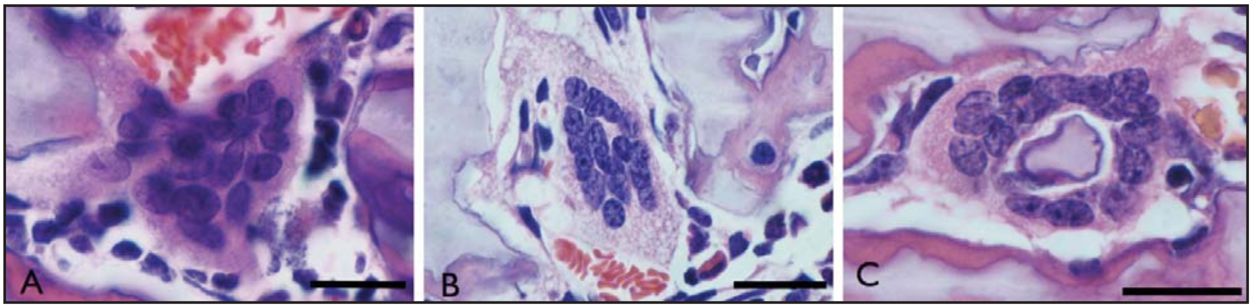


*Fig. 7: Giant osteoclasts. Present in all groups treated with alendronate (ALN+SR, ALN+VEH and ALN). A: partially active giant osteoclast in group ALN+SR in the trabecular zone formed during the period in which alendronate was administered. B: partially active osteoclast in group ALN+VEH, with cytoplasmic heterogeneity. C: osteoclast in group ALN, surrounding a mixed trabecula. Bar, 30 µm.*

The same occurred with the apoptotic forms of osteoclasts, which were present in the animals treated with ALN, in both tibia (Fig. 8 A) and maxillae (Fig. 8 B).

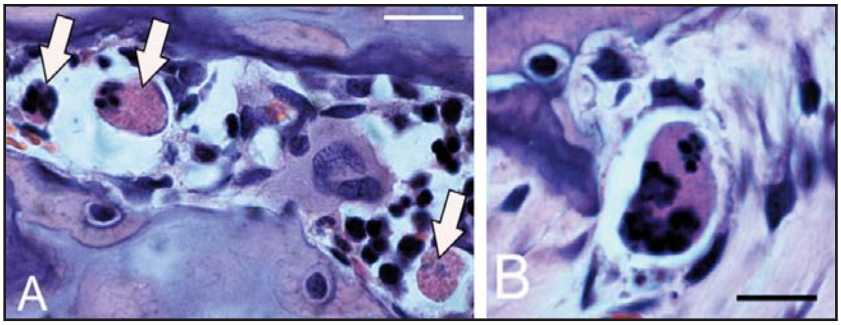


*Fig. 8: Apoptosis in osteoclasts. Apoptotic forms of osteoclasts were present in all animals in the groups that received ALN, both in tibia (arrows, A), and in interradicular bone (B). Bar, 20 µm.*

It was also noted that there were intensely basophilic zones on the bone surface of animals treated with bisphosphonates, both on tibiae and on maxillae, corresponding to apposition lines (Fig 9 A). This was observed both in the group that was euthanized after receiving alendronate for eight weeks, and in the groups which had a BP-free period (ALN+TW and ALN+SR). These basophilic surface areas were also apparent in the interradicular bone, where reversal lines could also be clearly seen (Fig. 9 B), and were located on the bone matrix delimiting the lacuno-canalicular system of some superficial osteocytes (Fig. 9 C and D).

**Static histomorphometry**

Fig. 10 shows the bone volume results for tibiae and hemimandibles.

Tibia cortical thickness (Ct.Th, µm) showed no significant variation among groups: ALN+SR: 545.66±32.16, ALN+TW: 554.06±47.23, VEH+SR: 534.12±55.78, VEH+TW: 522.65±33.9, ALN: 487.33 ±24.63, VEH: 478.35± 33.56. Anova p=0.58. Cortical area (Ct.Ar $\mu m^2$): ALN+SR: 5.05±0.47, ALN+TW: 5.02±0.40, VEH+SR: 4.78±0.29, VEH+TW: 4.74±0.37, ALN: 4.58 ±0.63, VEH: 4.71±0.20. Anova p=0.34.

**DISCUSSION**

The results of this study show that administering bisphosphonate (alendronate) followed by strontium ranelate produces the same effect on bone as administering bisphosphonate alone.

To date, there are few studies on animals using alendronate and strontium ranelate[16-18], and none using them sequentially.

Biomechanics showed that administering alendronate makes femurs stiffer, at the same time increasing maximum elastic load and fracture load, while administering strontium ranelate alone also improves femur fracture load. However, sequential administration of the two drugs showed no difference compared to administration of either bisphosphonate alone or strontium ranelate alone.

A paper by Chen et al.[17] partially agrees with our findings for long bones, as it reports that administering alendronate to ovariectomized rats improves the biomechanical properties of the femur and increases subchondral bone volume, but it also describes histomorphometric differences between groups that received strontium ranelate and the

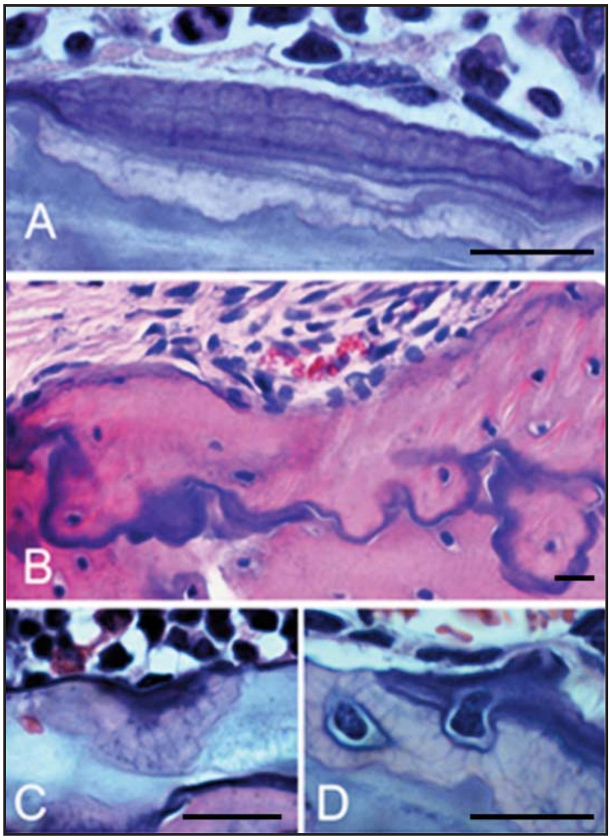


*Fig. 9: Intensely basophilic zones in animals treated with ALN in tibia and maxillae bone tissue. Appositional lines strongly stained with hematoxylin, in tibia (A), and in the reversal lines of maxillaries (B). Basophilia of bone matrix in bone tissue delimiting part of some superficial osteocyte lacunae (C and D). Bar, 20 µm.*

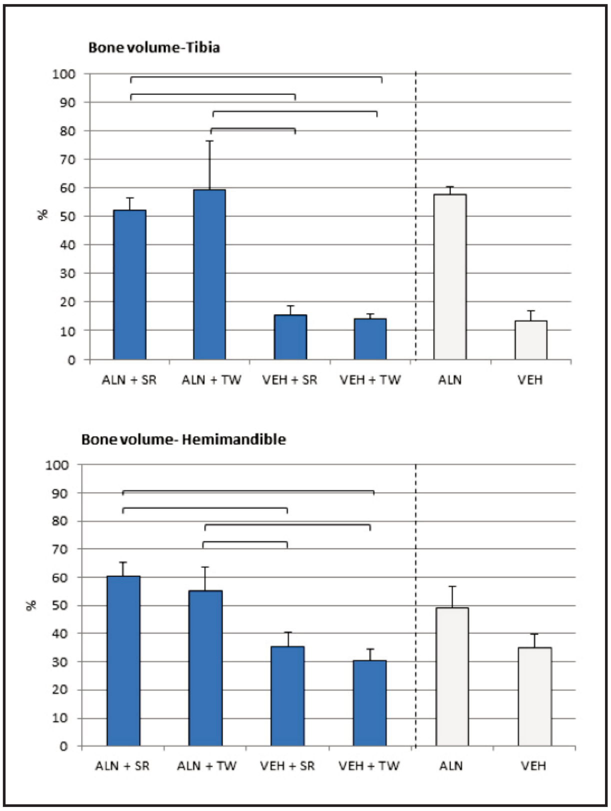


*Fig. 10: Bone volume in tibias and hemimandibles. For tibia there were statistically significant differences between animals that received ALN versus those that did not (ALN+SR and ALN+TW vs. VEH+SR and VEH+TW; Anova p<0.001, post-hoc Bonferroni). For hemimandibles, there were also differences between groups with and without ALN (ALN+SR and ALN+TW vs. VEH+SR and VEH+TW).*

control, which was not observed in our study. Sun et al.[16] used a model of bone loss associated to administration of glucocorticoids and also reported that alendronate and strontium ranelate were equally efficient at preventing bone loss (measured by densitometry and histomorphometry) and at improving biomechanical properties.

Interradicular bone showed the same behavior as long bones regarding bone volume, with less marked differences. Considering that in rat subchondral bone there is constant presence of trabeculae produced by endochondral ossification which does not cease throughout the animal's lifetime, any changes in the bone are more evident in interradicular bone, which has already completed its formation.

Once bisphosphonates are deposited in the bone matrix, they can only be released into circulation by bone resorption performed by osteoclasts. Considering that bisphosphonates affect the osteoclast, and therefore also resorption, the bisphosphonates themselves prolong the time they remain in the bone matrix. It is estimated that after suspending treatment, bone tissue releases quantities of bisphosphonate equivalent to 25% of the dose absorbed while actively receiving the drug[24]. Considering this aspect of bisphosphonates, in our experimental design we decided that the bisphosphonate-free period should be double the time during which it was administered, with the aim of observing its residual effects. It is worth highlighting that no change in bone volume was found between the group that received alendronate and was immediately euthanized (group ALN) and the groups euthanized after 4 months (ALN+SR and ALN+TW) (statistics not included in this paper), indicating a residual effect of ALN. This effect also was also apparent in the persistence of giant osteoclasts and apoptotic forms of osteoclasts in animals from the groups ALN+SR and ALN+ TW. Both effects are known to be produced by aminobiphosphonates[25-30].

The persistence of the effects of alendronate deposited in the matrix and the reduction in bone turnover that it produces is the most probable cause of the lack of effect of strontium administered after alendronate. It should be taken into account that strontium is deposited at bone formation sites where there are active BMUs in that phase of the remodeling cycle. As bisphosphonates cause a lower BMU activation frequency, they reduce the sites at which strontium may be deposited, as shown by Wu et al. in the model administering risedronate and strontium ranelate simultaneously, using Electron Probe Micro-Analysis[18]. Moreover, strontium ranelate is described as a dual agent: anti-catabolic and anabolic. Due to the fact that bisphosphonates inhibit remodeling, they may inhibit the anabolic effect of strontium. Although in our study the effects of strontium ranelate alone were subtle compared to the effects of bisphosphonates, they were not observed in the experimental group that received the drugs sequentially (ALN+SR) as a result of alendronate "slowing down" bone tissue. This experimental finding agrees with clinical findings reported in the literature[8-11] where bone response to the effect of strontium ranelate administered after alendronate is "attenuated" during the first 6 months. After this time, there is an improvement in densitometric parameters and fracture rate. Nevertheless, this improvement does not catch up with the response in bisphosphonate-naive patients who were treated with strontium ranelate.

Weinstein et al.[27] and our group[26] were the first to describe giant osteoclasts in patients receiving bisphosphonates or who had received bisphosphonates, in papers published in 2009. This finding is important to histopathological diagnosis, since the histological picture could be mistaken for Paget´s disease, hyperparathyroidism and even bone tumors, in which giant cells are often present.

The intensely basophilic zones observed on the bone surface and within the matrix in animals treated with bisphosphonates, both in tibia and maxillae, corresponding to apposition lines and reversal lines, are not a minor finding, considering the role of bone matrix components in various processes such as matrix mineralization and bone resorption[31]. Osteopontin is a non-collagenous protein synthesized by osteoblasts. During the bone formation stage, osteopontin is the first and last secretion of osteoblasts, and is therefore preferentially deposited on the reversal and appositional lines[32]. It is a phosphorylated acid protein, and therefore basophilic, one of its functions being to act as a bonding site between osteoclast and bone matrix. Any alteration in matrix composition at the level of the superficial appositional lines can condition osteoclast adhesion. One of the known effects of bisphosphonates is altering the organization of the osteoclast cytoskeleton by interfering in the mevalonate pathway and inhibiting prenylation of small GTPases[33]. The qualitative changes observed in the bone matrix require further study because they may be another potential point of interference of bisphosphonates with osteoclast adhesion to their bone substrate. Moreover, this topic requires further study to elucidate any potential bisphosphonate actions on the osteoblast lineage.

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## REFERENCES

1. Reginster JY, Neuprez A, Dardenne N, Beaudart C, Emonts P, Bruvere O. Efficacy and safety of currently marketed anti-osteoporosis medications. Best Pract Res Clin Endocrinol Metab 2014; 28:809-834.
2. National Institute for Health and Clinical Excellence. Alendronate, atidronate, risedronate, raloxifene, strontium ranelate and teriparatide for the secondary prevention of osteoporotic fragility fractures in postmenopausal women. URL:http://www.nice.org.uk/guidance/ta161/resources/guidance-alendronate-etidronate-risedronate-raloxifene-strontium-ranelate-and-teriparatide-for-thesecondary-prevention-of-osteoporotic-fragility-fractures-in-postmenopausal-women-amended-pdf
3. Salica D, Buceta A, Palacios S, Sanchez A. Consenso Iberoamericano de Osteoporosis SIBOMM 2013. Osteoporosis: Prevención, Diagnóstico y Tratamiento. Guía para el Diagnóstico, Prevención y Tratamiento de la Osteoporosis para la Argentina. URL: https://www.iofbonehealth.org/sites/default/files/PDFs/National%20Guidelines/Argentine_guidelines_2013-QSchurman_y_col-1QMedicina-2013.pdf
4. Marx RE. Pamidronate (Aredia) and zoledronate (Zometa) induced avascular necrosis of the jaws: a growing epidemic. J Oral Maxillofac Surg 2003; 61:1115-1117.
5. Cheung RK, Leung KK, Lee KC Chow TC. Sequential non-traumatic femoral shaft fractures in a patient on long-term alendronate. Hong Kong Med J 2007; 13:485-489.
6. Whitaker M, Guo J, Kehoe T, Benson G. Bisphosphonates for osteoporosis—where do we go from here? N Engl J Med 2012; 366:2048-2051.
7. Food and Drug Administration. Background document for meeting of Advisory Committee for Reproductive Health Drugs and Drug Safety and Risk Management Advisory Committee. Sep 9. URL:http://www.fda.gov/downloads/AdvisoryCommittees/CommitteesMeetingMaterials/Drugs/DrugSafetyandRiskManagementAdvisoryCommittee/UCM270958.pdf.
8. Middleton ET, Steel SA, Aye M, Doherty SM. The effect of prior bisphosphonate therapy on the subsequent BMD and bone turnover response to strontium ranelate. J Bone Miner Res 2010; 25:455-462.
9. Middleton ET, Steel SA, Aye M, Doherty SM. The effect of prior bisphosphonate therapy on the subsequent therapeutic effects of strontium ranelate over 2 years. Osteoporos Int 2012; 23:295-303.
10. Jobke B, Burghardt AJ, Muche B, Hahn M, Semler J, Amling M, Majumdar S, Busse B. Trabecular reorganization in consecutive iliac crest biopsies when switching from bisphosphonate to strontium ranelate treatment. PLoS One 2011; 6:e23638.
11. Brun LR, Galich AM, Vega E, Salerni H, Maffei L, Premrou V, Costanzo PR, Sarli MA et al Strontium ranelate effect on bone mineral density is modified by previous bisphosphonate treatment. Springerplus 2014; 3:676.
12. Fleisch H. Bisphosphonates: a new class of drugs in diseases of bone and calcium metabolism. Recent Results Cancer Res 1989; 116:1-28.
13. Rizzoli R, Laroche M, Krieg MA, Frieling I, Thomas T, Delmas P, Felsenberg D. Strontium ranelate and alendronate have differing effects on distal tibia bone microstructure in women with osteoporosis. Rheumatol Int 2010; 30:1341-1348.
14. Rizzoli R, Chapurlat RD, Laroche JM, Krieg MA, Thomas T, Frieling I, Boutroy S, Laib A, et al. Effects of strontium ranelate and alendronate on bone microstructure in women with osteoporosis. Results of a 2-year study. Osteoporos Int 2012; 23:305-315.
15. Lima H, Maia J, Bandeira F. Trajectories of bone remodeling markers and bone mineral density during treatment with strontium ranelate in postmenopausal women previously treated with bisphosphonates. Clin Med Insights Endocrinol Diabetes 2014; 7:7-11.
16. Sun P, Cai DH, Li QN, Chen H, Deng WM, He L, Yang L. Effects of alendronate and strontium ranelate on cancellous and cortical bone mass in glucocorticoid-treated adult rats. Calcif Tissue Int 2010; 86:495-501.
17. Chen B, Li Y, Yang X, Xie D. Comparable effects of alendronate and strontium ranelate on femur in ovariectomized rats. Calcif Tissue Int 2013; 93:481-486.

18. Wu Y, Adeeb SM, Duke MJ, Munoz-Paniagua D, Doschak MR. Compositional and material properties of rat bone after bisphosphonate and/or strontium ranelate drug treatment. J Pharm Pharmaceut Sci 2013; 16: 52-64.
19. Tuner CH, Burr DB. Basic biomechanical measurements of bone: a tutorial. Bone 1993; 14:595-608.
20. Bozzini C, Olivera MI, Huygens P, Alippi RM, Bozzini CE. Long-term exposure to hypobaric hypoxia in rat affects femur cross-sectional geometry and bone tissue material properties. Ann Anat 2009; 191:212-217
21. Hogan HA, Groves JA, Simpson HW. Long-term alcohol consumption in the rat affects cross sectional geometry and bone tissue material properties. Alcohol Clin Exp Res 1999; 23:1825-1833.
22. Parfitt AM, Drezner MK, Glorieux FH, Kanis JA, Malluche H, Meunier PJ, Ott SM, Recker RR. Bone histomorphometry: standardization of nomenclature, symbols, and units. Report of the ASBMR Histomorphometry Nomenclature Committee. J Bone Miner Res 1987; 2:595-610.
23. Dempster DW, Compston JE, Drezner MK, Glorieux FH, Kanis JA, Malluche H, Meunier PJ, Ott SM, et al.Standardized nomenclature, symbols, and units for bone histomorphometry: a 2012 update of the report of the ASBMR Histomorphometry Nomenclature Committee. J Bone Miner Res 2013; 28:2-17
24. Rodan G, Reszka A, Golub E, Rizzoli R. Bone safety of long-term bisphosphonate treatment. Curr Med Res Opin 2004; 20:1291-1300.
25. Jain N, Weinstein RS. Giant osteoclasts after long-term bisphosphonate therapy: diagnostic challenges. Nat Rev Rheumatol 2009; 5:341-346.
26. Escudero ND, Lacave M, Ubios AM, Mandalunis PM. Effect of monosodium olpadronate on osteoclasts and megakaryocytes: an in vivo study. J Musculoskelet Neuronal Interact. 2009; 9:109-120.
27. Weinstein RS, Roberson PK, Manolagas SC. Giant osteoclast formation and long-term oral bisphosphonate therapy. N Engl J Med 2009, 360:53-62.
28. Jobke B, Milovanovic P, Amling M, Busse B. Bisphosphonate-osteoclast: changes in osteoclast morphology and function induced by antiresorptive nitrogen-containing bisphosphonate treatment in osteoporosis patients. Bone 2014; 59:37-43.
29. Morrissey C, Roudier MP, Dowell A, True LD, Ketchanji M, Welty C, Corey E, Lange PH, et al. Effects of androgen deprivation therapy and bisphosphonate treatment on bone in patients with metastatic castration-resistant prostate cancer: results from the University of Washington Rapid Autopsy Series. J Bone Miner Res 2013; 28:333-340.
30. Mac-Way F, Trombetti A, Noel C, Lafage-Proust MH. Giant osteoclasts in patients under bisphosphonates. BMC Clin Pathol 2014; 8:14-31.
31. Davies JE. Bone bonding at natural and biomaterial surfaces. Biomaterials 2007; 28: 5058-5067.
32. McKee MD, Nanci A. Osteopontin at mineralized tissue interfaces in bone, teeth, and osseointegrated implants: ultrastructural distribution and implications for mineralized tissue formation, turnover, and repair. Microsc Res Tech. 1996; 33:141-164.
33. Benford HL, McGowan NW, Helfrich MH, Nuttall ME, Rogers MJ. Visualization of bisphosphonate-induced caspase-3 activity in apoptotic osteoclasts in vitro. Bone 2001;28: 465-473.

# SALIVARY PROTEIN CHARACTERISTICS FROM SALIVA OF CARIOUS LESION-FREE AND HIGH CARIES ADULTS

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***ABSTRACT***

*It has been argued that specific salivary proteins could have a protective effect against caries, but data from the many available studies are rather contradictory. The purpose of this study was to analyze whether there is a relationship between protein concentration, electrophoretic profile and concentration of salivary IgA and the presence or absence of caries in adults. Adults with high caries activity (HC) and without caries lesions (CF), assessed by ICDAS criteria, were asked to provide unstimulated saliva samples. Protein concentration (µg/mL) was determined using the Bradford method. Western blotting was used to detect IgA. Data were compared using Student's t test at p<0.05. Total protein concentration in CF was higher (50.65±7.5 µg/mL) than in HC individuals (26.80±2.5 µg/mL) (p=0.001). More protein bands were visualized in the gels from CF than the HC group (p=0.001). CF subjects showed higher salivary IgA concentration (11.27±0.5 µg) than HC individuals (1.71±0.2µg) (p=0.001).Salivary composition in high caries experience and caries-free young adults seems to differ in terms of the type and amount of proteins. Further research is needed to expand these findings.*

**Key words:** *Dental caries, saliva, salivary proteins and peptides, Immunoglobulin A, Blotting, Western, electrophoresis.*

## CARACTERÍSTICAS DE PROTEÍNAS SALIVALES DE ADULTOS LIBRES Y CON ALTO NÚMERO DE LESIONES DE CARIES

***RESUMEN***

*Se ha descrito que proteínas salivales específicas podrían tener un efecto protector sobre la caries, sin embargo, los datos de los-numerosos estudios disponibles son contradictorios. El propósito de este trabajo fue analizar si existe una relación entre la concentración total de proteínas, perfil electroforético y la concentración de IgA salival y la presencia o ausencia de lesiones de caries en adultos. Se obtuvieron muestras de flujo salival no estimulado de adultos con alta actividad de caries (HC) y sin lesiones de caries (CF), evaluados según criterios ICDAS. La concentración total de proteínas (mg / ml) se determinó utilizando el método de Bradford. Para detección de IgA se empleó Western Blot. Los datos se compararon mediante la prueba t student, estableciendo diferencias significativas si p<0,05. La concentración total de proteínas en CF fue mayor (50,65 ± 7,5 mg / ml) que en individuos HC (26,80 ± 2,5 mg / ml) (p=0,001). En los geles, se visualizó un mayor número de bandas de proteínas en CF que en el grupo HC (p=0,001). Los Sujetos CF mostraron mayor concentración de IgA salival (11,27 ± 0,5 µg) que los individuos HC (1,71 ± 0,2 µg) (p=0,001). La composición salival de sujetos adultos jóvenes con alta experiencia y libres de caries, parece ser diferente en función del tipo y la cantidad de proteínas. Se requiere de más investigación para profundizar estos resultados.*

**Palabras clave:** *Caries dental, saliva, proteínas y péptidos salivales, Inmunoglobulina A, Western blot, electroforesis.*

## INTRODUCTION

Saliva contains several types of proteins, which constitute less than 1% of its total composition[1]. Despite the low proportion, this organic fraction actively participates in the defense of the oral cavity against deleterious agents[2]. Among the approximately one thousand salivary proteins currently described, only some have been related with protective activity against oral diseases. For example, differences in the amount and type of salivary proteins have been reported in subjects with and without systemic diseases, including diabetes[3], cystic fibrosis[4] and oral cancer[5]. Similarly, different protein patterns have been suggested in dental caries. Peroxidase, lysozyme, lactoferrin and histatin have been described as providing protective activity against the disease.

Although the mechanisms involved in the putative anticariogenic activity of salivary proteins have not been fully identified, it has been proposed that some proteins, such as those from the peroxidase systems, lysozyme, lactoferrin and histatins, may interfere with sucrose metabolism or promote bacterial aggregation with the subsequent removal of cariogenic bacteria[6]. Another caries-protective mechanism involves mucins and proline-rich proteins. These proteins are adsorbed onto the surface of the dental hard tissues to form the acquired pellicle, which actively regulates the demineralization and remineralization process[7]. The interplay between specific proteins and certain bacterial species conditions the bacterial composition of the oral biofilm[8,9]. Due to these specific functions, salivary proteins could be one of the most important factors in protecting the host against caries[10]. Hence, it would be reasonable to assume that a specific protein composition of saliva could have an effect on caries onset. The many available studies regarding the role of salivary proteins in caries are rather contradictory[11]. In fact, it has been proposed that proline-rich proteins and salivary IgA may participate in the opsonization and impairment of bacterial adherence to the tooth surface in caries-free subjects [8,12]. Also, IgA may have an anti-caries effect due to inhibition of bacterial adherence, neutralization of some enzymes and bacterial toxins and to the synergistic activity with other salivary proteins such as lactoferrin or lysozyme[13]. Conversely, one study showed that higher lysozyme concentration from the parotid or submandibular saliva was unrelated to differential caries susceptibility[14]. Furthermore, other studies indicate that subjects with higher protein concentration[15] or IgA levels[13] in saliva had more carious lesions than those people with lower content, suggesting a protein-concentration effect.

The analysis of salivary protein composition can provide insight into the role played by these factors on the caries process. Due to the lack of certainty on this issue and the contradictory results reported in the literature, our aim was to analyze whether protein concentration, electrophoretic profile and salivary IgA concentration are related to the presence or absence of caries lesions in adult subjects.

## MATERIALS AND METHODS

### Subjects

From the patients attending to the Dental Clinics of the University of Talca, twenty young adults without clinical or radiographic caries lesions, missing teeth and restorations (DMFT = 0) (CF) were recruited. A second group of 20 subjects with high caries activity (HC), defined as having at least 3 lesions ICDAS codes 5 or 6 (mean 3.9 ± 0.7 lesions) was also invited to participate. Participants read and signed an informed consent. The study protocol and the consent form were approved by the Bioethics Committee of the University of Talca and the study was performed in accordance with the Declaration of Helsinki. Twelve women and 8 men (mean 24 ± 2 years old) were included in the CF group, while 11 men and 9 women 25 ± 3 years old were included in the HC group. Clinical procedures included general health questions and an intraoral examination. An experienced clinician, calibrated in the ICDAS criteria for carious lesion detection, performed all the examinations. Bitewing radiographs were obtained for each subject to determine the presence of proximal caries. All clinical examinations were conducted at a dental clinic (C8, Sirona Dental Systems GmbH, Bensheim, Germany). Participants received oral prophylaxis before the examination. Data from the exam were recorded in a specially-designed record. Subjects with oral or systemic conditions that could affect saliva flow or composition, subjects taking medication that decreases salivary flow and smokers were not included in the study[16].

### Saliva samples

In a separate session, each participant was requested to provide unstimulated saliva. The subject was comfortably seated, with their head bent forward to gently pour saliva secreted for 15 minutes into a sterile 15 mL tube (Kima, Arzergrande, Italy)[16,17]. Each volunteer was instructed to refrain from rinsing or eating at least 2 hours prior saliva collection. To minimize the influence of the circadian rhythms on salivary flow, all samples were collected in a single session under the same conditions and by the same operator between 9:00 and 11:00 AM[18].

To minimize protein loss, each tube contained 60 µL of a protease inhibitor (Protease Inhibitor Cocktail Set I, Merck, Darmstadt, Germany) samples were kept at -20°C in a container with dry ice for a maximum of two hours during transportation to the laboratory[2]. Each sample was centrifuged at 29,000 *g* for 20 min at 4°C (Eppendorf 5810, Eppendorf AG, Hamburg, Germany). Using 1 mL acetone and 40 µL DL-Dithiothreitol (DTT) (Thermo Fisher Scientific Inc., Pittsburgh, PA, USA), a 1 mL aliquot from the supernatant was precipitated. Samples were stored 2 h at -80°C and then centrifuged at 4°C at 16,000 g (Eppendorf 5415 C, Eppendorf AG Hamburg, Germany), washed 3 times with 1 mL 90% acetone and 4 mL DTT. The supernatant was discarded and the resulting pellet was re-suspended in 100 µL of a solution of 1M Tris-HCl pH 8 + DTT 10 mM and 10% PMSF (v/v). The resulting suspension was used for the quantification of total protein concentration, saliva electrophoresis and immunological identification of IgA.

**Salivary protein concentration**

Protein concentration (µg/mL) was assessed with 10 µL of the suspension from the previous step by the Bradford method[19] with spectrophotometer (Victor ™ X - Multilabel Plate Reader, Perkin Elmer, MA, USA) at 595 nm. Using a calibration curve with albumin (Merck, Darmstadt, Germany), the optical density (OD) was converted to µg/mL using the Gen5 Data Analysis Software (Biotek, WA, USA).

**Salivary protein electrophoresis**

After determining the protein concentration in each sample, a standardized amount of sample, equivalent to 30 µg of protein was loaded in each well of a10% denaturing polyacrylamide gel with a molecular weight marker in the first lane (Spectra Multicolor Broad Range Protein Ladder, Thermo Fisher Scientific Inc., Pittsburgh, PA, USA). The procedure was performed in electrophoresis chambers (Mini-PROTEAN, Bio-Rad, California, USA) at 90V for 30 min and then at120V until completion. Gels were stained with Coomassie Blue for 30 min and washed for 2 h in a de-staining solution based on acetic acid and methanol. The number of bands in each gel was obtained by a digital scanner (Perfection V300 Photo Scanner, Epson America Inc., USA) and analyzed with the Gel-Pro Analyzer software (Version 4.0.00.001 for Windows, Media Cybernetics Inc., Rockville, MD, USA).

**Western blotting for IgA**

To identify IgA, a specific polyclonal antibody (Rabbit Anti-IgA, Santa Cruz, Dallas, USA) was used in western blot membranes (Nitrocellulose membrane for hybridization 11,327 - 41BL, Sartorius, Madrid, Spain). Membranes were blocked for 1 h with 8% skim milk and washed three times with TBS (Tris-Buffered Saline). Membranes were incubated with the primary antibody (IgA 0.2 µg/mL) under overnight stirring at 4°C. Subsequently, the membrane was washed with TBS-Tween (TBST) for 4 min and incubated with a mouse anti-rabbit IgG secondary antibody conjugated with HRP (2 mL + 10 mL TBS) under agitation for 1 h. Each membrane was washed four times with TBST for 3 min and bands were evidenced by immersion in 10 mL of a developing solution for 3 min (Super Signal, West Pico Chemiluminescent Substrate, Thermo Fisher Scientific Inc., MA, USA). Images of the bands were obtained through a digital camera (Fine Pix S2980, Fuji Film, Tokyo, Japan) and analyzed using the Gel-Pro Analyzer software (Media Cybernetics Inc.). To assess intensity, resulting bands were quantified and compared with those obtained in a standard curve with known concentrations of IgA.

**Statistical Analysis**

Normal distribution of all data was tested with Kolmogorov-Smirnov ($p>0.05$). Student's *t*-test was used to compare the differences between the study groups regarding total protein concentration, number of electrophoretic bands and IgA concentration. The differences were considered statistically significant if $p<0.05$. All analyses were performed using SPSS v15.0 software for Windows (IBM Corporation, NY, USA).

## RESULTS

CF subjects showed higher ($p=0.001$) protein concentration in saliva (50.65 ± 7.5 µg / mL) than HC individuals (26.80 ± 2.5 µg/mL) (Fig.1). When the number of bands was evaluated, CF participants had an average of 12 ± 0.7 electrophoretic bands (Fig. 2A), which was higher ($p=0.001$) than HC subjects, who only had a mean of 7 ± 0.4 (Fig. 2B). The most distinctive difference in the electrophoretic pattern between groups occurred between 40 and 70 kDa. In that molecular weight range, bands in the CF group were more numerous and more intense than in the HC group. Likewise, a18 kDa band was observed in both groups, but with notoriously higher intensity in the CF subjects (Fig. 2).

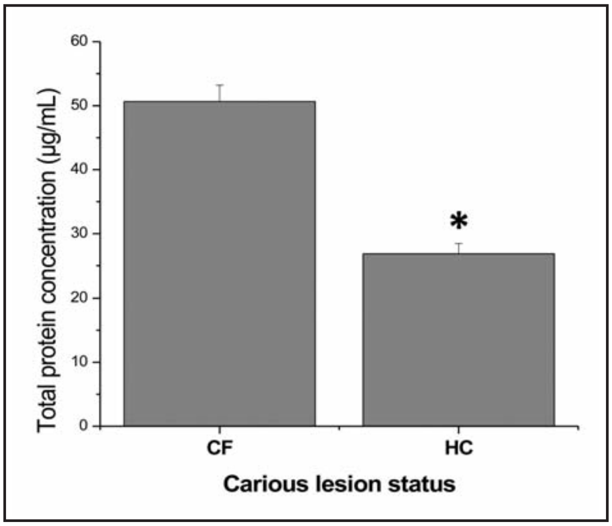


*Fig. 1: Total salivary protein concentration. Salivary protein concentration was assessed in CF and HC individuals. Bars show mean values (µg/mL) and error bars indicate standard deviation. * : p<0.05.*

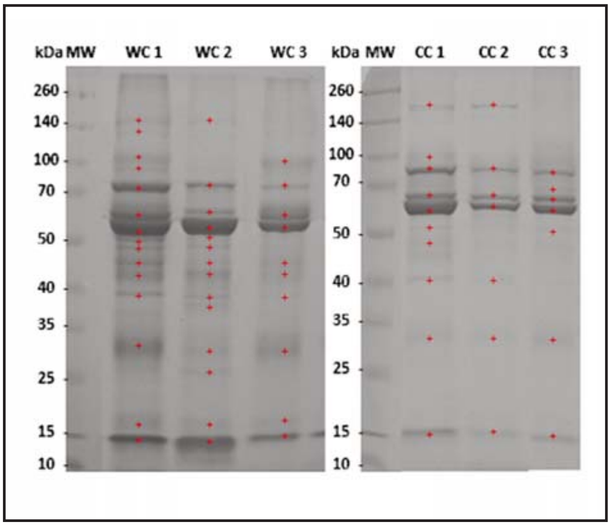


*Fig. 2: Number of electrophoretic bands in CF and HC subjects. Denaturing polyacrylamide gels were made for CF and HC participants and the resulting number of bands were counted and compared between the groups. Panel A shows the number of bars obtained in three CF individuals, while panel B depicts the number of protein bands from saliva from three HC subjects. Black marks highlight the area of the gel zone (between 40 and 70 KDa) where most of the variability took place. Black arrow points to the area of the gel where a 18 KDa band was visualized only in CF subjects.*

When IgA expression was compared between study groups by Western blotting, densitometry showed greater intensity (p=0.001) in the IgA bands (50 – 55 kDa: IgA heavy chain [20]) obtained in CF subjects than in HC subjects (Fig. 3A). When the concentration of salivary IgA was quantified by densitometric digital analysis, CF subjects showed higher concentration (11.27 ± 0.5 µg) than HC individuals (1.71 ± 0.2 µg) (p=0.001) (Fig. 3B).

## DISCUSSION

The chief finding in our study was that protein concentration, electrophoretic profile and concentration of salivary IgA differed between young adults with no caries experience and those with a high number of lesions. Total salivary protein concentration was higher in subjects without lesions. Very few studies have reported on this issue and their results are as yet inconclusive[11]. Although some studies have reported a difference in total concentration of salivary proteins between individuals with different caries experience[21-24], the methodologies used are heterogeneous and the results cannot easily be compared with ours. Given the lack of a standard protocol that could have been used as a gold standard, we tested several methods to optimize protein yield from saliva. After the pilot studies testing the final methods presented herein, we created a working protocol that may be used in further studies on the subject.

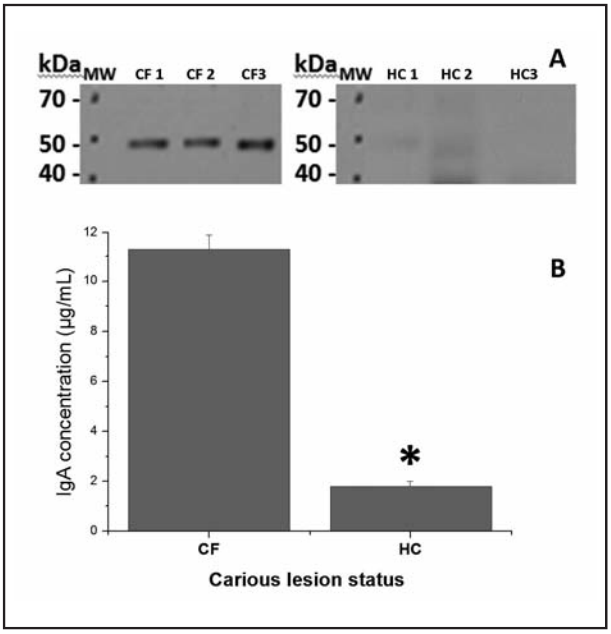


*Fig. 3: IgA identification by Western Blot in CF and HC participants. Panel A shows the resulting WB bands at about 50 KDa in CF and HC individuals. Band intensity was quantified using software and normalized against a standard curve. Panel B shows the resulting mean IgA concentrations in both CF and HC subjects. Error bars show standard deviation. *: p<0.05.*

When the electrophoretic pattern was compared between study groups, CF subjects had a higher number and variety of proteins in saliva. These differences are expressed most clearly in the bands between 40 and 70 kDa. Salivary proteins corresponding to this molecular weight include Amylase (46-60 kDa), IgA (70 kDa), Albumin (67.5kDa), Haptocorrin (60-80 kDa), α2 glycoprotein (41 kDa), α–Amylase (58 kDa), Catalase (55 kDa), Enolase 1 (47 kDa) and Transferrin (52 kDa)[25, 26]. Although electrophoresis is a rather basic analysis, this preliminary approach to a more detailed study of the protein component of saliva and its relation to caries confirms the hypothesis that there are differences in protein content in the saliva of sound individuals compared to those severely affected by caries. These encouraging results call for further research aimed at specific protein identification. In fact, the antibody Anti-IgA provides information on monomeric, dimeric and secretory IgA. We have not individualized the different origin of the proteins, as they can all be present in total unstimulated saliva. Further studies should be conducted to establish the specific characteristics of the antibody. Hence, molecular sequencing techniques may be used to identify and characterize the variants of the proteins between both groups. Precise identification of one or more proteins exclusively present incaries- free individuals opens up the field to novel strategies for caries prevention or treatment. In fact, new salivary peptides with high bactericidal power have been described, with very low potential for bacterial resistance[27].

Regarding IgA, our results are consistent with others that have shown that IgA is more abundant in caries-free subjects than in people with caries[28-31]. In this study, IgA concentration was nine times higher in CF subjects than in the HC group. IgA is the main immunoglobulin involved in the defensive response of saliva[28]. IgA may interfere with adhesion and neutralizes bacterial products alone or in association with other proteins such as lysozyme and lactoferrin[32]. Although a clear association between IgA concentration and presence of caries remains elusive[33], our findings are comparable to other studies reporting low incidence of carious lesions in subjects with higher IgA concentrations[34-36]. In this sense, IgA deficiency may represent an interesting novel risk factor for caries[31]. Furthermore, most of these studies have been conducted on children, so this report is one of the few that shows this relationship in adults.

It is important to mention that all the participants in this study had normal unstimulated salivary flow, as recommended[37]. This methodological approach avoids the potential source of bias as a result of taking samples from individuals with low salivary flow. Indeed, a previous study showed that a marked increase or decrease in salivary flow volume was related to a proportional change in salivary protein concentration due to a dilution effect on the salivary molecules[22]. Furthermore, some authors suggest the use of stimulated saliva for the analysis of its biochemical characteristics because it is easy to obtain and to standardize the procedures[38]. Nevertheless, in this study we used unstimulated saliva. While unstimulated saliva is in contact with the dental tissues most of the time in the mouth, stimulated saliva mainly occurs during food consumption[39]. Thus, it may be speculated that unstimulated salivary flow has a more permanent effect on caries. It is reasonable to assume that continuous contact with the hard tissues of the tooth implies a greater protective effect of unstimulated saliva than of stimulated secretion. Thus, the acquired pellicle, which allows bacterial adhesion to the tooth, may be formed mainly from unstimulated saliva. Other researchers have used saliva directly obtained from one or more salivary glands[40-42]. In this study, we decided to analyze whole saliva, as it better represents the actual clinical situation. The etiological dental biofilm is exposed to whole saliva and not only to the secretion that comes from the gland[25, 43]. Importantly, IgA may have been derived from the crevicular fluid and may have been pooled with that from the salivary glands. More precise techniques should explore this possibility.

In contrast to the results of a study conducted in a Colombian population[44] reporting that men with high number of caries have a differential electrophoretic band located at 17 kDa, we found a band at 18 kDa, which is consistently observed in the gels from CF subjects. Proteins described with that molecular weight include Cofilin (18 kDa), Cystatin (17 kDa), IgG–J chain (16 kDa), prolactin-induced protein (17 kDa) and extra-parotid glycoprotein (EP-GP) (18 kDa)[25, 26]. The differences between the studies are not surprising. As mentioned, protocols designed to obtain salivary proteins differ greatly among researchers. To develop a reliable protocol, we made several attempts and used alternative methods. The main difficulty to overcome was the very small protein concentration in saliva. Human saliva contains only 1% organic matter, of which only a small fraction corresponds to proteins. Procedures therefore clearly require optimization. For example, once obtained from saliva, proteins were precipitated with acetone and all procedures were performed immediately and under cold conditions to prevent degradation. Additionally, samples were treated at all times with protease inhibitors, also to prevent loss and peptide denaturation. Besides these technical factors during sample processing, the variability in the molecular weight of the bands between studies may derive from the fact that proteins can form complexes with each other or with polysaccharides[45]. Degradation may also explain differences reported in the literature[46]. As a result, bands with larger or smaller molecular size may arise. Undoubtedly, more sophisticated and precise techniques are required to clearly identify proteins that may be present in higher or lower amount in the groups studied. Despite the differences in protein concentration, electrophoretic profile and salivary IgA concentration between the two groups under testing, current state-of-the-art precludes using these variables as biomarkers for dental caries[11]. If more precise protein characterization develops in the future to allow a clear distinction between some proteins that may be only present in caries patients, saliva may well serve as an easily obtainable target for caries risk assessment. Proteomic analysis with mass spectrometry should be used in future research. Since saliva is a readily accessible secretion that can be obtained from the mouth without any surgical or invasive procedure, sampling is usually completely pain-free. Hence, samples may be obtained from small children without the complications inherent to other sampling sites of the body, such as the gut or the bloodstream. Furthermore, the cost for obtaining large samples is minimal, allowing for massive widespread application[47]. Although saliva is already being used for the detection of specific antibodies in some diseases (HIV, Hepatitis C), hormones or drugs, its use for diagnostic purposes is still limited due to the variability and polymorphism in the salivary proteome. Given the potential for accurate caries risk assessment from salivary proteins, further research is strongly encouraged.

Despite the limitations of the experimental setting, total protein concentration, number of electrophoretic bands and concentration of salivary IgA appear to be higher in adults with no caries experience than those who have a high number of carious lesions. Salivary composition, particularly IgA, may be a potential target for caries risk assessment.

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## REFERENCES

1. Carpenter GH. The secretion, components, and properties of saliva. Annu Rev Food Sci Technol 2013; 4: 267-276.
2. Denny P, Hagen FK, Hardt M, Liao L, Yan W, Arellanno M, Bassilian S, Bedi GS et al. The proteomes of human parotid and submandibular/sublingual gland salivas collected as the ductal secretions. J Proteome Res 2008; 7:1994-2006.
3. Rao PV, Reddy AP, Lu X, Dasari S, Krishnaprasad A, Biggs E, Roberts CT, Nagalla SR. Proteomic identification of salivary biomarkers of type-2 diabetes. J Proteome Res 2009; 8: 239-245.
4. Minarowski Ł, Sands D, Minarowska A, Karwowska A, Sulewska A, Gacko M, Chyczewska E. Thiocyanate concentration in saliva of cystic fibrosis patients. Folia Histochem Cytobiol 2008; 46: 245-246.
5. Hu S, Yen Y, Ann D, Wong DT. Implications of salivary proteomics in drug discovery and development: a focus on cancer drug discovery. Drug Discov Today 2007; 12: 911-916.

6. Lenander-Lumikari M, Loimaranta V. Saliva and dental caries. Adv Dent Res 2000; 14: 40-47.
7. Siqueira WL, Zhang W, Helmerhorst EJ, Gygi SP, Oppenheim FG. Identification of protein components in in vivo human acquired enamel pellicle using LC-ESI MS/MS. J Proteome Res2007; 6:2152-2160.
8. Vitorino R, Lobo MJ, Duarte JR, Ferrer-Correia AJ, Domingues PM, Amado FM. The role of salivary peptides in dental caries. Biomed Chromatogr 2005; 19: 214-222.
9. Siqueira WL, Helmerhorst EJ, Zhang W, Salih E, Oppenheim FG. Acquired enamel pellicle and its potential role in oral diagnostics. Ann N Y Acad Sci 2007; 1098: 504-509.
10. Vukosavljevic D, Custodio W, Siqueira WL. Salivary proteins as predictors and controls for oral health. J Cell Commun Signal 2011; 5: 271-275.
11. Martins C, Buczynski AK, Maia LC, Siqueira WL, Castro GF. Salivary proteins as a biomarker for dental caries-A systematic review. J Dent 2012; 41: 2-8.
12. Koga-Ito CY, Martins CA, Balducci I, Jorge AO. Correlation among mutans streptococci counts, dental caries, and IgA to Streptococcus mutans in saliva. Braz Oral Res 2004; 18: 350-355.
13. Ranadheer E, Nayak UA, Reddy NV, Rao VA. The relationship between salivary IgA levels and dental caries in children. J Indian Soc Pedod Prev Dent 2011; 29: 106-112.
14. Stuchell RN, Mandel ID. A comparative study of salivary lysozyme in caries-resistant and caries-susceptible adults. J Dent Res 1983; 62: 552-554.
15. Tulunoglu O, Demirtas S, Tulunoglu I. Total antioxidant levels of saliva in children related to caries, age, and gender. Int J Paediatr Dent 2006; 16: 186-191.
16. Löfgren CD, Isberg PE, Christersson C. Screening for oral dryness in relation to salivary flow rate addresses the need for functional tests of saliva. Oral Health Prev Dent 2006; 8: 243-252.
17. Heintze U, Frostell G, Lindgärde F. Secretion rate and buffer effect of resting and stimulated whole saliva in relation to general health. Swed Dent J 1986; 10: 213-219.
18. Banderas-Tarabay JA, Gonzalez-Begne M, Sanchez-Garduno M, Millán-Cortéz E, López-Rodríguez A, Vilchis-Velázquez A. The flow and concentration of proteins in human whole saliva. Salud Publica Mex 1997; 39: 433-441.
19. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 1976; 72: 248-254.
20. Kerr MA. The structure and function of human Ig A. Biochem J 1990; 271: 285-296.
21. Hao GF, Lin HC. Relationship of concentration of lactoferrin and lysozyme in saliva and dental caries in primary dentition. Zhonghua Kou Qiang Yi Xue Za Zhi 2009; 44: 82-84.
22. Banderas-Tarabay JA, Zacarías-D'Oleire IG, Garduño-Estrada R. Aceves-Luna E, González-Begné M. Electrophoretic analysis of whole saliva and prevalence of dental caries. A study in Mexican dental students. Arch Med Res 2002; 33: 499-505.
23. Zehetbauer S, Wojahn T, Hiller KA, Schmalz G, Ruhl S. Resemblance of salivary protein profiles between children with early childhood caries and caries-free controls. Eur J Oral Sci 2009; 117: 369-373.
24. Bhalla S, Tandon S, Satyamoorthy K. Salivary proteins and early childhood caries: A gel electrophoretic analysis. Contemp Clin Dent 2010; 1: 17-22.
25. Huang CM. Comparative proteomic analysis of human whole saliva. Arch Oral Biol 2004; 49: 951-962.
26. Schenkels LC, Veerman EC, Nieuw Amerongen AV. Biochemical composition of human saliva in relation to other mucosal fluids. Crit Rev Oral Biol Med 1995; 6: 161-175.
27. Van Nieuw Amerongen A, Bolscher JG, Veerman EC. Salivary proteins: protective and diagnostic value in cariology? Caries Res 2004; 38: 247-253.
28. Jorgensen GH, Arnlaugsson S, Theodors A, Ludviksson BR. Immunoglobulin A deficiency and oral health status: a case-control study. J Clin Periodontol 2010; 37: 1-8.
29. Kuriakose S, Sundaresan C, Mathai V, Khosla E, Gaffoor FM. .A comparative study of salivary buffering capacity, flow rate, resting pH, and salivary Immunoglobulin A in children with rampant caries and caries-resistant children. J Indian Soc Pedod Prev Dent 2013; 31: 69-73.
30. Shifa S, Muthu MS, Amarlal D, Rathna Prabhu V. Quantitative assessment of IgA levels in the unstimulated whole saliva of caries-free and caries-active children. J Indian Soc Pedod Prev Dent 2008; 26: 158-161.
31. Tar I, Kiss C, Maródi L, Márton IJ. Oral and dental conditions of children with selective IgA deficiency. Pediatr Allergy Immunol 2008; 19: 33-36.
32. Marcotte H, Lavoie MC. Oral microbial ecology and the role of salivary immunoglobulin A. Microbiol Mol Biol Rev 1998; 62: 71-109.
33. Fidalgo TK, Freitas-Fernandes LB, Ammari M, Mattos CT, de Souza IP, Maia LC.The relationship between unspecific s-IgA and dental caries: A systematic review and meta-analysis. J Dent. 2014; 42: 1372-1381.
34. Bratthall D, Serinirach R, Hamberg K, Widerström L. Immunoglobulin A reaction to oral streptococci in saliva of subjects with different combinations of caries and levels of mutans streptococci. Oral Microbiol Immunol 1997; 12: 212-218.
35. Sanui T, Gregory RL. Analysis of Streptococcus mutans biofilm proteins recognized by salivary immunoglobulin A. Oral Microbiol Immunol 2009; 24: 361-368.
36. Nogueira RD, Alves AC, Napimoga MH, Smith DJ, Mattos-Graner RO. Characterization of salivary immunoglobulin A responses in children heavily exposed to the oral bacterium Streptococcus mutans: influence of specific antigen recognition in infection. Infect Immun 2005; 73: 5675-5684.
37. Dawes C. Salivary flow patterns and the health of hard and soft oral tissues. J Am Dent Assoc 2008; 139 Suppl, 18S-24S.
38. Aiuchi H, Kitasako Y, Fukuda Y. Nakashima S, Burrow MF, Tagami J. Relationship between quantitative assessments of salivary buffering capacity and ion activity product for hydroxyapatite in relation to cariogenic potential. Aust Dent J 2008; 53: 167-171.
39. Stookey GK. The effect of saliva on dental caries. J Am Dent Assoc 2008; 139 Suppl, 11S-17S.
40. Ambatipudi KS, Hagen FK, Delahunty CM, Han X, Shafi R, Hryhorenko J, Gregoire S, Marquis RE et al. Human

common salivary protein 1 (CSP-1) promotes binding of Streptococcus mutans to experimental salivary pellicle and glucans formed on hydroxyapatite surface. J Proteome Res 2010; 9: 6605-6614.
41. Lamy E, Mau M. Saliva proteomics as an emerging, non-invasive tool to study livestock physiology, nutrition and diseases. J Proteomics 2012; 75: 4251-4258.
42. Anderson LC, Lamberts BL, Bruton WF. Salivary protein polymorphisms in caries-free and caries-active adults. J Dent Res 1982; 61: 393-396.
43. Jensen JL, Lamkin MS, Oppenheim FG. Adsorption of human salivary proteins to hydroxyapatite: a comparison between whole saliva and glandular salivary secretions. J Dent Res 1992; 71: 1569-1576.
44. Roa NS, Chaves M, Gómez M, Jaramillo LM. Association of salivary proteins with dental caries in a Colombian population. Acta Odontol Latinoam 2008; 21: 69-75.
45. Morzel M, Palicki O, Chabanet C, Lucchi G, Ducoroy P, Chambon C, Nicklaus S. Saliva electrophoretic protein profiles in infants: changes with age and impact of teeth eruption and diet transition. Arch Oral Biol 2011; 56: 634-642.
46. Rabilloud T, Vaezzadeh AR, Potier N, Lelong C, Leize-Wagner E, Chevallet M. Power and limitations of electrophoretic separations in proteomics strategies. Mass Spectrom Rev 2009; 28: 816-843.
47. Castagnola M, Cabras T, Vitali A, Sanna MT, Messana I. Biotechnological implications of the salivary proteome. Trends Biotechnol 2011; 29: 409-418.

# INFLUENCE OF ORAL HEALTH ON QUALITY OF LIFE IN PREGNANT WOMEN

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***ABSTRACT***

*This study evaluated the relationships between oral conditions and oral health-related quality of life (OHRQoL), as well as related factors. A cross-sectional study was performed on 119 postnatal women who had sought pre-natal care during pregnancy in the public health system of São Paulo State, Brazil. The women received oral clinical exams and were interviewed using the questions on the OHIP-14. A second survey with information about their socio-economic status, pregnancy and health habits was administered. The highest OHIP-14 scores were found in the area of physical pain, with an average score of 10.6. Average DMFT rate for the population was 12.2 (±6.1), with the majority having DMFT ≥4.5 (89.9%). Most of the women needed some type of dental prosthesis (59.7%), had some type of periodontal disease (90.8%), tooth decay (73.9%), missing teeth (64.7%) and were in need of oral treatment (68.1%). The OHIP-14 scores were significantly associated with age (p=0.02), first pregnancy (p<0.001), need for dental prosthesis (p<0.001), presence of dental caries (p<0.001) and missing teeth (p=0.01). In the multivariate analysis, the worst OHRQoL was significantly associated with the presence of caries (p=0.03). The results suggest an association between the worst oral condition and poorer quality of life during pregnancy. This risk group should be prioritized in the health services in order to treat and recover the oral health of pregnant women, promoting better oral health conditions and better quality of life for their children.*

***Key words:*** *Oral health, quality of life, pregnancy.*

## INFLUÊNCIA DA SAÚDE BUCAL NA QUALIDADE DE VIDA DE GESTANTES

***RESUMO***

*O presente estudo avaliou as relações entre condições bucais e o impacto da saúde bucal na qualidade de vida de gestantes, bem como fatores relacionados. Um estudo transversal foi realizado em 119 mulheres que, durante a gravidez, tinham procurado atendimento pré-natal no sistema público de saúde do Estado de São Paulo, Brasil. Foram realizados exames clínicos bucais e as gestantes foram entrevistadas utilizando o questionário OHIP-14, forma abreviada, e um segundo inquérito, com informações sobre os seus hábitos de status sócio-econômico, gravidez e saúde foi administrado. As maiores pontuações OHIP-14 foram encontrados na área de dor física, com uma pontuação média de 10,6. A taxa média de CPO-D para a população foi de 12,2 (± 6,1), com a maioria tendo um CPOD de ≥4.5 (89,9%). A maioria das mulheres precisava de algum tipo de prótese dentária (59,7%), tiveram algum tipo de doença periodontal (90,8%), apresentaram cárie dentária (73,9%), falta de dentes (64,7%) e estavam na necessidade de tratamento odontlógico (68,1% ). Os escores do OHIP-14 estiveram significativamente associados com a idade (p = 0,02), primeira gravidez (p <0,001), necessidade de prótese dentária (p <0,001), presença de cárie dentária (p <0,001)) e falta de dentes (p = 0,01). Na análise multivariada, o pior impacto da saúde bucal sobre a qualidade de vida de gestantes esteve significativamente associada com a presença de cárie (p = 0,03). Os resultados sugeriram que a pior condição bucal esteve relacionada com pior qualidade de vida durante a gravidez. Este grupo de risco deve ser priorizado nos serviços de saúde, a fim de tratar e recuperar a saúde bucal destas grávidas, promovendo melhores condições de saúde bucal e da qualidade de vida de seus filhos.*

***Palavras chave:*** *Saúde bucal, qualidade de vida, gravidez.*

## INTRODUCTION

The classic diagnosis of oral health focuses only on professional clinical evaluation of the patient. It does not assign any importance to other factors that directly affect oral health, such as quality of life, income, schooling level, habits, and an individual's perception of their own health[1]. If quality of life indicators and self-perception of oral health are taken into account in the oral health diagnosis, the estimated need for treatment may be greater and assessment criteria may be more realistic[2].

To evaluate the impact of oral health on quality of life, many studies have used the OHIP-14 questionnaire (Oral health impact profile questionnaire –

short form), developed by Slade and Spencer[2-9]. The 14 questions in the questionnaire were effective in terms of revealing associations between clinical, social and demographic factors[10]. The OHIP-14 has already been tested and validated for use in the Portuguese language and Brazilian culture and, in addition, this version of the OHIP-14 had good psychometric properties, similar to those shown by the original version[6]. The OHIP-14 enables evaluation of the unfavorable impacts of the oral condition on a patient`s well-being and quality of life. It also reveals subjective experiences associated with oral health. Using an indicator like the OHIP-14 can facilitate dental service planning by enabling prioritization of care for people whose oral health has high impact on their quality of life[1] and thus directly affects the oral health and quality of life of their children[8,11]. Pregnant women are considered to be a special category of patients because they are at higher risk for oral diseases and are undergoing physical, biological and hormonal changes that may create adverse conditions in the oral environment and their psychosocial state[2].

The quality of life of pregnant women affects maternal health as well as fetal and infant health[12,13]. Studies have demonstrated that the absence of teeth can damage both nutrient intake and psychosocial behavior [3,12].

Few studies have investigated the impact of oral health on a pregnant woman's quality of life[3-5,8,12,14]. Oral pain during pregnancy has been found to have a negative effect on Brazilian women's quality of life and causes difficulty in maintaining emotional balance, eating and oral hygiene during pregnancy, and may harm the fetus[12]. A study of pregnant Indian women found that increasing age, multiparity, tooth decay and periodontal disease adversely affected OHRQoL[3]. Another study undertaken on pregnant Indian women showed that they had more periodontal problems than non-pregnant women, and that OHIP-14 scores were significantly higher for pregnant women[5]. A survey conducted on pregnant women in Uganda using the OIDP (Oral Impacts on Daily Performance) to assess OHRQoL showed that there was a strong association between the score and loss of teeth, but no association with periodontal disease[4]. An evaluation of Argentina's low-income pregnant women using the OHIP-49 found that even with high prevalence of caries and gum disease in the study population, oral health status was not verified to have any impact on quality of life, although it can be an important variable for the demand for services[14]. A study conducted in Shanghai, China showed that the negative oral impacts experienced by pregnant women were mainly related to functional limitation and physical pain, and the loss of teeth was associated with OHRQoL[8]. There is no consensus on whether oral health status during pregnancy causes further impact on quality of life, due to the few studies on this topic. The aim of this study was therefore to evaluate the relationships between oral conditions and OHRQoL, as well as related factors.

## MATERIAL AND METHODS

### Study Design and Ethics

This research formed part of "The Impact of Care in the Practice of Maternal Breastfeeding and Oral Health on the Mother-Child Binomal" conducted by the Graduate Program in Preventive and Social Dentistry of São Paulo State University (UNESP) at all public health units of two medium-sized cities in the State of São Paulo, Brazil. The study conforms to the Strobe guidelines for cross-sectional studies[15]. A research project was submitted and approved by the Ethics Committee on Research Involving Humans of the Araçatuba School of Dentistry-UNESP.

### Participants

The total calculated sample consisted of 120 pregnant women, a number obtained by calculation through finite populations[16]. To calculate sample size, the OHIP-14 score obtained in a similar study conducted on Brazilian pregnant women, according to the literature[12], was considered, with significance level $\alpha = 0.05$, absolute sampling error 6 4% and finite population during the study period (August-October 2007).

Written informed consent was obtained from all participants. Included were women who sought prenatal care at the public health units of the Brazilian Health System (*Sistema Único de Saúde - SUS*) from August to October 2011 and women in the last trimester of pregnancy. Pregnant women who refused to undergo clinical examination were excluded (n = 1). The final sample consisted of 119 pregnant women, who represented 95% of the pregnant population of the municipalities in the study period.

**Data sources/ measurement**
A pilot study was performed on pregnant women to calibrate examiners and check for possible errors in data collection. During the pilot study, the methods of data collection, administration of the clinical exam and statistical data analyses were tested.
Women in the study received clinical oral exams and were interviewed using two forms, one containing the OHIP-14 questions to evaluate the OHRQoL and a second questionnaire, which was pre-tested during the pilot study and contained questions about socioeconomic status, pregnancy and health habits.

**Variables**
Socio-economic status included household monthly income (0–1 BMW – [Brazilian Minimal Wage]; More than 1 BMW) – one BMW was equivalent to US$150.05 in 2011 (standard value) and years of schooling (0–8 years; 9 or more years). Demographic data included ethnicity (White; Non-White - Brown; Black); age (up to 21 years; 22 years old or older); employment (yes; no) and marital status (living with partner; no partner). Questions about pregnancy and habits were included: first pregnancy (yes; no); number of pregnancies; presence of systemic diseases (yes; no) and unplanned pregnancy (yes; no).
Clinical oral exams were performed by a previously-calibrated team, according to WHO (World Health Organization)[17] criteria (Kappa test = 0.91), using a flat mouth mirror and a CPI (Community Periodontal Index) probe for the epidemiological survey under natural light, with the examiner and the patient seated. Dental conditions were recorded, such as the WHO standardizations for the crown (codes – 0: sound; 1: decayed; 2: filled, with decay; 3: filled, no decay; 4: missing, as a result of caries; 5: missing, any other reason; 6: fissure sealant; 7: bridge abutment, special crown or veneer/implant; 8: unerupted tooth; T: trauma; 9: not recorded), and for the treatment needed (codes– 0: none; 1: one surface filling; 2: two or more surface fillings; 3: crown for any reason; 4: veneer or laminate; 5: pulp care and restoration; 6: extraction; 7: white spot remineralization; 8: fissure sealant; 9: not recorded) 20. The DMFT index (number of teeth that are decayed (D), missing (M), or filled (F) in an individual, applied to permanent dentition) was calculated. Additionally, periodontal condition was assessed by the CPI score (codes – 0: healthy; 1: bleeding; 2: calculus; 3: shallow pockets 4-5mm; 4: deep pockets > 6mm; X: excluded), and the need for prostheses was recorded (codes – 0: no prosthesis needed; 1: need for one-unit prosthesis; 2: need for multi-unit prosthesis; 3: need for a combination of one- and/or multi-unit prostheses; 4: need for full prosthesis; 9: not recorded)[17].
The OHIP-14 was used to measure the social impact of problems that may compromise oral health. The questions asked whether any of the problems evaluated by the OHIP-14 had occurred during the previous six months and the response choices were: Often, Never, Rarely, Sometimes, Repeatedly or Always. The Portuguese version of the OHIP-14 questionnaire was not changed or altered[12].
To increase the reliability of the results in the pilot study population, the questionnaires were reapplied after an interval of seven days and test-retest reliability was analyzed by calculating the Pearson correlation coefficient (0.87; $p < 0.01$) and Cronbach's alpha test (0.93). The results showed stability and internal consistency, demonstrating that the examiner was capable of applying the instruments successfully.
In the pilot study, we realized that we needed to develop an explanation for the options in the questionnaire. This included adding the following details: Never – never in the past 6 months; Rarely – once or twice in the past 6 months; Sometimes – Every month or every week in the past 6 months; Repeatedly – Nearly every day or twice or more times per week and Always– All the time, daily during the past 6 months.
The OHIP-14 deals with the following domains: functional limitations (questions 1 and 2), physical pain (questions 3 and 4), psychological discomfort (questions 5 and 6), physical disability (questions 7 and 8), psychological disability (questions 9 and 10), social disability (questions 11 and 12), and handicap performing daily activities (questions 13 and 14)[18].
To calculate the impact of oral health on a pregnant women's quality of life, the original OHIP-14 scoring was assigned to each question, according to the response provided: never – 0; rarely – 1; sometimes – 2; frequently – 3; always – 4. Final scores for all questions could thus range from 0 to 56 points. The higher scores indicated a greater perception of OHRQoL[19].

**Statistical methods**

All the questionnaires were reviewed, entered and analyzed employing the Epi Info 7 program[20] and the Bioestat program 5.3, freely available in Brazil[21]. The variables (social and demographic status and the women's clinical oral health condition) were described via frequency distributions for categorical variables and the average for continuous variables. The Chi-square test was used to evaluate the associations between the categorical variables. The adopted statistical significant p-value was equal to or lower than 0.05.

Clinical variables were evaluated according to the method adapted by Cohen-Carneiro et al.[19], which associates OHIP scores with the following clinical parameters: need for prostheses (yes, type of oral prosthesis corresponds to codes 1 to 4; not needed, code 0); need for dental treatment (yes, presence of at least one type of treatment needed, classified as 1 to 8; no, all the teeth with codes 0 or 9); presence of untreated decayed teeth (yes, "D" component of the DMFT index different from zero; no, "D" component equal to zero); missing teeth (yes, "M" component of the DMFT different from zero; no, "M" component equal to zero); periodontal disease (yes, if there was any kind of change - codes 1, 2, 3, 4 according to the CPI score). The non-parametric Mann-Whitney test was used to compare the OHIP scores with the dichotomous nominal values described above.

Spearman's Rank Correlation Coefficient was used to evaluate the relationship among CPI, DMFT, age, number pregnancies and OHIP scores.

The variables that had a p-value of $\leq 0.20$ were included in the analysis of multiple logistical regression. The results were presented using frequencies and an Odds Ratio (OR) with a 95% CI.

## RESULTS

The majority of the study population consisted of mothers of average age 24.7 (±5.9) years, not working, with more than eight years of schooling, living with their partners, with low household income and not in their first pregnancy.

Table 1 shows the numerical and percentage distributions of the scores for the OHIP-14 responses. The higher scores are concentrated around the second area of the questionnaire, which asked about physical pain. The average OHIP-14 score for the population was 10.6(±14.4).

Most of the women in this study (Table 2) had DMTF (decayed, missing and filled teeth index) ≥4.5 (89.9%) and some type of periodontal disease - CPI≠0 (90.8%). Average DMFT for the population was 12.2(±6.1). Most women needed some kind of dental prosthesis (59.7%), showed some kind of periodontal

**Table 1: Numerical distribution of pregnant women, according to OHIP-14 performance items and total prevalence scores for domains.**

| Items | | 0 Never n (%) | 1 Rarely n (%) | 2 Sometimes n (%) | 3 Repeatedly n (%) | 4 Always n (%) |
|---|---|---|---|---|---|---|
| Functional limitation | Articulation | 105 (88.2) | 1(0.8) | 2 (1.7) | 2 (1.7) | 9 (7.6) |
| | Sense of taste | 94 (79.0) | 3 (2.5) | 7 (5.9) | 1 (0.8) | 14 (11.8) |
| Physical pain | Pain | 55 (46.2) | 5 (4.2) | 20 (16.8) | 9 (7.6) | 30 (25.2) |
| | Eating | 72 (60.5) | 1 (0.8) | 11 (9.3) | 2 (1.7) | 33 (27.7) |
| Psychological discomfort | Self-conscious | 95 (79.8) | 0 (0.0) | 7 (5.9) | 2 (1.7) | 15 (12.6) |
| | Felt tense | 83 (69.8) | 2 (1.7) | 8 (6.7) | 8 (6.7) | 18 (15.1) |
| Physical disability | Diet unsatisfactory | 88 (73.9) | 4 (3.4) | 5 (4.2) | 5 (4.2) | 17 (14.3) |
| | Interrupt meals | 89 (74.8) | 1 (0.8) | 6 (5.0) | 4 (3.4) | 19 (16.0) |
| Psychological disability | Relax | 85 (71.5) | 3 (2.5) | 6 (5.0) | 2 (1.7) | 23 (19.3) |
| | Embarrassed | 95 (79.9) | 1 (0.8) | 6 (5.0) | 1 (0.8) | 16 (13.5) |
| Social disability | Irritable | 97 (81.5) | 0 (0.0) | 3 (2.5) | 2 (1.7) | 17 (14.3) |
| | Usual work | 107 (89.9) | 1 (0.8) | 4 (3.4) | 2 (1.7) | 5 (4.2) |
| Handicap performing daily activities | Less satisfied | 96 (80.7) | 3 (2.5) | 4 (3.4) | 3 (2.5) | 13 (10,9) |
| | Unable to function | 114 (95.8) | 0 (0.0) | 3 (2.5) | 0 (0.0) | 2 (1.7) |

change (90.8%), had tooth decay (73.9%), missing teeth (64.7%) and were in need of treatment (68.1%). Table 2 shows the association between the OHIP-14 scores and variables. There was a statistically significant relationship between the OHIP-14 scores and age, first pregnancy, need for prosthesis, presence of decayed tissue and missing teeth.

Spearman's correlation was analyzed to investigate the relationship between the clinical indicators of periodontal condition, the DMFT index, age, the number of prior pregnancies and the women's OHIP-14 scores (Table 3). There was a statistically significant correlation between the DMFT score and all of the domains of the OHIP-14 and for the total OHIP-14 score (p<0.001). Moreover, age and the number of pregnancies were significantly correlated (p = 0.05 and p<0.001, respectively).

In the multivariate analysis (table 4), the worst OHRQoL was significantly associated with the presence of caries (p=0.03).

**Table 2: The association between mean OHIP-14 scores and variables.**

| | Variables | n | Mean (±SD) | p |
|---|---|---|---|---|
| Age | Up to 21 years old | 39 | 6.2 (11.1) | 0.02 |
| | 22 years or older | 80 | 12.7 (15.4) | |
| Employed | No | 65 | 11.0 (14.6) | 0.52 |
| | Yes | 54 | 10.1 (14.3) | |
| Skin color | White | 49 | 12.1 (13.7) | 0.70 |
| | Nonwhite | 70 | 9.6 (15.3) | |
| Marital status | With a partner | 93 | 11.7 (15.1) | 0.12 |
| | No partner | 26 | 6.7 (11.1) | |
| Years of Schooling | Up to 8 years of schooling | 52 | 12.4 (12.8) | 0.19 |
| | More than 8 years of schooling | 67 | 8.3 (15.4) | |
| Household income | Up to twice the BMW* | 77 | 12.0 (15.4) | 0.22 |
| | More than twice the BMW* | 42 | 8.1 (12.2) | |
| First pregnancy | No | 61 | 15.0 (16.4) | <0.001 |
| | Yes | 58 | 5.9 (10.2) | |
| Presence of systemic diseases | No | 86 | 8.7 (12.9) | 0.08 |
| | Yes | 33 | 15.7 (16.9) | |
| Unplanned pregnancy | No | 44 | 11.7 (12.0) | 0.75 |
| | Yes | 75 | 8.8 (15.6) | |
| Need of dental prosthesis | No | 48 | 5.0 (9.2) | <0.001 |
| | Yes | 71 | 14.4 (16.1) | |
| DMFT Value | DMTF $\leq$ 4.4 | 12 | 5.0 (10.3) | 0.18 |
| | DMTF $\geq$ 4.5 | 107 | 11.2 (14.1) | |
| Periodontal disease | No – CPI = 0 | 11 | 5.3 (8.4) | 0.27 |
| | Yes - CPI $\neq$ 0 | 108 | 11.1 (14.8) | |
| Presence of tooth decay | "D" component of the DMFT = 0 | 31 | 5.2 (10.7) | <0.001 |
| | "D" component of the DMFT $\neq$ 0 | 88 | 12.5 (15.1) | |
| Need of some form of treatment | No | 38 | 11.1 (13.5) | 0.87 |
| | Yes | 81 | 10.4 (14.9) | |
| Missing teeth in the mouth | "M" component of the DMFT = 0 | 42 | 5.6 (10.0) | <0.001 |
| | "M" component of the DMFT $\neq$ 0 | 77 | 13.3 (15.7) | |

SD - Standard deviation
BMW - U$150.05

**Table 3: Correlation* between variables and OHIP-14 performance items and total prevalence scores.**

| Variables | Age | Number of pregnancy | DMFT | CPI | PIP |
|---|---|---|---|---|---|
| Functional limitation | 0.1167 | 0.2066 | 0.2954 (<0.001) | 0.1301 | -0.0517 (0.58) |
| rs (p) | (0.20) | (0.02) | 0.1859 | (0.15) | -0.0624 (0.50) |
| Physical pain | 0.1317 | 0.3236 (<0.001) | (0.04) | 0.8031 | -0.0276 |
| rs (p) | (0.15) | 0.3195 | 0.2340 | (0.37) | (0.77) |
| Psychological discomfort | 0.1292 | (<0.001) | (0.01) | 0.0883 | -0.0458 (0.62) |
| rs (p) | (0.16) | 0.2999 (<0.001) | 0.2634 (<0.001) | (0.34) | -0.0234 |
| Physical disability | 0.1822 | 0.3232 | 0.2882 (<0.001) | 0.1260 | (0.80) |
| rs (p) | (0.04) | (<0.001) | 0.2017 | (0.17) | 0.0174 |
| Psychological disability | 0.1380 | 0.2179 | (0.02) | 0.1004 | (0.85) |
| rs (p) | (0.13) | (0.02) | 0.1851 | (0.28) | -0.0920 (0.32) |
| Social Disability | 0.0319 | 0.3551 (<0.001) | (0.04) | 0.0430 | 0.0517 |
| rs (p) | (0.73) | 0.3952 (<0.001) | 0.2954 | (0.64) | (0.58) |
| Handicap in performing daily activities | 0.1730 | | (<0.001) | 0.0603 | |
| rs (p) | (0.06) | | | (0.51) | |
| Total OHIP | 0.1754 | | | 0.1105 | |
| rs (p) | (0.05) | | | (0.23) | |

rs -coefficient
p - Value of significance: $p < 0.05$
Spearman's rho test
*Spearman's correlation analysis

**Table 4: Multiple logistical regression analysis between variables and OHRQoL.**

| Variables | | p | Coefficient | SD | OR | CI (95%) |
|---|---|---|---|---|---|---|
| | Age | 0.75 | 0.0146 | 0.05 | 1.01 | 0.93 – 1.11 |
| | Working | 0.92 | -0.0432 | 0.46 | 0.95 | 0.39 – 2.88 |
| | Marital Status | 0.06 | 1.0175 | 0.56 | 2.77 | 0.92 – 8.29 |
| | Years of schooling | 0.99 | -0.0020 | 0.99 | 0.99 | 0.38 – 2.62 |
| | Household income | 0.70 | 0.0082 | 0.02 | 1.01 | 0.97 – 1.05 |
| OHRQoL | First pregnancy | 0.34 | -0.4576 | 0.48 | 0.63 | 0.24 – 1.64 |
| | Presence of systemic diseases | 0.48 | 0.3246 | 0.46 | 1.38 | 0.56 – 3.41 |
| | Need of dental prosthesis | 0.52 | 0.3574 | 0.56 | 1.42 | 0.48 – 4.30 |
| | DMFT Value | 0.83 | 0.0094 | 0.04 | 1.01 | 0.92 – 1.10 |
| | Presence of tooth decay | 0.03* | 1.1229 | 0.53 | 3.07 | 1.08 – 8.73 |
| | Missing teeth in the mouth | 0.76 | 0.1894 | 0.61 | 1.21 | 0.37 – 3.9 |

SD - Standard deviation
OR – Odds ratio

## DISCUSSION

This study's main findings were to verify that a pregnant woman's oral health condition interferes with her quality of life and that women with poorer oral health conditions (presence of caries) had poorer OHIP-14 scores, which was also verified by the clinical oral exams. The average score of the OHIP-14 in this study was 10.6, a value higher than those found for Chinese women (7.9)[8], pregnant women in India (7.0)[3,5] and pregnant women in Brazil (3.8)[6]. A national survey of oral health conducted in 2010 in Brazil showed that average OIDP in non-pregnant women aged 15 to 44 years ranges from 1.19 to 1.44[22].

One limitation of this study was sample size. Another limitation was related to study design, since it was a cross-sectional study and therefore may have some biases such as memory or social desirability and inability to provide more evidence on the results. Longitudinal studies are needed. Multi-site studies should be performed on a larger sample representative of the population of pregnant Brazilian women with more heterogeneous characteristics.

The most commonly employed method for evaluating oral health condition is professional clinical evaluation. However, this method assigns little or no importance to crucial factors such as how the state of the mouth affects a person`s daily life. In order to provide better care for patients, it is necessary to use subjective indicators of oral health to better capture the specific needs of individuals[1].

It is widely accepted that oral problems can cause a significant impact on physical, social and mental wellbeing during pregnancy. The results of our study demonstrate that the impact of oral health on quality of life, as reflected by the OHIP-14 scores, was significantly worse for those patients who also had a clinical issue. This confirmed the work of Acharya et al.[3] and Acharya and Bath[5]. It constitutes a matter of concern because pregnant women's quality of life and health condition are known to have a direct effect on their children's quality of life and health condition[11].

Increasing age, multiple pregnancies, DMFT index, presence of tooth decay, need for treatment and dental prosthesis, and missing teeth were all associated with a poorer impact on the women's quality of life. These associations are in agreement with the findings of Acharya et al.[3].

Women not in their first pregnancy had higher oral health impact scores than women in their first pregnancy, suggesting that the number of pregnancies may be an important predictor for this impact. This finding may explain why, during pregnancy, a woman is at increased risk of mouth disease due to changes in her habits, such as eating more sugary foods, less tooth brushing because of an increase in nausea and vomiting, and/or hormonal changes caused by pregnancy that increase the inflammatory response[23].

The study shows that factors such as presence of caries were important predictors of the impact of oral health on quality of life in the multivariate analysis. The DMFT index was associated to all the domains of the OHIP. This pattern suggests that the presence of caries and missing teeth can cause dental pain, thereby leading the patient to be constrained by her oral health condition and prompting her to socialize less with relatives, friends, and acquaintances[24]. The loss of a tooth as a result of caries and periodontal disease also had a negative impact on the OHRQoL in another study[8].

We found no such correlation between the OHIP-14 score and periodontal disease as pointed out in the literature[3,5], perhaps because the present study had high prevalence of the disease (90.8%). It is also important to note that the CPI index was used, which although easy to use and enabling comparison of data with international studies to be indexed and indicated by the WHO on periodontal disease[4], is partially performed with regard to some teeth indices and not performed on all teeth (full mouth)[8].

These women are an essential part of the family unit regarding oral health, since after childbirth they are also responsible for their children's oral health. Studies have shown that the worse the mother's oral health, the worse is their children's [24,25].

The definition of a specific population's need for dental treatment is an important step in planning health policies, using subjective indications, such as applying the OHIP-14 questionnaire, which complements the clinical exam and allows health professionals to better understand a person's perception of his or her oral health and perceived need for treatment. This knowledge also helps healthcare professionals to formulate effective programs and health services.

## CONCLUSION

These results suggest that poor oral conditions have a negative influence on quality of life during pregnancy. This risk group should be prioritized in the health services in order to treat and recover the oral health of pregnant women, thereby also promoting better oral health conditions and quality of life of their children.

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## REFERENCES

1. Miotto MH, Barcellos LA, Velten, DB. Evaluation of the impact on quality of life caused by oral health problems in adults and the elderly in a southeastern Brazilian city. Cien Saude Colet 2012; 17: 397-406.
2. Sheiham A, Tsakos G. Assessing needs through socio-dental approach. In: Pinto VG editor. Public Oral Health. São Paulo, Brazil: Ed. Santos; 2008.
3. Acharya S, Bhat P, Acharya S. Factors affecting oral health-related quality of life among pregnant women. Int J Dent Hyg 2009; 7: 102-107.
4. Wandera MN, Engebretsen IM, Rwenyonyi CM, Tumwine J, Astrom AN. Periodontal status, tooth loss and selfreported periodontal problems effects on oral impacts on daily performances, OIDP, in pregnant women in Uganda: a cross-sectional study. Health Qual Life Outcomes 2009; 7: 89.
5. Acharya S, Bhat PV. Oral-Health-Related Quality of Life during Pregnancy. J Public Health Dent 2009; 69: 74-77.
6. Oliveira BH, Nadanovsky P. Psychometric properties of the Brazilian version of the Oral Health Impact Profile–short form. Community Dent Oral Epidemiol 2005; 33: 307-314.
7. Slade GD, Spencer AJ. Development and evaluation of the oral health impact profile. Community Dent Health 1994; 11: 3-11.
8. Lu H, Xu W, Wong MC, Wei TY, Feng XP. Impact of periodontal conditions on the quality of life of pregnant women: a cross-sectional study. Health Qual Life Outcomes 2015; 13:67.
9. Gonzales-Sullcahuamán JA, Ferreira FM, de Menezes JV, Paiva SM, Fraiz FC. Oral health-related quality of life among brazilian dental students. Acta Odontol Latinoam 2013; 26:76-83.
10. Kuo HC, Chen JH, Wu JH, Chou TM, Yang YH. Application of the Oral Health Impact Profile (OHIP) among Taiwanese elderly. Qual Life Res 2011; 20:1707-1713.
11. Johnson M, George A, Dahlen H, Ajwani S, Bhole S, Blinkhorn A, Ellis S, Yeo A. The midwifery initiated oral health-dental service protocol: an intervention to improve oral health outcomes for pregnant women. BMC Oral Health 2015; 15:2.
12. Oliveira BH, Nadanovsky P. The impact of oral pain on quality of life during pregnancy in low-income Brazilian women. J Orofac Pain 2006; 20: 297-305.
13. Volpato FC, Jeremias F, Spolidório DM, Silva SRC, Valsecki Junior A, Rosell FL. Effects of oral environment stabilization procedures on Streptococcus mutans counts in pregnant women. Braz. Dent J 2011; 22: 280-284.
14. Cornejo C, Rossi G, Rama A, Gomez-Gutierrez N, Alvaredo G, Squassi A, Klemonskis G. Oral health status and oral health-related quality of life in pregnant women from socially deprived populations. Acta Odontol Latinoam 2013; 26: 68-74.
15. Strobe Statement - Checklist of items that should be included in reports of cross-sectional studies. URL: http://www.strobe-statement.org/fileadmin/Strobe/uploads/checklists/STROBE_checklist_v4_cross-sectional.pdf.
16. Hulley SB. Outlining the clinical research: an epidemiological approach. 3th edition Porto Alegre, Brazil: Ed. Artmed; 2008.
17. World Health Organization. Oral health surveys: basic methods. 4th edition. 2009. 93p.
18. Slade GD. Derivation and validation of a short-form oral health impact profile. Community Dent Oral Epidemiol 1997; 25: 284-290.
19. Cohen-Carneiro F, Rebelo MA, Souza-Santos R, Ambrosano GM, Salino AV, Pontes DG.. Psychometric properties of the OHIP-14 and prevalence and severity of oral health impacts in a rural riverine population in Amazonas State, Brazil. Cad Saude Publica 2010; 26: 1122-1130.
20. Center for Disease Control and Prevention. Program Epi Info™ Version 7.0. 2012. URL: http://www.cdc.gov/epiinfo/.
21. Ayres M, Ayres Júnior M, Ayres DL, Santos AS. Bioestat 5.0: statistical applications in the biological and medical sciences areas. Belém: Mamirauá Civil Society; 2007. URL: http://www.mamiraua.org.br/download/index.php?dirpath=./BioEstat%205%20Portugues&order=0.
22. Brazil. Ministry of Health. Department of Health Care. Secretariat of Health Surveillance. SB Brazil 2010: National Oral Health Survey: Main results. Brasilia: Ministry of Health. 2012.
23. Moimaz SAS, Rocha NB, Saliba O, Garbin CAS. The access of pregnant to dentistry treatment. Rev Odontol Univ Cid São Paulo 2007; 19: 39-45. https://www.researchgate.net/publication/242164447_O_acessO_de_gestantes_aO_tratamentO_OdOntOlogicO_The_access_of_pregnan_Ts_To_den_Tis_Try_Trea_TmenT
24. Zanata RL, Navarro MF, Pereira JC, Franco EB, Lauris JR, Barbosa SH. Effect of caries preventive measures directed to expectant mothers on caries experience in their children. Braz. Dent. J. 2003; 14: 75-81.
25. Shearer DM, Thomson WM, Broadbent JM, Poulton R. Does maternal oral health predict child oral health-related quality of life in adulthood? Health Qual Life Outcomes 2011; 9: 50. DOI: 10.1186/1477-7525-9-50.

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