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Referencias: (1) de Albuquerque RF Jr., Head TW, Mian H, Muller K, Sanches K. Ito IY. Reduction of salivary S. Aureus and mutans group streptococci by a preprocedural chlorexidine rinse and maximal inhibitiry dilutions of chlorexidine and cetylpyridinium, Quitessence Int. 2004 Sept; 35 (8): 635-40. (2) Willians C, Mostler K, Simone AJ, Crawford R, Patel S, Petrone Me, Chakins P. Devizio W, volpe AR, Proskin HM. Efficacy of a dentrifice containing zinc citrate for the control of plaque and gengivitis: A 6-month clinical study in adults. Compendium. 19 (Suppl):4-15. (3) Antibacterial effects of a 2% zinc citrate toothpaste versus a regular toothpaste with fluoride alone on the supragengival plaque bacteria after múltiple use. Data on Flie; Colgate Palmolive Company Study design: 6 months, double blind clinucal study in harmony with ADA guidelines with 99 subjects completed the study.

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

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Phagocytic activity of monocytes and neutrophils in patients with periodontitis, whether or not associated to type 2 diabetes

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ABSTRACT

Phagocytic functions by neutrophils/ monocytes and biochemical parameters were assessed in peripheral blood of patients with periodontitis, whether or not associated to type 2 diabetes, or patients with type 2 diabetes, or systemically healthy people. Fifty-eight participants were divided into four groups: Control – systemically and periodontally healthy patients (C, n=16), Periodontitis (P, n=14), Type 2 Diabetes (DM, n=11) and Periodontitis associated with type 2 diabetes (DMP, n=17). Blood samples were used to analyze phagocytic activity and the production of superoxide anion using optical microscopy. Significantly lower phagocytic activity of neutrophils was observed in non-opsonized samples (p = 0.008, Kruskal-Wallis) of the periodontitis group and in opsonized samples (p = 0.029, Kruskal-Wallis) of the periodontitis associated with type 2 diabetes group when these groups were compared to the healthy individuals when a 20:1 yeast: phagocyte stimulus was used. Periodontitis patients, whether associated (p = 0.0007, sensitized; Kruskal-Wallis, 20:1) or not with diabetes (p = 0.018 and 0.0007, in the proportions 5:1 and 20:1 yeast: monocyte respectively in sensitized samples; Kruskal-Wallis) also showed lower phagocytic function of monocytes compared to the control group. There was no significant difference in the production of superoxide anion among the evaluated groups. Severe clinical attachment loss was associated with lower levels of HDL in periodontitis patients and a higher percentage of A1C in diabetes with periodontitis patients (p< 0.05; Pearson and Spearman correlations, respectively). Patients with both associated diseases had higher levels of triglycerides and CRP (p < 0.001, Kruskal-Wallis) compared to patients with diabetes only. The results of the present study suggest that periodontitis negatively interferes with the innate immune response and may represent a major risk of systemic complications such as cardiovascular disease in diabetic patients or even in healthy individuals.

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Keywords: *leukocytes – phagocytosis - superoxide anion - biochemical markers -periodontitis - diabetes mellitus.*

Atividade fagocítica de monócitos e neutrófilos em pacientes com periodontite, associada ou não ao diabetes tipo 2

RESUMO

As funções fagocíticas de neutrófilos/monócitos e parâmetros bioquímicos foram avaliados no sangue periférico de pacientes com periodontite com ou sem diabetes do tipo 2, ou em pacientes com diabetes tipo 2, ou em pessoas saudáveis sistemicamente. 58 participantes foram divididos em quatro grupos: Controle - pacientes sistemicamente e periodontalmente saudáveis (C, n = 16), Periodontite (P, n = 14), Diabetes Tipo 2 (DM, n = 11) e Periodontite associada a diabetes tipo 2 (DMP, n = 17). Amostras de sangue foram usadas para analisar a atividade fagocítica e a produção de ânion superóxido por microscopia óptica. Observou-se menor atividade fagocítica dos neutrófilos em amostras não opsonizadas (p = 0,008, Kruskal-Wallis) do grupo periodontite e em amostras opsonizadas (p = 0,029, Kruskal-Wallis) do grupo periodontite associada ao diabetes tipo 2 quando esses grupos foram comparados aos indivíduos saudáveis sob um estímulo de levedura:monócito de 20:1. Pacientes com periodontite associada (p = 0,0007, sensibilizados; Kruskal-Wallis, 20: 1) ou não com diabetes (p = 0,018 e 0,0007, nas proporções 5: 1 e 20: 1 de levedura: monócito, respectivamente, em amostras sensibilizadas; Kruskal- Wallis) também demonstraram menor função fagocítica dos monócitos em comparação com o grupo controle. Não houve diferença significativa na produção de ânion superóxido entre os grupos avaliados. A perda de inserção clínica grave foi associada a níveis mais baixos de HDL na periodontite e maior percentual de A1C nos pacientes com periodontite associada ao diabetes (p <0,05; correlações de Person e Spearman, respectivamente). Os pacientes com ambas as doenças associadas apresentaram

níveis mais altos de triglicerídeos e PCR (p <0,001, Kruskal-Wallis) em comparação aos pacientes com somente diabetes. Os resultados do presente estudo sugerem que a periodontite interfere negativamente na resposta imune inata e pode representar um risco maior para complicações sistêmicas, como a doença cardiovascular, em pacientes com diabetes ou mesmo em indivíduos saudáveis.

Palavras-chave: *leucócitos - fagocitose - ânion superóxido - marcadores bioquímicos - periodontite - diabetes mellitus.*

INTRODUCTION

Phagocytosis is performed by the host's phagocytic cells to eliminate invading microorganisms and stimulate other immune responses. Phagocytosis is initiated by the interaction of cell surface receptors with ligands found on the microorganisms, such as lipopolysaccharides, or host-derived opsonins, such as complement or IgG antibodies[1].

The phagocytic cell killing response to periodontal pathogen invasion results in the formation of reactive oxygen species (ROS), such as superoxide anion, which contribute to local periodontal tissue destruction when released in larger amounts[2].

During periodontitis, ROS end-metabolites can be translocated to distant organs via blood circulation, leading to tissue damage[3].

It is currently clear that periodontitis is associated with some systemic diseases such as diabetes mellitus (DM)[4]. Diabetic patients with inadequate glycemic control are more likely to develop severe periodontitis. Emerging evidence also indicates that the severity of periodontitis is related to higher mortality rates and significantly correlates with dyslipidemia, oxidative stress[3] and cardio-renal complications of diabetes[4].

Considering the infectious and inflammatory nature of periodontal diseases, the aim of this study was to analyze the influence of periodontitis alone or in association with type 2 diabetes on the phagocytic functions of monocytes and neutrophils and on the biochemical parameters of peripheral blood.

MATERIALS AND METHODS

Study groups

The study protocols, procedures and consent form were approved by the Human Research Ethics Committee, University of Brasília (UnB) (process number 46609515.7.0000.0030), in accordance with Brazilian legislation, which complies with the Declaration of Helsinki, as revised in 2013. All participants were individually informed about the study and agreed to participate by signing a written informed consent form.

Fifty-eight non-smokers (23 males and 35 females), ≥ 30 years old, were included in the study, which was further divided into four groups: periodontitis group (P), type 2 Diabetes Mellitus group (DM), periodontitis and type 2 DM group (DMP) and Controls (C). They were recruited from the Periodontology Clinic at the University Hospital of Brasília (HUB), Distrito Federal, Brazil.

Participants were interviewed to obtain medical and demographic information, including age, sex and tobacco use.

An experienced examiner (PFN) conducted the periodontal clinical examination, which consisted of six sites recorded for each tooth, excluding third molars, measured using a periodontal probe (UNC 15 screening probe). The following parameters were recorded: probing depth (PD), clinical attachment loss (CAL), visible plaque index (PI) and gingival bleeding on probing (BOP) index.

The calibration and measurements of periodontal clinical parameters were repeated within 24 hours, showing agreement of over 80%. BOP was calculated by the Kappa coefficients, and intra-examiner agreement was > 0.85.

The periodontitis group (P) consisted of subjects without systemic diseases and with at least 12 teeth. The following inclusion criteria were adopted: clinical diagnosis of periodontitis at ≥ 2 non-adjacent teeth (CAL ≥ 3mm, PD ≥ 4mm, ≥ stage II; grade A).

The diabetes mellitus with periodontitis group (DMP) consisted of individuals previously diagnosed with type 2 DM with diagnosis of periodontitis. The same criteria as for the periodontitis group were used, with the inclusion of grades B or C periodontal conditions. Both controlled (A1c < 7%) and uncontrolled diabetic patients (A1c ≥ 7%) were included.

The diabetes group (DM), consisted of subjects with previous diagnosis of type 2 diabetes, regardless of glycemic control status, without periodontal disease and who had at least 12 teeth.

The control group (C) consisted of orally and systemically healthy individuals, with at least 20 teeth. The periodontal health criteria used for both DM and C groups were the absence of interproximal or buccal CAL ≥ 3mm, PD ≤ 3 mm, BOP < 10% and no radiographic evidence of bone loss.

The following exclusion criteria were adopted: anyone who smoked within the past 5 years, periodontal treatment within the last 12 months, antimicrobial therapy for systemic conditions or topical oral use within the last 12 months, use of medications such as anti-inflammatory, corticoid and immunosuppressive therapy; pregnant or lactating women; autoimmune, infectious, allergic, renal and gastrointestinal diseases; cancer; morbid obesity [body mass index (BMI) > 40kg/m^2] or malnutrition (BMI < 18.5 kg/m^2)[5].

Periodontal disease was classified according to the new classification of periodontal diseases and conditions[6].

Blood sampling for biochemical analysis

Blood samples were taken after eight hours' fasting. Samples were collected and analyzed at Laboratory Sabin, Brasilia – Distrito Federal, Brazil. The following parameters were evaluated: Hemogram, lipid profile, fasting glycemia, glycated hemoglobin (A1c) and C-reactive protein. (CRP).

Evaluation of phagocytosis

A technique previously described[7] was adapted to assess phagocytosis of dead *Saccharomyces cerevisiae* via pattern-recognition receptors or facilitated by opsonins.

Whole blood (40 µL/area) was placed on clean glass slides containing eight marked areas 7 mm in diameter each and incubated in a wet chamber for 45 minutes at 37°C. The slides were then rinsed with 0.15M PBS, pH 7.2 at 37°C, to remove non-adherent cells. After washing, monocytes and neutrophils remained adhered to the slide approximately in the same proportion as they were in the whole blood. Adherent cells were incubated with a suspension 0.625x10^5 or 2.5x10^5 *S. cerevisiae* in 20 µL Hanks-tris (Sigma, USA), pH 7.2, with 10% heat-inactivated fetal calf serum (FCS) (Gibco) for 30 min in a wet chamber at 37°C. Slides were then rinsed with 0.15M PBS at 37°C to eliminate non-phagocytosed *S. cerevisiae* and the final wash was done with 30% FCS in Hanks-tris. The slides were then fixed with absolute methanol and stained with 10% Giemsa solution. The number of *S. cerevisiae* phagocytosed by 200 neutrophils or monocytes in single preparations was assessed by optical microscopy. Microscopic fields distributed throughout the slide were randomly selected and all phagocytes in each particular field were examined by a single blinded examinator (ACGC). The phagocytic index was calculated as the average number of phagocytosed *S. cerevisiae* per phagocytosing neutrophil or monocyte, multiplied by the percentage of these cells engaged in phagocytosis[7].

Yeasts to be phagocytosed by phagocytes were used with or without previous incubation with fresh serum from the donor for 30 min at 37°C. In the former case, yeast cells were considered sensitized, because complement molecules present in serum opsonized them and phagocytosis occurred through phagocytes CR1 and CR3 receptors that bind to C1 and C3 components of complement adhered to the surface of the yeasts[7]. The yeast cells that were not pre-incubated with fresh serum from the donor, but were incubated with inactivated fetal bovine serum, were considered as non-opsonized, and their phagocytosis occurred via the pattern-recognition receptors (PRR) of neutrophils or monocytes[8].

Evaluation of superoxide anion production

The superoxide anion production was analyzed by the Nitro blue tetrazolium (NBT) test based on an adaptation of a technique previously described[9]. This technique evaluates the microbicidal mechanism of phagocytes by their ability to generate toxic oxygen radicals capable of reducing the compound NBT to an insoluble form, called formazan, which can be identified under optical microscopy by a blue color in the cytoplasm of the cell.

The quantity of NBT reduced is directly proportional to the amount of oxygen radicals produced by phagocytes, and these molecules are among the principal microbicidal agents produced by phagocytic cells[2].

Briefly, the phagocytes adhered to the slide, as previously described, were incubated with 0.05% NBT solution in Hanks-tris (Sigma, USA) for 20 minutes at 37°C in a humidified chamber.

The slides were then washed, fixed with methanol and stained with a solution of 1.4% safranin and 28.6% glycerol in distilled water. NBT reduction was also stimulated by sensitized *S. cerevisiae*. The

percentage of phagocytes with reduced NBT in the cytoplasm was assessed by a blinded researcher (ACGC) using optical microscopy.

Statistical analysis

Sample size was determined for a desired power of 90% and an alpha level of significance of 0.05 using Sigma Stat software, based on the annual number of patients seen at the periodontal clinic (HUB). Estimated sample size was 60.

Prism® software (GraphPad, USA) was used in the data analysis. Normality of variables was previously verified by the Kolmogorov-Smirnov test. Differences among three or more groups were verified by analysis of variance (ANOVA), followed by the Turkey test or Kruskal-Wallis test, followed by Dunn's method, to variables with Gaussian or non-Gaussian distribution and similar or different variances, respectively. Pearson or Spearman correlation coefficients were used to estimate the correlation between periodontal clinical attachment loss versus hematologic parameters, for variables with Gaussian or non-Gaussian distribution, respectively. The level of significance was set at $p < 0.05$.

RESULTS

Clinical and demographic data

All individuals that volunteered to participate were chosen to be part of a convenience sample. A total 58 participants, 23 (39.7%) males and 35 (60.3%) females, were enrolled at the study.

Four groups were assessed as follows: 1) Periodontitis group (P) including 14 patients (8F; 6M), age 45±9; 2) Diabetes type 2 with periodontitis group (DMP) including 17 patients, age 53±7, 7 females (F) and 10 males (M); 3) Diabetes type 2 group (DM), without periodontal diseases, including 11 patients (9F; 2M), age 51±10; 4) Control group (C) including 16 healthy volunteers (11F; 5M), age 42±9.

The control group had a significantly higher number of teeth (mean 26.6; $p = 0.0108$; Kruskal-Wallis followed by Dunn's comparison) in relation to patients with diabetes (mean 21.6 or 21.3, with or without periodontitis) or periodontitis (mean 22.8), but without significance.

Mean body mass index (BMI) of DM (31 kg/m^2) and DMP patients (30 kg/m^2) was higher than the control (26 kg/m^2) or periodontitis group (28 kg/m^2 $p = 0.0137$, ANOVA).

Patients with periodontitis (with or without diabetes) had significantly higher probing depth (PD), clinical attachment loss (CAL), plaque index (PI) and bleeding on probing (BOP) when compared to groups without periodontal disease ($p < 0.0001$; Kruskal-Wallis) (Table 1).

Laboratory parameters

All values obtained from the hematological examinations were within the normality or reference values, except for the glycemic indices for both diabetes groups or triglycerides in the DMP group. Although triglyceride values in subjects with diabetes were within normal ranges, this was not observed in subjects with diabetes associated with periodontitis, whose concentrations were above established standards (Table 2).

Correlation analysis showed that there was a moderate inverse correlation between CAL and HDL ($r = -0.5870$, Pearson) in periodontitis patients (Fig. 1) and a moderate positive correlation between CAL and A1C in DMP patients ($r = 0.6618$, Spearman, Fig. 2). No more significant correlations were found between CAL and the other hematologic parameters in periodontitis patients (with or without diabetes) (Table 3).

C-reactive protein (CRP) levels

Among patients with diabetes, those with periodontitis as co-morbidity had higher and significant CRP levels (5/0.3-9.5) compared to the control group (0.3/0.02-1.4) or diabetes without periodontitis group (0.4/0.02-2.9) (Fig. 3).

Phagocytic activity by neutrophils

There was no difference in the phagocytic index among the groups, when sensitized or non-sensitized yeasts were used in the proportion 5:1 yeasts per neutrophil ($p<0.05$). However, the number of sensitized phagocytized yeasts per neutrophil was significantly lower in periodontitis (1.6±0.5) and DMP groups (1.8±0.6) compared to controls (2.4±0.7), ($p=0.007$, ANOVA).

When the stimulus was used in the proportion

Table 1 - Clinical periodontal data from the participants of the study.

	C	*P*	*DM*	*DM-P*	p-Value
CAL+ (mm)	2.8 ± 0.2	6.3 ± 1.9	2.9 ± 0.2	5.5 ± 1.6	< 0.0001
PD+ (mm)	2.1 ± 0.4	6.1 ± 1.7	2.4 ± 0.3	5.5 ± 1.2	< 0.0001
PI (%)	12.1 ± 2.9	79 ± 27.4	13.8 ± 1.4	77 ± 23.3	< 0.0001
BOP (%)	7.5 ± 1.3	48 ± 26.1	8.9 ± 1.9	38 ± 25.8	< 0.0001
Extension (%)	0	51 ± 29.8	0	42 ± 26.3	< 0.0001

Kruskal-Wallis test. + Deeper pockets or major CAL per tooth. Values are expressed as mean ± standard deviation; p-value significant when < 0.05. Legends: C = control group; P = periodontitis group; DM = diabetes mellitus group; DMP = diabetes mellitus with periodontitis group; PI = plaque index; BOP = bleeding on probing; CAL = clinical attachment loss; PD = probing depth.

Table 2 - Biochemical profile of individuals according to study groups.

Groups		C	P	DM	DMP	RV
		(Mean ± SD)				
Lipids	Total cholesterol (mg/mL)	188±34	192±35	178±28	195±39	<190
	HDL (mg/mL)	50±10	53±10	47.8±10.8	47±8	> 40
	LDL (mg/mL)	113±31	113±25	95.5±24.7	107±42	< 130
	Triglycerides (mg/mL)	124±55	131±75	186±150	214±102	< 150
Blood glucose	A1c (%)	5±0	5±0	8±2*#	7±1*#	4 to 6
	Glucose (%)	88±9	97±12	139±50*	138±43*#	70 to 99
Hemogram	Hematocrit (%)	42±4	69±102	41±4	66±94	36.0 a 54.0 ♂ 33.0 a 47.8 ♀
	Total leukocytes (mm^3)	6359±1510	6746±1942	5866±1503	7918±2738	3600 to 11000

Kruskal-Wallis test. *Values differ from control group (p < 0.05); # Values differ from periodontitis group (p < 0.05). Legends: C = control group; P = periodontitis group; DM = diabetes mellitus group; DMP = diabetes mellitus with periodontitis group; HDL = high density lipoprotein; LDL = low density lipoprotein; A1c = glycated hemoglobin. RV = Reference values for normality with fasting from 8 to 12 hours.

20:1 yeasts per neutrophil, the phagocytic index in periodontitis patients (3.5±2.4) was lower compared to controls (16.6±16.9) and DM patients (26.7±22.1), in the non-sensitized samples (p=0.008, Kruskal-Wallis). These results can be related to a lower frequency of neutrophils involved with phagocytosis in the periodontitis group (7±15.8) compared to the DM group (12.5±9.8), (p=0.04, Kruskal-Wallis).

Healthy individuals also had a higher phagocytic index (223.5±56.1) when compared to patients with diabetes associated to periodontitis (126.8±101.1) in the opsonized samples, in the proportion of 20 yeasts per neutrophil (p=0.029, Kruskal-Wallis). There was a higher number (p=0.008, ANOVA) of phagocytized *S. cerevisiae* per neutrophil in controls (2.9±0.7) compared to patients with DMP (2±0.7) or periodontitis alone (1.9±0.7). The results are summarized in Fig. 4.

Phagocytic activity of monocytes

There was no difference in the phagocytic index (PhI) among the groups, when non-sensitized yeasts were used in the proportion 5:1 yeasts per monocyte (p<0.05). However, PhI was lower in periodontitis patients (82.4±59.2) compared to the controls (132.9±27.5), when sensitized yeasts were used (p=0.018, Kruskal-Wallis). There was also a significantly lower percentage of cells engaged in phagocytosis in periodontitis (51.5±17.9) and DMP (51.8±21.7) groups compared to the control group (73.5±8.1) in sensitized samples (p=0.001, Kruskal-Wallis).

There was no significant difference in the phagocytic

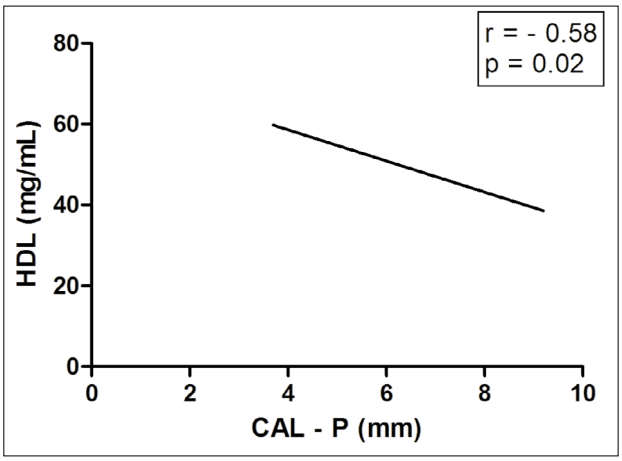

Fig. 1: Pearson correlation analysis between clinical attachment loss (CAL) and serum HDL levels during periodontitis (P). p < 0.05.

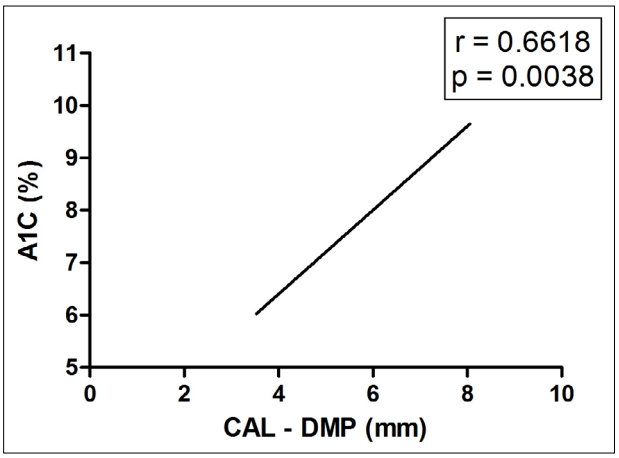

Fig. 2: Spearman correlation analysis between clinical attachment loss (CAL) and serum glycated hemoglobin (A1C) levels during periodontitis associated to diabetes (DMP). p < 0.05.

Table 3. Correlation analysis between clinical attachment loss and biochemical parameters.

P	CAL X TC	CAL X HDL	CAL X LDL	CAL X TG	CAL X BG	CAL x A1C	CAL X CRP	CAL X TL
r	-0.00006+	-0.5870+**	0.1747+	0.06601#	-0.1239+	0.1920+	0.2999#	0.001375+
p	0.9998	0.0273*	0.5503	0.8226	0.6731	0.5108	0.3195	0.9963
DMP	**CAL X TC**	**CAL X HDL**	**CAL X LDL**	**CAL X TG**	**CAL X BG**	**CAL x A1C**	**CAL X CRP**	**CAL X TL**
r	-0.3272S	-0.2458+	-0.2762S	0.2191+	0.4047#	0.6618+**	0.3603#	0.1043#
p	0.1999	0.3416	0.2831	0.3982	0.1071	0.0038*	0.1554	0.6904

P = Periodontitis patients; DMP = Diabetes with periodontitis patients; CAL = Clinical attachment loss; TC = Total cholesterol; HDL = High density lipoproteins; LDL = low density lipoproteins; TG = Triglycerides; BG = Blood glucose; A1C = Glycated hemoglobin; CRP = C reactive protein; TL = Total leukocytes. + Pearson correlation. # Spearman correlation. ** significant correlation. * p < 0.05.

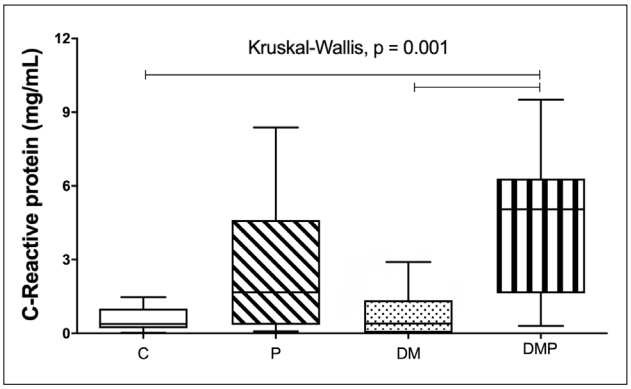

Fig. 3: CRP levels among the different study groups. Kruskal-Wallis test. Legends: C = control group; P = periodontitis group; DM = diabetes mellitus group; DMP = diabetes mellitus with periodontitis group. p < 0.05. Results are expressed as median with maximum and minimum quartiles.

index among groups when non-sensitized yeasts were used in the 20:1 proportion of yeasts per monocyte (p<0.05). A lower PhI was found in both periodontitis and DMP groups compared to controls only when sensitized yeast was used (p=0.0007, Kruskal-Wallis).

There was a significantly lower percentage of monocyte cells engaged in phagocytosis in DMP (52.4±23.9) and periodontitis groups (53.6±15.4) when the 20:1 proportion of yeasts per monocyte was used (p=0.0001, Kruskal-Wallis). The mean number of phagocytized yeasts from the periodontitis group was lower (1.7±0.4) than the controls (2.4±0.7) (p=0.02, ANOVA). All results concerning phagocytosis by monocytes are summarized in Fig. 5.

Superoxide radical

Neither periodontal disease nor diabetes influenced the capacity of basal or stimulated production of superoxide when compared to the control or diabetes groups, as shown in Fig. 6.

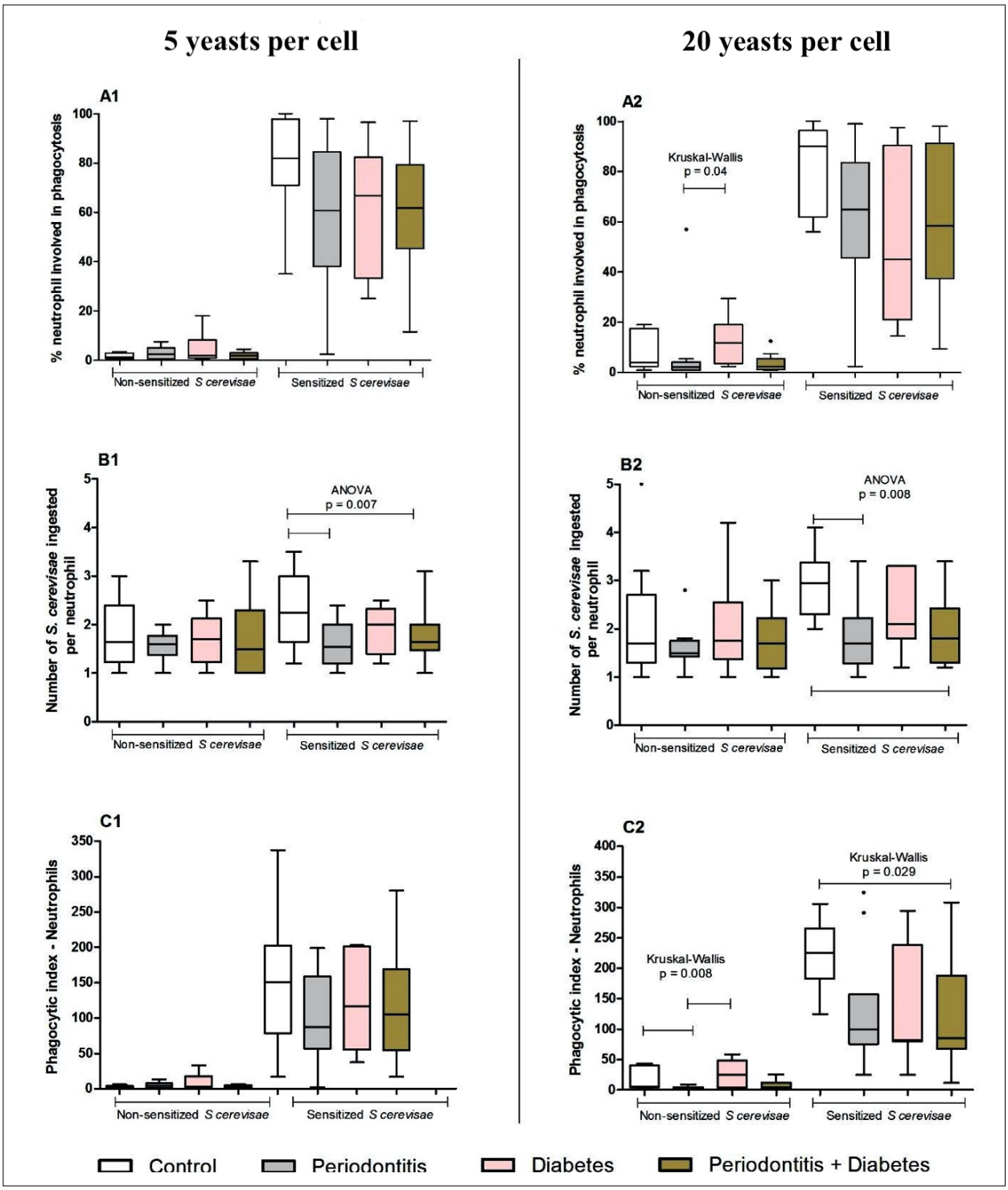

Fig. 4: Phagocytic capacity of neutrophils obtained from the peripheral blood of individuals in the control (C, n.16), periodontitis (P, n.14), diabetes (DM, n.11) and periodontitis + diabetes (DMP, n.17) groups evaluated by the phagocytic index (C1 and C2), which is the product of percentage of cells involved in phagocytosis (A1 and A2) by mean yeasts (sensitized or not with the individual's own serum) ingested per cell (B1 and B2). The medians, quartiles, maximum and minimum values are shown from a single experiment, representative of six independent experiments (three with 5 yeasts and three with 20 yeasts per cell, from 58 samples). The experiments were performed in duplicate. ANOVA or Kruskal-Wallis were performed according to the indication in the figure. $p < 0.05$.

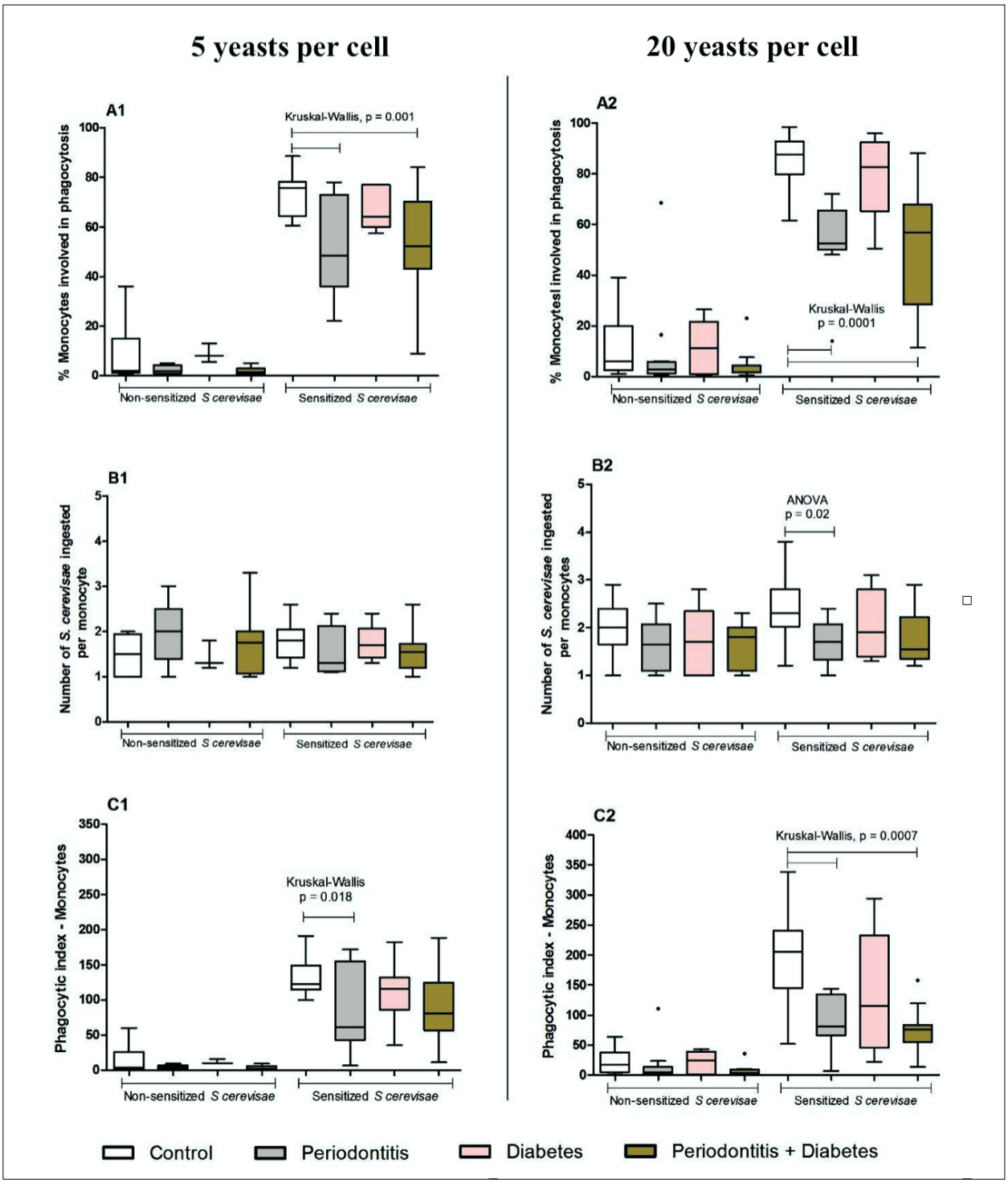

Fig. 5: Phagocytic capacity of monocytes obtained from the peripheral blood of individuals in the control (C, n.16), periodontitis (P, n.14), diabetes (DM, n.11) and periodontitis + diabetes (DMP, n.17) groups evaluated by the phagocytic index (C1 and C2), which is the product of percentage of cells involved in phagocytosis (A1 and A2) by mean yeasts (sensitized or not with the individual's own serum) ingested per cell (B1 and B2). The medians, quartiles, maximum and minimum values are shown from a single experiment, representative of six independent experiments (three with 5 yeasts and three with 20 yeasts per cell, from 58 samples). The experiments were performed in duplicate. ANOVA or Kruskal-Wallis were performed according to the indication in the figure. $p < 0.05$.

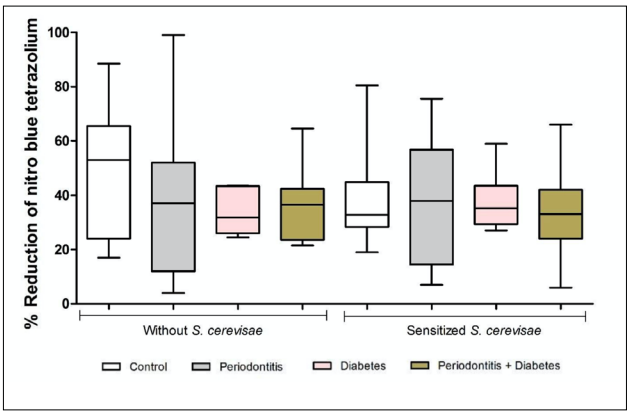

Fig. 6: Percentage of reduction of NBT (nitro blue tetrazolium) test by peripheral blood leukocytes from individuals in the control (C, n.16), periodontitis (P, n.14), diabetes (DM, n.11) and periodontitis + diabetes (DMP, n.17) groups. The results showed that both basal (Kruskal-Wallis test) and S. cerevisiae-stimulated (ANOVA) production did not differ between groups. The medians, quartiles and extreme values are shown from a single experiment, representative of two independent experiments, from 58 samples. The experiments were performed in duplicate. $p < 0.05$.

DISCUSSION

This study analyzed the association between periodontitis and diabetes, since to date, the mechanisms by which periodontitis promotes metabolic dysfunction are not fully understood.

Regarding the clinical characteristics of the study population, healthy individuals were found to have more teeth than periodontitis and diabetes patients with or without periodontitis. This probably reflects the fact that general healthcare is, in addition to other factors, associated with oral healthcare, since both DM2 and periodontitis are often associated with risk factors such as health-threatening habits and lifestyle[1-4,6].

The patients in our study mainly presented stage III periodontitis, grade A, with severe, generalized extension of the disease (reaching more than 30% of the teeth present on the oral cavity).

The total surface area of this periodontal inflammatory field is estimated to be the size of the palm of the hand. Immediate medical intervention would be necessary if there were a lesion like this on the skin. However, periodontitis is frequently ignored by health professionals, even though it may be associated with a range of systemic diseases and conditions[10].

Systemically healthy subjects, including those with periodontitis, had a maximum body mass index (BMI) of 28 kg/m^2, while the groups with diabetes had BMI higher than 30 kg/m^2. This indicates that most of the participants were already overweight or in class 1 obesity according to the World Health Organization[5]. It is worth mentioning that BMI between 17 and 25 kg /m^2 provides a better survival rate and that the relative risk of mortality increases in individuals outside this range[5].

Although we did not find differences in the hemogram and the leukogram parameters among all categories of patients and controls, other studies have reported increased levels of total leukocyte counts[11], in contrast to our results regarding patients with periodontitis, and lower levels of erythrocytes and hemoglobin in severe chronic periodontitis patients compared to controls[12].

Interestingly, we found that the higher the clinical attachment loss, the lower was the HDL level detected in patients with periodontitis. This suggests that severity of periodontitis could indirectly be an important risk factor for cardiovascular disease by influence of blood lipid concentrations such as lower levels of HDL.

The lower HDL levels in circulation during periodontitis might be due to the fact that local chronic inflammation leads to the release of LPS by Gram-negative bacteria and cytokines as IL-1β and TNF-α in blood stream, which have the capacity to influence lipid metabolism[13]. Furthermore, low HDL levels may also indirectly contribute to inflammatory processes because HDL has anti-inflammatory properties and can decrease the adhesivity of endothelial cells[13].

In contrast to participants with DM who had triglyceride levels similar to controls, those with DMP presented higher levels of triglycerides when compared to healthy individuals, even exceeding reference values. This may be an indication that periodontal disease is capable of predisposing patients with DM to a higher risk of complications such as the development of cardiovascular diseases, since the literature reports a positive association between high levels of triglycerides and higher cardiac risk[14].

Periodontitis has been associated with a more reduced glycemic control in diabetes[10]. Nevertheless, our study did not find differences between subjects with DMP and DM alone concerning the glycemic levels (blood glucose or % A1c). However, after performing the correlation analysis, we found that the higher the clinical attachment loss, the

higher was the A1C level in patients with diabetes associated to periodontitis.

A cohort longitudinal study in Germany[15] conducted over 15 years in patients with severe periodontitis showed that A1c levels, just after five years from their baseline levels, were significantly higher than in healthy individuals. Additionally, after a 10-year follow-up period[16], another study reported a greater increase in the prevalence of periodontal disease among patients who developed glucose intolerance than among those who had not. This poorer control in glycemic indices increases the risk for the incidence of diabetes in non-diabetic patients, insulin resistance in patients with DM and disease complications, including mortality[17.]

Regarding innate immune defense, leukocytes are the body's main mechanism against microbial invasion. At the beginning of a bacterial infection, neutrophils are the predominant cells involved in host defense, and they also play an important role in the course of pathogenesis and inflammation due to infectious diseases[18]. Leukocytes respond to virulence factors with the secretion of pro-inflammatory mediators such as cytokines, chemokines, reactive oxygen species (ROS) and C-reactive protein (CRP)[19]. CRP is mainly produced by the liver, and cardiovascular disease has CRP as an independent predictor of its occurrence[20].

The present study found no significant differences in leukocyte counts between periodontitis patients and controls; however, other studies have reported different results[21, 22]. Patients with periodontitis may present a significantly higher number of leukocytes compared to healthy people, which may indicate an increased risk of myocardial infection[21]. A study conducted by Pejčić et al.[22] concluded that an increase in total leukocyte count in patients with periodontitis, especially in its severe form, may be an indicator of possible exposure to a systemic disease, and may represent an important warning for physicians to refer their patients to a dentist.

Phagocytes, such as neutrophils and monocytes, stand out as leukocytes that act in the initial defense response against periodontal pathogens. Notwithstanding, Carneiro et al.[23] observed a significant reduction of phagocyte functions in individuals with periodontitis. In other studies, authors reported that non-surgical periodontal treatment significantly improved the clinical periodontal status of subjects with periodontal disease and resulted in significantly increased phagocytosis of neutrophils[24] and monocytes[25] to levels observed in control subjects.

The elevated number of white blood cells has been associated with the risk of coronary heart disease, cardiovascular disease, atherosclerosis, thrombosis and myocardial ischemia. In this context, the inflammatory nature of chronic infections such as periodontitis could be associated with leukocytosis and major CRP levels in the bloodstream[26].

CRP has been considered an independent inflammatory biomarker[20.] Elevated or even moderate levels of CRP can predict future vascular events and are associated with an increased risk of cardiovascular diseases such as heart strokes and myocardial infarctions among apparently healthy people with acceptable levels of LDL[27,28].

Other previous studies have shown that serum inflammatory biomarkers, such as leukocytes and CRP, are elevated in diabetes[28] and periodontitis patients[29]. When periodontitis is associated with diabetes, these levels are further elevated[28].

In line with these findings, our study showed that serum CRP levels increased when diabetes was associated with periodontitis compared to DM alone. The risk of coronary heart disease is estimated to be 1.5- to 2-fold higher in people with periodontitis than in those without the disease[30].

However, our results did not show any difference in CRP levels between periodontitis patients and healthy subjects. This agrees with other studies[31] that also found no difference in CRP between controls and periodontitis patients.

Acute phase proteins and leukocytosis are induced by pro-inflammatory cytokines or bacterial components released into the circulation. Probably in response to endotoxins such as lipopolysaccharides found in periodontal microorganisms, there is an increase in the production of proinflammatory cytokines that can alter lipid metabolism and insulin resistance[32].

However, the current knowledge about the association between the two diseases shows that CRP serum levels decrease with the administration of mechanical periodontal treatment in periodontal disease[33], supporting the view that severe periodontitis induces systemic micro-inflamation.

Concerning phagocytic function, the present study showed that when the antigen is in a low proportion (5:1) in relation to neutrophil cells, there is no difference in phagocytosis between patient groups or healthy individuals.

The increase in the stimulus (5 to 20 yeasts per neutrophil) causes the reduction in cell phagocytic capacity when phagocytosis is mediated by cell surface receptors, in individuals with periodontitis in relation to the controls. In the same conditions, when phagocytosis is facilitated by opsonins, there is a reduction in the phagocytic activity when diabetes is associated with periodontitis compared to controls.

This impairment in neutrophil phagocytic activity seems to be related to the reduction in the number of phagocytized yeasts by neutrophils and not to the total percentage of neutrophils involved in phagocytosis.

In contrast, in monocytes, there was a reduction in phagocytic activity even when the antigen was used in a low proportion (5:1), when periodontitis group was compared to the controls. However, this reduction was only observed when phagocytosis occurred by opsonin receptors.

When an increased stimulus per monocyte (20:1) was used, there was a reduction in their phagocytic capacity, both in the periodontitis group and in diabetes associated to periodontitis group in relation to the controls, only when this function occurred by opsonins.

This impairment in monocyte phagocytic activity appears to be related to the reduction both in the number of neutrophil-phagocytized yeasts and in the frequency of cells involved in phagocytosis.

There are few studies that have analyzed the phagocytic activity of neutrophils or monocytes from the peripheral circulation. These studies report contradictory results, with reduction[23] or even increase[34] of this function in individuals with periodontitis. Our findings agree with those of Carneiro et al.[23], who showed a deficiency in this function in individuals with severe periodontitis, indicating that periodontal disease may modify immune responses such as phagocytic activities, even in systemic healthy individuals.

Periodontitis might –by mechanisms not yet elucidated– promote opsonic defects and impair the phagocytic activity of neutrophils and, especially, of monocytes. These data may reinforce the notion that periodontitis, per se, is capable of impairing innate defense mechanisms, which could contribute to the development of diabetic complications

The superoxide anion (O2-) has been identified as one of the most important phagocyte-derived oxidants associated with periodontitis[35]. Neutrophils and monocytes are examples of cells that produce superoxide anions against bacterial agents[9]. The present study did not find any alteration in the production of superoxide among all groups. However, there is no consensus among studies that have evaluated reactive oxygen species, including superoxide, in the systemic circulation of individuals with periodontitis. Although one study found similar results[36], others reported enhanced[37] production of superoxide anions. The production of ROS by phagocytic cells is enhanced in periodontal pockets[37]. Excessive local ROS can affect the oxidative status of tissues, activating and sustaining phagocytes to kill phagocytized pathogens. This may be harmful not only to the microorganisms but also to the host cells[38].

It is important to emphasize that ROS is one of the factors that may have an impact on insulin resistance, based on two aspects. One is the association of obesity and diabetes with markers of oxidative stress[39] and the other could be related to the evidence that direct treatment with agents that induce ROS accumulation can induce insulin resistance[40].

This study showed that periodontitis may change some biochemical parameters from peripheral blood, when associated or not with diabetes and, consequently, it might impair general health, contributing to the development of systemic complications in individuals with pre-diagnosed type 2 diabetes or even increasing the risk of acquired diseases such as cardiovascular disease in healthy individuals.

The reduction of phagocytosis found in the present study in patients with periodontitis, whether or not associated to diabetes, reinforces the theory that periodontitis may predispose healthy individuals to a series of systemic complications.

A possible limitation of this study is the extrapolation of an in vitro laboratory finding to a clinical condition that involves a multiplicity of interplaying functional factors. Despite this limitation, our findings reinforce the concept that periodontitis may promote significant immunological changes in cells from human peripheral blood, whose impact on the systemic condition of patients with or without diabetes mellitus, needs to be better understood.

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DECLARATION OF CONFLICTING INTERESTS
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Retention of cemented zirconia copings on TiBase abutments

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ABSTRACT
This study evaluated the influence of resin cements and glass ionomers on tensile strength and types of failure of zirconia copings cemented on titanium base abutments. Forty-two samples were prepared, which were formed by a Cone Morse implant, a titanium abutment with the fixing screw, and a zirconia structure made using a CAD/CAM system. The samples (n = 42) were randomly distributed according to the cementing agent: resin-modified glass ionomer cement (RelyX Luting 2), self-adhesive resin cement (RelyX U200), and self-curing resin cement (Multilink N). After cementation of the copings, half of the samples from each group (n = 7) were randomly selected and subjected to thermocycling (5000 cycles). A tensile load test was performed on a universal testing machine until failure occurred (1 mm). In addition, the type of failure was analyzed using the two-way analysis of variance test and Tukey's post-hoc test (α = 0.05). Lower tensile load was observed for the glass ionomer cement (p < 0.001) regardless of the evaluation period. After thermocycling, a significant reduction in tensile load values was verified for both evaluated cements (p = 0.047). For the resin cements, failures were predominantly of the screw fracture type (82.1%) already with the use of glass ionomer cement, and 28.5% of the failures were of an adhesive type between the zirconia coping and the cement. Resin cements have better stability under tensile load compared to resin glass ionomers when cementing zirconia copings on titanium base abutments..
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Keywords: *prostheses and implants - dental implant - ceramics - resin cements - glass ionomer cements - dental implant abutment interface.*

Retenção de copings de zirconia cimentados sobre pilares tibase

RESUMO
Este estudo avaliou influência dos cimentos resinosos e a base de ionômero de vidro na resistência à tração e os tipos de falhas de copings de zircônia cimentados sobre pilares TiBase. Foram confeccionadas 42 amostras, sendo estas formadas por implante cone morse, pilar de titânio (TiBase) com o parafuso de fixação e uma estrutura de zircônia (coping de Zr) confeccionado através do sistema CAD/CAM. As amostras (n =42) foram aleatoriamente distribuídas de acordo com o agente de cimentação: (cimento de ionômero de vidro modificado por resina [RelyXTMLuting2]; cimento resinoso autoadesivo [RelyXTM U200] e cimento resinoso autopolimerizavel (Multilink® N). Após cimentação dos copings, metade das amostras de cada grupo (n = 7) foram aleatoriamente selecionadas e submetidas a termociclagem (5000 ciclos). O Teste de resistência a tração foi realizado em uma máquina de ensaio universal, até que ocorresse a falha (1 mm/min). Adicionalmente, o tipo de falha foi analizado. Os dados foram analisados pelo teste ANOVA two-way e post teste de Tukey's (α = 0.05). Menor média de resistência a tração foi observada para o cimento de ionômero de vidro (p < 0,001) independente do período de avaliação. Após a termociclagem, foi verificada uma redução significativa nos valores de resistênca a tração, para os cimentos avaliados (p=0,047). Para os cimentos resinosos, as falhas foram predominantemente do tipo fratura do parafuso (82,1%) já com o uso do cimento de ionômero de vidro, 28,5% das falhas foram de tipo adesiva entre o coping de Zr e o cimento. Cimentos resinosos apresentam melhor estabilidade na resistência a tração em comparação a ionmeros de vidro resinosos na cimentação de copings de zircônia sobre pilares TiBase.

Palavras-chave: *proteses e implantes - implante dentário - cerâmicas - cimentos de resina - cimentos de ionômeros de vidro - interface pilar Implante dentário.*

INTRODUCTION

There has been great technological progress in implantology in recent years. The search for functional and aesthetic results has fostered the development of techniques and materials with high aesthetics and biodynamic performance[1].

In this context, research on infrastructures using metal-free materials has increased significantly, concomitantly with the advancement of digital dentistry[1,2]. Digital systems for planning and clinical execution have become increasingly frequent in the dental routine[1-4].

Among the digital systems, CAD/CAM (computer-aided design/computer-aided manufacturing) enables planning and execution with the aid of a computer, facilitating the introduction of zirconia (Zr)-based ceramics in implant dentistry[1-4].

Zr is a material with aesthetic properties of high resistance and good health relationship with peri-implant tissues[2,3]. In addition, CAD/CAM enables automation and standardization of the manufacturing process of prosthetic infrastructures, thereby making it possible to manufacture prostheses with high-quality adaptation in a short time[1]. However, the high strength of a Zr-based infrastructure can generate wear when adapted directly to the prosthetic platform of osseointegrated implants[4-6]. It is therefore recommended to use a titanium base (TiBase) between the Zr and the implant [6,7], especially when the implant is functional[6, 8-11].

Thus, TiBases that fit the prosthetic platforms of the implants are made with a coronary portion suitable for receiving Zr infrastructures (Zr copings). After being made, the Zr copings are cemented to the TiBase abutment, and the set is fixed to the osseointegrated implant[6-8]. However, there are currently several types of dental cements with different indications and characteristics, raising questions about which cement is the most appropriate and has the best retention and stability[9]. Resin and ionomeric cements have been indicated as materials with high retention and resistance index in the aging process[7, 10-13], and are therefore indicated for the cementation of Zr copings on the TiBase abutments due to their adhesion and mechanical resistance[7,14]. Although adhesion characteristics between TiBase abutments and various restorative materials have been reported[13-15], it has not yet been well established which type of cement is the most suitable for cementing Zr copings on TiBase abutments, and especially their stability over time and the temperature changes that occur in the oral environment.

The aim of the present study was therefore to evaluate the tensile strength of Zr copings cemented on TiBase abutments with resin-modified glass ionomer cement and various resin cements after thermocycling. The following null hypotheses were tested: (1) there is no difference in tensile strength between the different cements tested, and (2) thermocycling did not influence the tensile strength values of the Zr copings cemented on the TiBase abutments.

MATERIALS AND METHODS

Experimental design

Forty-two samples were selected, composed of the set: Strong SW® Cone Morse implant (S.I.N, São Paulo SP, Brazil), TiBase abutment (Duotech® S.I.N, São Paulo SP, Brasil). The set was fixed on a steel base to receive the threads of the type of implant used.

For each TiBase abutment, a Zr coping was manufactured in a standardized manner using a CAD/CAM system, 6 mm in height and 6 mm in thickness. The coping was manufactured in a cylindrical shape, with an indentation of 4 mm in thickness and 2 mm in height towards the abutment, thus enabling adaptation of the traction device support in this region[14,15].

The samples (n = 42) were randomly distributed according to the two study variables: 1) cementing agent: (resin-modified glass ionomer cement [RelyX Luting 2], self-adhesive resin cement [RelyX U200], and self-curing resin cement [Multilink N]); and 2) Thermocycling: without thermocycling (after 24 h of cementation), with thermocycling.

The abutments were digitalized using a bench scanner (inEos X5, Dentsply Sirona, São Paulo, SP, Brazil), and the coping project was designed (CAD software inLab, Dentsply Sirona)[15].

The thickness of the cement film was designed to be 40 µm, except at the margins, which were designed to achieve total sealing to the abutment[1,16].

Surface treatment and cementation

After milling, all Zr copings underwent a blasting process with 50 µm aluminum oxide (Al_2O_3) particles at a pressure of 1.0 bar for 10 s[17]. The

coping was fixed and manually blasted at an angle of 45° and a distance of 3 cm from the jet firing point. Then the copings were washed ultrasonically with distilled water for 180 s, cleaned in 96% ethanol, and dried with an air jet.

After blasting, all copings were subjected to the surface treatment recommended by the cement manufacturer (Table 1). Subsequently, the cement was manipulated, inserted into the coping, and manually fixed to the TiBase. Initially, digital pressure was applied, and excess cement was removed. Then each sample was subjected to a constant pressure of 20 N in the center of the occlusal surface for 10 min[16-19].

Photopolymerization followed the time recommended by the cement manufacturer (Table 1) and was carried out with a high-intensity LED light device at 1200 mW/cm^2 (VALO Cordless, Indaiatuba, SP, Brazil). A radiometer (Demetron L.E.D, Radiometer, Kerr Sybron Dental Specialties, Middleton, WI, USA) was used to check the light intensity for each of the five specimens.

The specimens were stored in distilled water at 37°C for 24 h. Half the specimens in each group (n = 7) were subjected to 5,000 cycles of thermocycling in water baths, at temperatures of 5°C, 37°C, and 55°C, with residence times of 30 s and rest times of 15 s[14].

Tensile test

The tensile test was performed on a universal testing machine (EMIC DL 2000, Paraná, Brazil) at a speed of 1 mm/min until cementation failure or screw fracture occurred.

A load cell of 5000 N was used, in which a steel device was fixed for traction with a retention area for fitting the specimen (Fig. 1). The maximum force used at the time of the cementation failure (T) was recorded in newton (N)[14,15].

After the test, the specimens were analyzed according to the location of the cement residue in each component[16], using an optical microscope (SZH-131, Olympus Ltd., Tokyo, Japan) at a magnification of 10x.

The type of failure was classified as: adhesive failure between the Zr surface and the cement; mixed failure, when there was a failure in both interfaces (between the cement and the Zr and between the cement and the TiBase); or fracture of the screw (when the screw fractured before cement failure).

Statistical analysis

After verifying the normality of the data (Shapiro Wilk, $p > 0.05$), all data were analyzed using two-way analysis of variance (cementing agent and thermocycling). The Tukey post hoc test was used to compare between pairs in all analyses. The effect of size was calculated using eta squared ($\eta 2$) for the independent variables.

Statistical analysis was performed using IBM SPSS Statistics for Windows v.26 (IBM., Armonk, NY,

Table 1. Cements used, basic compositions, and form of use

Cement / Manufacturer / Lot	Composition	Instructions for use
RelyX Luting 2; 3M; Lot number: 1929100186	Water, HEMA, fluoroaluminosilicate glass, reducing agents, titanium dioxide, dispersing agent, BisGMA, HEMA, water, potassium persulfate, polyacrylate methacrylate, BHT, nonreactive Zr-Si charge, solubility modifiers	1. Mixing of catalyst and base pastes for 20 s. 2. Insertion in Zr coping. 3. Polymerization for 5 s on each face.
RelyX U200; 3M; Lot number: 1921800715	Glass powder treated with silane, 2-propenoic acid, 2-methyl, 1,1 '- [1-(hydroxymethyl) -1,2-ethanodiyl], TEG-DMA and silane treated with silane, glass fiber, persulfate sodium and t-butyl per-3,5,5-trimethylhexanoate.	1. Application of the Single Bond Universal 3M in the Zr structure. 2. Mixing of catalyst and base pastes for 30 s. 3. Insertion in Zr coping 4. Polymerization for 20s on each face.
Multilink N; IVOCLAR; Lot number: Y32874	Bis-GMA, HEMA, UDMA, 2-dimethylethyl aminoethyl methacrylate, dibenzoyl peroxide (organic), and inorganic fillers: barium glass, ytterbium trifluoride, spheroid mixed oxide and titanium oxide, catalysts, stabilizers, and pigments	1. Application of Monobond N in Zr coping. 2. Mix of the catalyst and base pastes for 20 s. 3. Insertion in Zr coping. 4. Polymerization for 20 s on each face.

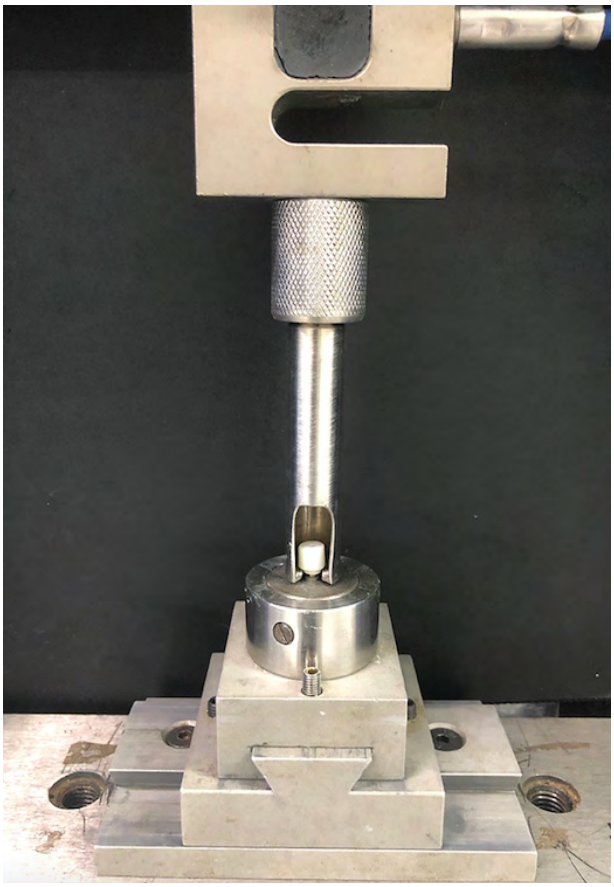

Fig. 1: Steel cylinder set attached to the load cell and sample at the bottom. Zirconia coping with traction insert.

USA). Statistical tests were performed considering a 5% significance level.

RESULTS

Analysis of tensile bond strength

The mean values (standard deviation) demonstrated that the type of cement significantly influenced the recorded tensile load ($p < 0.001$) (Table 2). The glass ionomer (RelyX Luting 2) showed the lowest value of tensile load ($p < 0.05$), whereas the resin cements (RelyX U200 and MultilinkN) did not show a statistically significant difference ($p > 0.05$).

Influence of temperature change on tensile bond strength of cement

The interaction between the cements and thermocycling was significant ($p = 0.163$). When subjected to temperature changes (thermocycling), a reduction in the mean values of cement tensile strength (95% confidence interval) was observed ($p = 0.047$). The effect size was 0.760 for the cement type, and 0.105 for the thermocycling variable, i.e., thermocycling was responsible for approximately 10% of the bond strength variation, and the cement type was responsible for 76% (Fig. 2).

Table 2. Average values (standard deviations) of the tensile bond load; unit of measurement in newton (N)

Group	Without thermocycling	Thermocycling	Total
RelyX Luting 2	752.20 (140.93)	569.69 (105.31)	660.94 (152.49)[a]
RelyX U200	1150.48 (105.19)	1170.62 (80.94)	1160.55 (90.77)[b]
MultilinkN	1174.44 (36.16)	1073.84 (254.03)	1124.14 (181.96)[b]
Total	**1025.71 (221.46)[A]**	**938.05 (312.29)[B]**	

[A,B] Superscript capital letters = significant difference between without thermocycling and with thermocycling.
[a,b] Superscript lowercase letters = significant difference between groups.

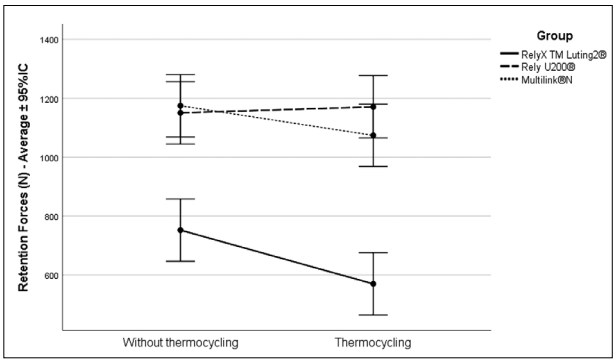

Fig. 2: Average values (95% confidence interval) of tensile load in groups.

Failure analysis after the tensile test

When cementation was performed with RelyX Luting 2, the failures were predominantly adhesive, between the Zr coping and the cement (85.7%). RelyX U200 presented failures predominantly due to screw fracture (85.7%), with a minimum number of adhesive failures between the Zr coping and cement (14.3%). In MultilinkN, all samples that were not thermocycled showed failures due to screw fracture (100%), while in the group that underwent thermocycling 28.5% were adhesive failures between the Zr coping and cement (Fig. 3).

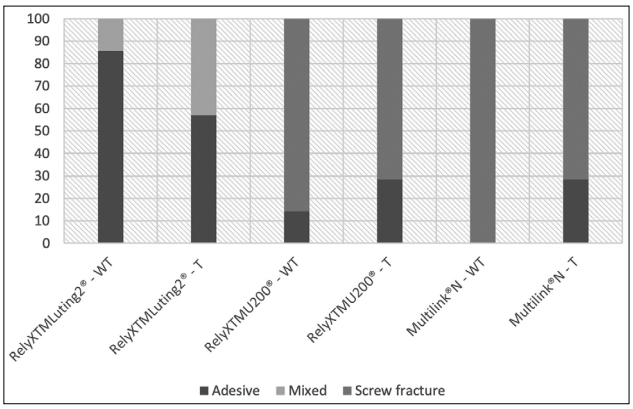

Fig. 3: Type of failure found in the evaluated groups (values in %).WT: Without thermocycling, T: Thermocycling

DISCUSSION

The results of tensile strength and longevity of the Zr coping cementation on the TiBase abutment found in this study are important to guide clinicians in choosing the type of cementation material. The results showed that the glass ionomer cement promoted lower tensile strength values than the resin cements, leading to the rejection of the first proposed null hypothesis. Another result was the significant decrease in tensile strength after thermocycling for the cements used, leading us to reject the second null hypothesis.

Resin cements are known to promote higher values of tensile strength than glass ionomer cements[19]. Resin cements have high shear strength to resist the internal forces that can cause the structure of the material to slide against itself. At the same time, they can withstand tensile forces, enabling the hydrophobic matrix of a composite cement to adhere to hydrophilic substrates[20]. Thus, better behavior of resin cement is also observed when Zr copings are cemented on TiBase abutments[8], reaching higher levels of retention force.

Comparison of the tensile bond strength values of resin cements showed averages of 1160.55 and 1124.14 N respectively for the RelyX U200 and MultilinkN cements, and 660.94 N for the ionomer (RelyX Luting 2). Resin cements are composed of resinous monomers that provide adequate chemical adhesion as well as micromechanical adhesion to dental and metallic structures when compared to resin-modified glass ionomer cement[19]. Moreover, there is consensus in the literature that treating the Zr surface with ceramic primers can promote better adhesion of the organic particles of the cement with the inorganic particles of Zr[13-22]. This may explain the higher values of the retention forces for the resin cements tested in relation to the glass ionomer cement modified by resin.

Another aspect is the surface treatment of Zr. Hansen et al.[21] evaluated the importance of preparing Zr with jets of AL_2O_3 before cementation. Different authors have concluded that the use of Zr surface treatment significantly increases the quality of retention after cementation[7,8,23,24]. However, other studies have shown that abrasive air particles with AL_2O_3 can cause damage to the surface of Zr, causing flaws and microcracks, compromising the mechanical strength of Zr[25,26].

The literature also reports that a lack of application of adhesive systems between Zr structures and resin cements could decrease the bond strength of Zr structures on implants[8,10,12]. However, the increase in adhesion can only be improved by using surface treatments such as blasting, silanization, and use of adhesives next to resin cements containing phosphate groups[26]. The resin cements used in this study include phosphate groups in their composition, which together with the surface treatment could explain the better performance of both resin cements.

The high holding force could also be the result of using a titanium abutment with retentive areas. The pillar used in this study had areas of roughness, which possibly guaranteed greater mechanical interlocking with cement. In fact, Güngör et al.[14] used smooth abutments for cementing Zr structures and concluded that thermocycling added to the mechanical cyclic load and resulted in a significant decrease in tensile strength

Another aspect studied was the effect of aging by thermocycling. Thermocycling is well accepted as a form of aging, since in addition to hydrolytic degradation promoted by water, temperature changes, repeated expansion/contraction, and other stresses within a sample have a significant impact on tensile strength[27].

In this study, the average retention for the tested cements was 1025.71 N for the groups without thermocycling and 938.05 N for the thermocycled groups, showing a significant decrease. In the individual analysis, the effect of thermocycling was found to be less on resin cements than on the resin-modified glass ionomer cement . Saleh et al.[26] also used thermocycling for aging and found that resin cements are more resistant than other types of

cements to its effects and maintain the integrity of the cement line.

Among the causes of this decrease are increased sorption and solubility, and less interaction with organic components. The absence of a resinous monomer in this type of cement can aggravate the low resistance to aging processes, fostering a weak connection between the cement and the Zr structure. This could also have promoted an increase in adhesive-type failures in this group . However, in resin cement, although thermocycling decreased the values of tensile strength, this did not promote a significant difference. This may be due to the use of adhesive systems that, in addition to the surface alteration of Zr with AL_2O_3 blasting, made the adhesion more stable even after an aging process, as shown in Figure 2 in the confidence interval line[15,28].

In the analysis of the type of failure, it was found that for the resin cements, the failures were predominantly of the screw fracture type (82.1%), while with the use of glass ionomer cement, 28.5% of the failures were of the adhesive type between the Zr coping and cement. The increase in retentive strength in the groups where resin cements were used caused the predominance of failures due to screw fractures. The region most susceptible to fracture of the pillar screw is located at the junction between the threads and the neck[29], and it was in this region where all fractures occurred in this experiment. Studies report that this type of fracture can be minimized by using thicker screws and with an apical indexer that helps in the stabilization of the prosthetic component[30].

Another feature that could have caused an increase in such failures is the thickness of the cement film. In the present work, it was standardized at 40 µm for all samples. According to the literature, when thicknesses of up to a maximum of 60 µm are used, better results are obtained in tensile strength tests[8,10]. This thickness of cement film, added with a retentive titanium pillar, probably caused the high retentive force.

Malthazan et al[8] tested two resinous cementing agents, finding the best retention rates with preconditioning of the coping and application of a ceramic primer. The maximum average observed in one of the groups in their study was 598.6 N, using a smooth titanium pillar 7.8 mm high and 3.4 mm wide and a 30 µm cementation space.

Although studies have evaluated different materials for cementing Zr structures on TiBase abutments[7,11,12,14,21], the cementation step is important to ensure the stability and longevity of the implant-supported prosthesis system, and the lack of a predictable protocol for cementation of Zr copings on TiBase abutments is still a limitation in this area. The results in this study will help the appropriate choice of cement for zirconia restorations on zirconia or TiBase abutments.

The methodology used in this study endeavored to simulate what is performed clinically in a standardized way, but the clinical condition of each patient can influence the final outcome and longevity of the restorations. It is known that *in vitro* studies provide limited information regarding the clinical performance of Zr copings on TiBase abutments; therefore, randomized clinical trials that show real clinical situations of the behavior of these cements in the oral cavity have yet to be conducted.

In conclusion, resin cement has better adhesive stability when cementing the Zr coping to the TiBase abutment. Thermocycling decreases the retentive strength of resin-modified ionomer cement.

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DECLARATION OF CONFLICTING INTERESTS
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Mechanical and bonding properties of different combinations of nanohybrid and bulk-fill composites

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ABSTRACT

The aim of this research was to determine compressive and shear bond strength of blocks prepared with bulk-fill and nanofill composite resin combinations. Materials used were Filtek Bulk Fill (FBF) and Z350 (both 3M-ESPE) and Surefil SDR flow (SFF) - Dentsply. To determine shear bond strength, cylindrical specimens 10 mm thick were prepared with composite consisting of thicknesses of 6 mm of one material and 4 mm of the other, in the following combinations: G1: FBF- FBF; G2: Z350-Z350, G3: FBF-Z350, G4: Z350-SFF and G5: SFF-SFF. Materials were cured using a 1100 mw/cm^2 light for 20 seconds for each layer. Samples were stored for 24 hours at 37 °C in distilled water and shear bond strength was determined. To assess compressive strength, cylindrical samples 4 mm diameter and 6 mm thick consisting of 4 mm + 2 mm were used in the same combinations as described above, stored in distilled water at 37 °C for 24 hours, after which compressive strength was determined. Both tests were performed with a Universal testing machine at a cross head speed of 1 mm/min. Results were analyzed with ANOVA and Tukey's test.

Means and standard deviations in MPa for each group were the following: Shear bond strength: G1: 435.87 (65.86), G2: 233.6 (108.15), G3: 279.2 (22.05), G4:449.1 (109.35) and G5: 196.6 (51.16). Compressive strength: G1:160.07(4.27), G2: 149.49 (14.06), G3: 156.10 (29.99), G4: 199-30(39.28), G5: 171.23 (28.71). Evaluation with ANOVA showed no significant differences among combinations for compressive strength ($p>0.05$) and significant differences for bond strength ($p<0.05$). Tukey's test showed three homogeneous groups.

Under these experimental conditions, it can be concluded that the study combinations have adequate mechanical behavior, equivalent to materials used individually. However, shear bond strength was affected by the combinations analyzed.

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Keywords: *composite resins - shear strength - compressive strength.*

Propiedades mecánicas y adhesión entre diferentes combinaciones composite Nanohíbrido- Bulkfill

RESUMEN

El objetivo de este trabajo fue determinar la resistencia compresiva (RC) y la resistencia adhesiva al corte (RAC) en bloques preparados con combinaciones de composites bulk-fill y nanoparticulados. Los materiales usados fueron Filtek Bulk Fill (FBF) y Z350 (ambos de 3M-ESPE) y Surefil SDR flow (SFF) - Dentsply. Para medir la RAC, se prepararon probetas cilindricas de 10 mm de espesor consistentes en 6 mm de un material y 4 mm del otro con las siguientes combinaciones: G1: FBF- FBF; G2: Z350-Z350, G3: FBF-Z350, G4: Z350-SFF y G5: SFF-SFF. Se curaron a 1100 mw/cm^2 durante 20 segundos cada capa. Se conservaron 24 horas a 37 °C en agua destilada antes de determinar la RAC. Para medir la RC se prepararon probetas de 4 mm de diametro y 6 mm de espesor (4 mm + 2 mm de cada material), con las mismas combinaciones. Se conservaron en agua destilada a 37 °C durante 24 horas y se midió la RC. Ambos ensayos se realizaron con una máquina universal para ensayos mecánicos a 1 mm/min de velocidad de desplazamiento de cabezal. Los resultados se evaluaron con ANOVA y prueba de Tukey. Las medias y desviaciones estándar (MPa) para cada grupo fueron: RAC: G1: 435.87 (65.86), G2: 233.6 (108.15), G3: 279.2 (22.05), G4:449.1 (109.35) y G5: 196.6 (51.16). RC: G1:160.07(4.27), G2: 149.49 (14.06), G3: 156.10 (29.99), G4: 199-30(39.28), G5: 171.23 (28.71). ANOVA no mostró diferencias estadísticamente significativas para RC ($p>0.05$) y diferencias significativas para RAC ($p<0.05$). La prueba de Tukey mostró tres grupos homogéneos.

En las condiciones experimentales de este trabajo puede concluirse que las combinaciones evaluadas tienen un comportamiento mecánico adecuado equivalente al de los materiales individuales. Sin embargo, la adhesión entre materiales se vio afectada por las combinaciones realizadas.

Palabras clave: *resinas compuestas - resistencia al corte - resistencia compresiva.*

INTRODUCTION

Composites have become the material of choice in restorative dentistry because of their adequate mechanical behavior, aesthetic properties and above all, conservation of healthy tissue. According to Ferracane et al.[1], one of the deficiencies of conventional composites with regard to the polymerization reaction is that the volume of the material contracts by about 3%[2]. This contraction is transmitted to the interface between dental tissue and restorative material and may cause marginal filtration, secondary caries, loss of the restoration, cuspal deflection[3], and enamel micro-cracks[4], leading to postoperative sensitivity, usually during chewing. Various techniques and composites have been developed to minimize polymerization contraction and its clinical effects. The incremental technique is widely recommended for minimizing these problems. Placing layers 2 millimeters thick enables light to reach deeper zones, thereby achieving an adequate degree of conversion. However, this technique has some disadvantages such as inclusion of air bubbles, increased risk of contamination between layers, and longer working time[5,6]. Most improvements have focused on modifying the filling, which has improved mechanical properties, mainly resistance to wear. Despite this progress, average lifespan of these composite restorations was found to be only 10 years[7]. Initially, composite matrix was exclusively based on the chemistry of methacrylate, more specifically BisGMA, TEGDMA, BisEMA and UDMA, and in the past 20 years, approximately, alternative monomers have been developed with the aim of reducing polymerization contraction and stress, emphasizing the association between the development of stress and the formation of gaps between the restoration and the tooth structure[8]. One alternative attempted was to apply ring opening polymerization or monomers of very high molecular weight. In the former, the only material developed was Filtek LS (3M), based on the silorane chemistry. Both strategies were successful in reducing the contraction coefficient to 1%[9] and above all, reducing polymerization stress, according to in vitro assessments[10]. Although there are a few available clinical trials, results reported are contradictory. Popoff et al. found similar clinical behavior after one year with silorane-based and dimethacrylate-based resins in restorations[11]. However, they note that studies should have longer follow-up periods. Gonçalves et al. conducted an 18-month double blind, randomized study, finding that marginal integrity was worse in restorations with silorane-based composites than in those with dimethylacrylate, finding no benefit in using this kind of composites in the restoration of Class II lesions[12].

More recently, greater importance has been assigned to improving resistance to breakdown in the oral medium –including hydrolysis of ester groups present in the methacrylates– caused by saliva and bacterial enzymes, and to preventing biofilm formation on the surface and interface of a composite restoration[13]. The most recent strategy has been to develop materials requiring fewer steps in their protocol for use, such as bulk-fill and self-adhesive composites. Bulk-fill composites have become increasingly popular for general practices. Although they can be classified in several different ways, they are best identified according to in-depth polymerization capacity, since they may have high or low viscosity, higher and lower ceramic load, and a great variety of mechanical properties[14]. The term bulk-fill has thus been used by manufacturers to refer to composites that can be inserted and polymerized in a single block of 4-5 mm. Some examples of the modifications made to conventional composite are use of monomers with high molecular weight such as AUDMA (Aromatic urethane dimethacrylate) and monomers known as AFM (addition-fragmentation monomers)[15]. Regarding ceramic filling, the percentage in volume was reduced in both bulk-fill flowable composites and bulk-fill composites with regular viscosity, with the percentage of filling in volume being even lower in the former. This reduction in percentage of filling reduces the difference in the refraction index between the matrix and the ceramic filling, which increases translucency for the material, enabling in-depth curing[16]. Moreover, the elastic modulus of these materials is lower than in conventional composites, and the rigidity of bulk-fill flowable composites is even lower than that of regular viscosity bulk-fill composites. This is why bulk-fill flowable composite manufacturers recommend placing an additional 2 mm layer of a conventional composite in zones exposed to greater stress[17]. The main properties that have

been studied are degree of conversion and depth of cure[18]. However, no papers were found studying bond strength between composite Z350 3M ESPE –Filtek Bulk Fill 3M ESPE and Z350 3M ESPE– Surefil SDR flowable or the mechanical properties of these combinations. Thus, the aim of this study was to determine shear bond strength and compressive strength of different combinations of bulk-fill composites and conventional composites.

MATERIALS AND METHODS

The following materials were used: Filtek Bulk Fill (FBF) and Z350 (both 3M-ESPE), and Surefil SDR flow (SFF) (Dentsply). To determine shear bond strength, test specimens (n=3) 10 mm thick and 4 mm in diameter were prepared in cylindrical molds. This 10 mm thickness consisted of 6 mm of one material and 4 mm of another in the following combinations: G1: FBF- FBF; G2: Z350-Z350, G3: FBF-Z350, G4: Z350-SFF and G5: SFF-SFF. They were polymerized using a Coltolux LED light-curing unit (Coltene) with intensity 1100 mw/cm2 for 20 seconds per layer, following the thickness is recommended by the manufacturers in their respective instructions. An extra-fine marker (Edding 1880 Drawliner 0.1) was used to draw a line between the two parts. Specimens were embedded in self-curing acrylic resin cylinders (Subiton SL, Argentina) to make them easier to handle during mechanical assays. Specimens were stored for 24 hours at 37 °C in distilled water. The blocks prepared were placed on a support and load was applied at the level of the interface between the two materials with a metal needle until fracture occurred. Bond strength was determined using a universal testing machine for mechanical assays (1100. Instron Corporation) at a crosshead speed of 1 mm/minute. Fig. 1 shows the system used for determining shear bond strength. To determine compressive strength, cylindrical specimens (n=4) 4 mm in diameter and 6 mm thick were prepared. These 6 mm corresponded to 4 mm + 2 mm of the same combinations as described above. Specimens were stored in distilled water at 37 °C for 24 hours. Compressive strength was determined by placing an axial load using the same machine at the same crosshead speed as described for bond strength. Results were analyzed using ANOVA and Tukey's test with significance level $p<0.05$.

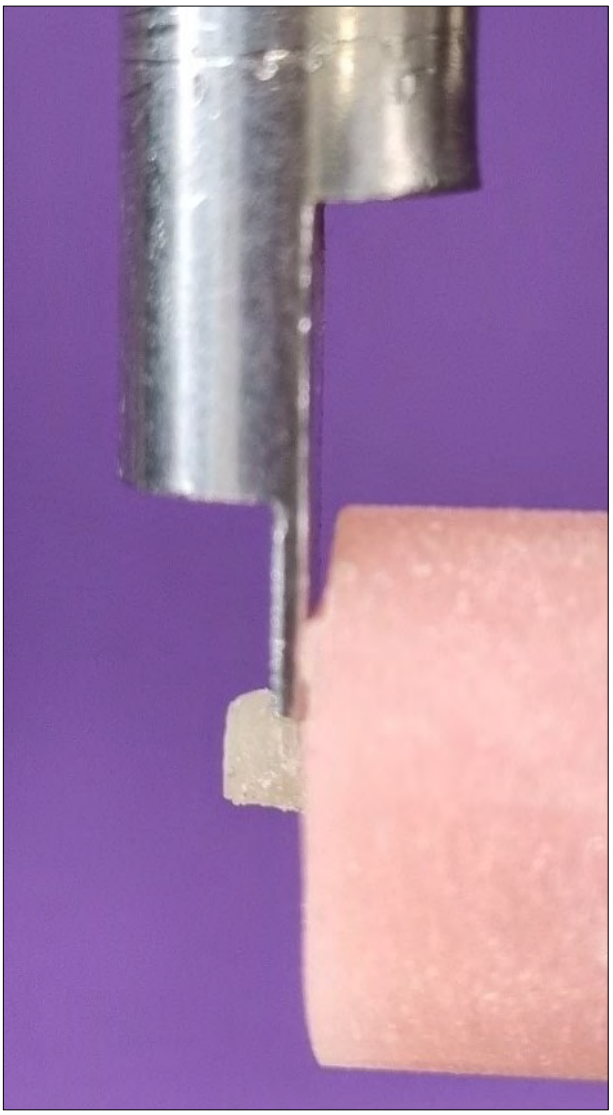

Fig. 1: Figure showing measurement of bond strength between parts made of different composites.

RESULTS

Mean and standard deviation in MPa for each group were the following: Bond strength: G1: 435.87 (65.86), G2: 233.6 (108.15), G3: 279.2 (22.05), G4: 449.1 (109.35) and G5: 196.6 (51.16); Compressive strength: G1: 138.61 (18.92), G2: 156.06 (9.71), G3: 167.18 (35.89), G4: 199.3 (39. 28) and G5: 171.23 (28.71). Figs. 2 and 3 show mean and standard deviation for each. Evaluation with ANOVA showed no significant difference between combinations for compressive strength ($p>0.05$) but did show significant differences in bond strength ($p<0.05$). Tukey's Test showed three homogeneous groups for comparison of shear bond strength, suggesting statistically significant difference between G4 and G5 (between combination G4: Z350-SFF and

combination G5: SFF-SFF. This is shown in Fig. 2 with the same letters indicating homogeneous groups.

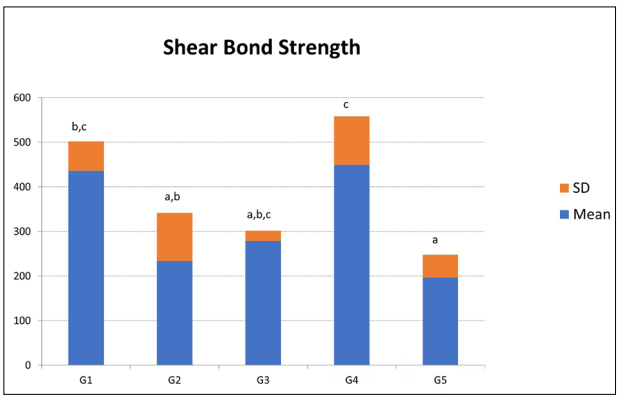

Fig. 2: Shear bond strength results. Mean (MPa) and standard deviation are shown for each group. Letters above the bars show results of comparisons using Tukey's test (the same letter in different groups indicates that the difference between them is not statistically significant).

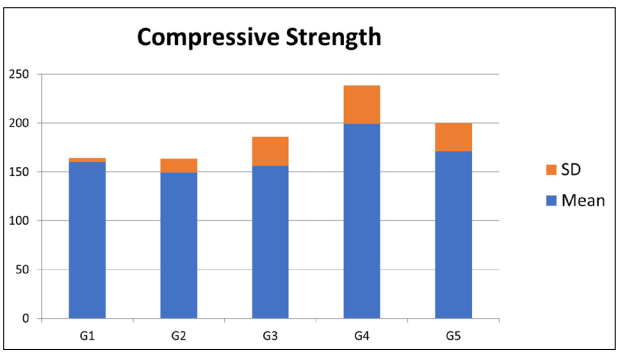

Fig. 3: Compressive strength results. Mean (MPa) and standard deviation are shown for each group.

DISCUSSION

The introduction on the market of bulk-fill composites raised two major questions: one regarding their ability to be photopolymerized adequately at the depths stated by the manufacturer, and the other regarding whether their mechanical properties might be deficient. As mentioned in the review by Camila Nuñez et al. regarding the advantage of shorter operative times, it would be interesting to evaluate whether the single block technique really saves clinical time[19]. Tiba et al. report that using bulk-fill flowable composites, which require an additional 2 mm occlusal layer of conventional composite, necessarily involves filling cavities with at least two increments, with different composites, which may not differ much from the operative times required for 4 mm cavities filled with conventional composites[20]. As there is some evidence of the lower mechanical properties in bulk-fill flowable and regular viscosity composites, dentists often choose to use them as a base and cover them with conventional composites. The in vitro studies evaluating marginal seal have found results which are comparable to those using conventional composites. However, the same is not true for the evaluation of their mechanical properties[21,22]. Esteves Lins et al. evaluated the mechanical properties of different composites, finding results in which they were unable to associate composite type –in terms of form of insertion– with compressive strength, having found similar values with no statistically significant difference compared to those inserted as a block[23].

This is consistent with the behavior observed in the current study, where there was no significant difference between the different combinations. This may also be attributed to the fact that this type of material does not differ from conventional materials in ceramic load only, but in a combination of factors[24]. De Assis et al. reached similar conclusions[25].

According to Haughen et. al., the composite FBF presents a specific monomer with high molecular weight without free hydroxy groups in order not to increase its viscosity, in addition to lower ceramic content. Although this should provide better light penetration, some of the particles are silica or silanized zirconia, which have a high refractive index, leading to lower light transmission than in other bulk fill composites such as SFF, which could cause a lower degree of conversion[14]. In our study, group 4 (Z350- SFF) had the highest mean bond strength, even though different materials had been combined. This could be associated to the lower degree of conversion of monomers in the organic matrix of Z350[26], which may leave a larger number of double bonds available for bonding to SFF. In turn, SFF has the lowest percentage of ceramic filling in volume (45 vol%), which may also contribute to providing a larger quantity of available monomers for bonding between the two materials.

Under the experimental conditions in this study, it can be concluded that the combinations of materials evaluated have similar mechanical behavior. However, bonding between them was affected in the combinations.

DECLARATION OF CONFLICTING INTERESTS:
The authors declare no potential conflicts of interest regarding the research, authorship, and/or publication of this article.

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Influence of resin cement and thermocycling on milled lithium disilicate ceramic microshear bond strength

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ABSTRACT
The aim of this study was to compare the microshear bond strength of different resin cements to CAD/CAM-created lithium disilicate ceramics after 24 hours and after 1 year (10,000 thermocycles). Forty (40) ceramic bars were subjected to pretreatment comprising airborne abrasion with aluminum oxide particles, etching with 10% hydrofluoric acid and Monobond N application. Bars were divided into 4 groups (n = 10), based on cement type: light-cured Variolink Esthetic LC (VLC) and dual-cured Variolink N (VN) at two different times: after 24 hours and after 1 year. Silicone molds were used to prepare cement cylinders on a ceramic surface. The set was stored in distilled water at 37ºC, for 24 hours or subjected to 10,000 thermocycles. The molds were removed and microshear bond strength was tested. Data were submitted to two-way analysis of variance and Tukey's test (α = 0.05). Based on the comparison between cement values at different aging times (p = 0.035), VN after 24 hours (27.10 ± 0.92) and after 1 year (20.62 ± 1.25) presented significantly higher values than VLC after 24 hours (14.79 ± 0.76) and after 1 year (6.61 ± 0.81). Bond strength recorded for both cements after 24 hours (VN: 27.10 ± 0.92 and VLC: 14.79 ± 0.76) was significantly higher than the one recorded after 1 year (VN: 20.62 ± 1.25 and VLC: 6.61 ± 0.81). The thermocycling reduced the values observed for both investigated cements; bond strength was greater for dual-cure resin cement than for light-cured resin cement..

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Keywords: *ceramics - strength, sherar - cementation.*

Influência do cimento resinoso e da termociclagem na resistência de união à cerâmica de dissilicato de lítio fresada

RESUMO
O objetivo nesse estudo foi comparar a resistência de união de diferentes cimentos resinosos à cerâmica de dissilicato de lítio processada por CAD/CAM após 24 horas e após 1 ano (10.000 termociclos). Foram utilizadas 40 barras cerâmicas com tratamento prévio por jateamento com óxido de alumínio, condicionamento com ácido fluorídrico 10% e aplicação do Monobond N. As barras foram divididas em 4 grupos (n=10) de acordo com o tipo de cimento: fotopolimerizável Variolink Esthetic LC (VLC) e dual Variolink N (VN) em diferentes tempos: após 24 horas e 1 ano. Matrizes de silicone foram usadas para preparar cilindros de cimento na superfície cerâmica. O conjunto foi armazenado em água destilada a 37ºC por 24 horas ou submetidos protocolo de termociclagem com 10.000 ciclos. As matrizes foram removidas e o teste de microcisalhamento realizado. Os dados foram avaliados por análise de variância a dois fatores e teste de Tukey (α=0,05). Ao comparar os valores dos cimentos em diferentes tempos de envelhecimento (p=0,035), observou-se que VN, após 24 horas (27,10 ± 0,92) e 1 ano (20,62 ± 1,25) apresentou valores significativamente superior a VLC após 24 horas (14,79 ± 0,76) e 1 ano (6,61 ± 0,81). A resistência para ambos os cimentos após 24 horas (VN: 27,10 ± 0,92 e VLC: 14,79 ± 0,76) foi significativamente superior que 1 ano (VN: 20,62 ± 1,25 e VLC: 6,61 ± 0,81). A termociclagem promoveu diminuição nos valores de ambos os cimentos estudados e o cimento resinoso dual mostrou maior resistência de união que o cimento resinoso fotopolimerizável..

Palavras-chave: *cerâmicas - resistência ao cisalhamento - cimentação.*

INTRODUCTION

Ceramic restorations are widely used in dental treatments[1]. They present thermal expansion coefficient compatible to teeth, biocompatibility, resistance to wear, favorable aesthetic results and clinical longevity[2]. Technological innovations associated with advances in restorative material have improved production methods and mechanical properties of new materials, affecting their use in dental professionals' daily routine[3].

Lithium disilicate ceramic stands out among these new materials due to its aesthetic effectiveness, mechanical properties, optical characteristics and accuracy. In addition, its manufacturing process involves more convenient production time and lower cost[3]. This material was developed to extend the indications of glass ceramics beyond the anterior teeth[4], and is recommended for inlays, onlays, veneers, anterior and posterior crowns, and implant-supported crowns[1]. It can be obtained by heat press technique (IPS e.max Press) based on the lost wax technique[4,5]. In addition, partially pre-crystallized blocks (IPS e.max CAD) have been introduced on the market to enable this material to be used with CAD/CAM technology[1,5]. Regarding its microstructure, topography, roughness and fractal dimension, milled lithium disilicate ceramics is smoother and more homogeneous, and has greater topographic feature complexity than the material that is used with the pressed technique[5]. Highly aesthetic restorations can be based on prefabricated ceramic blocks, with fewer defects and porosities than restorations based on conventional techniques[1,5].

Dual or light-cured resin cements are the main materials used for CAD/CAM-created lithium disilicate ceramic cementation[6]. According to the cementation procedure, the internal surface of ceramics is pretreated with hydrofluoric acid (HF) and/or subjected to airborne abrasion with aluminum oxide particles and silane, in order to enable mechanical retention and chemical bonding between cement and ceramics[6-9].

The success of ceramic restorations is directly related to the cementation process[10]. Although the effectiveness of the bond between the dental surface and resin cements has been extensively investigated, little information is currently available in the literature about the influence of simulated aging time and cement type on the microshear bond strength of milled lithium disilicate ceramics.

Thus, the aim of the current study was to compare the bond strength of two resin cements – dual-cured and light-cured – in milled lithium disilicate ceramics, after 24 hours and thermocycling (after 1-year simulated aging). The null hypotheses tested were: (1) cement type and (2) thermocycling would not influence the microshear bond strength of CAD/CAM-created lithium disilicate ceramics.

MATERIALS AND METHODS

A lithium disilicate ceramic block (IPS e.max CAD CAM, Ivoclar Vivadent, Schaan, Liechtenstein) in shade HT A1 was sectioned with diamond disc at low speed (Isomet Diamond Wafering Blades, Buehler Ltd., Lake Bluff, IL, USA) and subjected to crystallization firing process (Programat CS2, Ivoclar Vivadent), according to the manufacturer's recommendations.

Forty ceramic bars (6.5-mm long x 5-mm wide x 1-mm thick) were used. The bars were polished with 400, 100, 1200 and 1500-grit wet silicon carbide abrasive paper for 2 minutes (Norton Abrasivos, Recife, PE, Brazil) and ultrasound-cleaned in distilled water for 5 minutes. Subsequently, they were air abraded with 50-µm Al_2O_3 particles for 15 seconds at pressure of 2.5 bar (Micro-etcher ERC, Danville Engineering, San Ramon, CA, USA) and ultrasound-cleaned again in distilled water for 5 minutes. Finally, the bars were etched with 10% hydrofluoric acid for 20 seconds (Dentsply, Petrópolis, RJ, Brazil).

Monobond N (Ivoclar Vivadent Ltda, Barueri, SP, Brazil) was applied on the surfaces of ceramic bars, according to the manufacturer's instructions. Table 1 shows the resin cements used and composition. Four treatment groups (n = 10) were determined based on resin cement type: light-cured Variolink Esthetic LC (VLC; Ivoclar Vivadent Ltda, Barueri, SP, Brazil) and dual-cured Variolink N (VN; Ivoclar Vivadent Ltda, Barueri, SP, Brazil); at two different times: after 24 hours and after 1 year (10,000 thermocycles, between two water baths, at 5 °C and 55 °C with a dwell time of 30 seconds at each temperature extreme, and 2-second interval between immersions)[10]. The following experimental groups were formed: VLC after 24 hours, VN after 24 hours, VLC after 1 year and VN after 1 year.

Two transparent cylindrical molds (Tygon tubing - TyG-03, Saint Gobain Performance Plastic, Maime Lakes, FL, USA) - 1 mm (height) x 0.75 mm

Table 1. Resin cements and composition according to the manufacturers.

Resin Cement	Acronym	Composition
Variolink Esthetic LC (Ivoclar Vivadent Ltda, Barueri, SP, Brasil)	VLC	Urethane dimethacrylate, further methacrylate monomers, ytterbium trifluoride and spheroid mixed oxide, initiators, stabilizers and pigments
Variolink N (Ivoclar Vivadent Ltda, Barueri, SP, Brasil)	VN	Bis-GMA, urethane dimethacrylate, triethylene glycol dimethacrylate, barium glass, ytterbium trifluoride, Ba-Al fluorosilicate glass, spheroid mixed oxide, initiators, stabilizers and pigments

Bis-GMA = bisphenol-A glycidyl methacrylate.

(internal diameter) were placed on the surface of each bar[11] (Fig. 1A). Resin cements were prepared according to the manufacturer's instructions. A #5 exploratory probe (HuFriedy, Chicago, IL, USA) was used to apply the resin cements inside the molds. Each resin cement cylinder was light-cured for 20 seconds (Valo - Ultradent Products Inc., South Jordan, UT, USA) at 1,000 mW/cm^2 (Fig. 1B). A ceramic structure (IPS e.max CAD/CAM) was attached to the tip of the light-curing unit (0.5 mm in thickness) to attenuate light transmission and simulate the prosthetic structure. Next, samples were stored in distilled water at 37 ºC. After 24 hours or the thermocycling protocol (TC), the molds were carefully cut on opposite sides with the aid of scalpel blade and removed in order to expose two cement cylinders presenting adhesion area of 0.38mm^2 (Fig. 1C). Subsequently, these cylinders were subjected to microshear bond test. To do so, the bars were fixed to a universal testing machine (DL2000; Emic, São José dos Pinhais, PR, Brazil) and load was applied to the base of the cylinders by using steel wire (diameter 0.2 mm) at speed of 0.5 mm/min until fracture to determine the microshear bond strength[12] (Fig. 1D). Bond strength values were calculated, and data expressed in MPa. Each group included 10 bars and each bar had two cylinders, providing a total 20 cylinders per group. The mean for the two cylinders of each bar was considered as 1 specimen.

Fractured specimens were analyzed qualitatively under optical microscopy (Olympus Corp., Tokyo, Japan) at 40x magnification. Failure modes were classified as: I - adhesive (resin cement-ceramics interface); II - cohesive (resin cement) and III - mixed (combination of adhesive and cohesive fractures in resin cement).

Statistical analysis was performed in Minitab 16 software (Minitab, State College, PA, USA). Failure mode results were subjected to nonparametric one-way analysis of variance (ANOVA) and Kruskal-Wallis test (α = 0.05). Microshear bond strength results were subjected to Shapiro-Wilk normality test, which was followed by two-way analysis of variance – ANOVA (resin cement x time) and Tukey's test (α = 0.05).

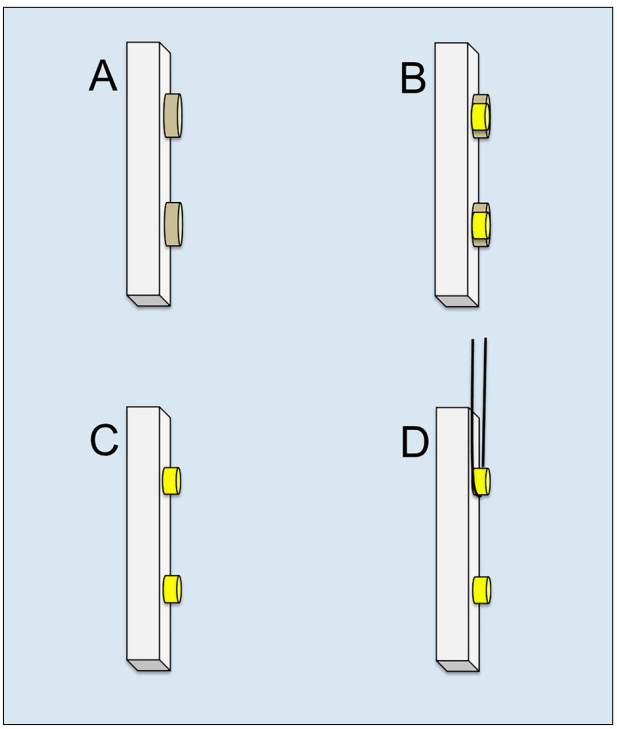

Fig. 1: Specimen preparation – (A) two cylindrical molds positioned on the surface of lithium disilicate ceramic; (B) resin cements filling the internal space of the molds; (C) resin cement cylinders; (D) microshear bond strength test.

RESULTS

Mean values of microshear bond strength test (MPa) are listed in Table 2. Comparison of resin cements bond strength values at different simulated aging

Table 2. Mean microshear bond strength values (MPa) and standard deviation recorded for all groups.

Resin Cement	Aging time	
	24 hours	1 year
VN	27.10 (0.92) A, a	20.62 (1.25) A, b
VLC	14.79 (0.76) B, a	6.61 (0.81) B, b

Mean values followed by different uppercase letters in the columns and by lowercase letters on the lines were significantly different from each other, at p = 0.035 (Tukey's test). Standard deviations are shown in parentheses. VN: Variolink N. VLC: Variolink Esthetic LC.

times showed that the VN resin cement presented significantly higher resistance values than the VLC resin cement, after 24 hours and 1 year (p = 0.035). Based on the comparison between simulated aging times, by taking into consideration the same resin cement type, mean resistance values recorded for both resin cements after 24 hours were significantly higher than the ones recorded at 1-year simulated aging (p = 0.035).

Table 3 lists the failure modes observed for all groups. All groups showed a prevalence of adhesive failures with similarity among the groups (p = 0.725) and none of them showed cohesive failure.

Table 3. Failure mode (%) recorded for all groups.

Groups	Adhesive	Cohesive	Mixed
VLC 24 hours	95.0 A	0	5.0 A
VN 24 hours	90.0 A	0	10.0 A
VLC 1 year	85.0 A	0	15.0 A
VN 1 year	85.0 A	0	15.0 A

Mean values followed by same uppercase letters in the columns did not differ significantly at p = 0.725 (Kruskal-Wallis test). The failure mode was classified as follows: adhesive (ceramic-resin cement interface), cohesive (resin cement) and mixed (involving adhesive and cohesive failures). VN: Variolink N. VLC: Variolink Esthetic LC.

DISCUSSION

Milled lithium disilicate ceramic is a restorative material. Ceramic blocks of it are manufactured through a pressure casting procedure and supplied in the so-called pre-crystallized blue state. Ceramics in this state present metasilicate and lithium disilicate cores and can be easily milled. After the milling process is complete, restorations are sintered in a ceramic oven and the ceramic is then vitrified[1,4]. Although its composition is similar to that of ceramics obtained by the heat press technique, its production process has great influence on final ceramic features: CAD/CAM-created lithium disilicate ceramic has a smoother, more homogeneous appearance and more complex topographic features[5]. Drumond *et al.*[13] report that the ceramic manufacturing technique (Press or CAD/CAM) can influence resin cement bond strength and its conversion degree. This suggests that the manufacturing method of the ceramic used may have influenced the current results, since ceramic surface features may interact with cement to promote adhesion between them[5].

The cementation procedure plays a significant role in the clinical success of fixed indirect restorations, and depends on the durability and quality of the adhesion among the tooth, the luting agent and the ceramic[6,7]. Mechanical treatments and/or chemical conditioning agents are used to create micromechanical and/or chemical bond to resin cement[8,9]. For this, the standard protocol for adhesive cementation of lithium disilicate ceramics requires blasting with aluminum oxide, conditioning with HF and silanization the ceramic surface[8]. Blasting comprises ceramic surface abrasion with 50 µm aluminum oxide particles to improve mechanical retention[8,9]. Air particle abrasion is a standard procedure performed by dental laboratories before delivering ceramic restorations to dentists[8]. HF removes the glass matrix from the ceramic surface by reacting it to silicon dioxide. This process increases ceramic surface roughness and, consequently, generates micromechanical interlock between resin cement and ceramics[6,9]. Silane coupling agents present bifunctional features. In addition, they have an inorganic group capable of reacting to the silica present on ceramic surface by a condensation reaction, and an organic group that can chemically bond to methacrylate-based resin cements, which enables the chemical bond between ceramic and cement and increases cement wettability[6,9]. Thus, the standard surface treatment protocol applied to lithium disilicate glass ceramic cementation was used in all groups in the present study.

Lower bond strength values have been associated with resin cements presenting lower mechanical properties[14,15]. Resin cements are a polymeric matrix embedded with inorganic fillers. Filler size and content are important factors defining the mechanical behavior of cements. Inorganic fraction increase leads to a decrease in polymerization contraction, and an increase in properties such as elastic modulus, and compressive and flexural

strength[16]. According to Spazzin *et al.*[15], the dual-cure resin cement Variolink II presented the best results in flexural strength and elastic modulus, whereas the Variolink Veneer (light-cure) resin cement showed the worst mechanical performance among the tested cements. These findings can be explained by the inorganic filler content observed in the investigated resin cements: Variolink Venner has low filler content, whereas Variolink II resin cement has high filler content[15]. These cements are predecessors to the resin cements used and have similar composition.

The resin cements investigated in the current study were subjected to indirect light-curing, with light transmission through ceramic in order to simulate the clinical procedure. Light transmission through indirect restoration is influenced by its thickness, opacity and shade[17]. In addition, a significant amount of light is lost during polymerization through ceramic due to absorption, dispersion or transmission, which can impair the final polymerization of dual or light-cured resin cements, change their properties and affect the longevity of ceramic restorations[18]. However, extended light-curing time to both light[19] and dual-cure[17] resin cements leads to greater polymerization depth, conversion degree, hardness and, therefore, to better mechanical and aesthetic properties[17]. Nonetheless, monomer conversion through dual-cure mode can be greater than that observed through light-cure mode[15]. Therefore, this difference may also be inferred to have influenced the results obtained.

Laboratory studies provide clinically relevant data[20]. To do so, they must simulate different oral environment conditions in order to replicate failure modes similar to those clinically observed[20], and subject materials to different aging conditions in order to estimate their long-term behavior[9,21]. The TC protocol must be applied to simulate hydrothermal aging *in vitro*[21] similar to that of the oral environment[20]. The temperature range adopted herein (5 ºC to 55 ºC) is indicated by ISO standards as appropriate[20], since these variations can result from eating habits, drinking and breathing[22]. Although the frequency of cycles *in vivo* remains undetermined, some authors[21,22] assume that these cycles occur between 20 and 50 times a day, and that about 10,000 cycles can represent one-year function *in vivo*. Temperature changes, along with repetitive stress, are caused by contraction and expansion that occur at the bond interface, since TC has significant negative effect on bond strength. In general, TC seems to decrease resistance values[21]. The present study tested the bond strength of two cements *in vitro* in the short- and long-term. TC aging had negative effect on bond strength. Samples from groups without TC showed higher values than the aged groups. This unfavorable effect can be attributed to mechanical stresses generated by thermal variations capable of inducing crack propagation along the bond interface[22] due to differences in thermal expansion coefficient among different materials[9,10].

Findings in the present study are only clinically acceptable (13-30 Mpa)[12] for dual-cure resin cement after one-year simulated aging, regardless of the microshear bond strength values. According to previous studies[14,23], dual-cure cements have shown reduced bond strength over time. On the other hand, Peumans *et al.*[10] reported increased strength values for dual-cure resin cement Variolink II after 10,000 TC cycles, and such increase remained stable after 40,000 thermocycles. This suggests that resin cement itself plays an important role in the final bond strength between cement and ceramics[10].

Bond strength tests are simple methods to compare the behavior of different materials *in vitro*, and different methods have been used for this purpose. Stress distribution in the microshear test can occur more uniformly, since samples do not suffer previous stress caused by sections performed prior to the test, in comparison to the microtensile test. Thus, its use is recommended for ceramic samples[2,24]. Adhesive-type fracture mode is indicative of adhesion between cement and ceramics, and differs from other fracture modes[12]. Adhesive failure was the most prevalent failure type observed in all groups tested in the present study. This is an additional advantage of the microshear test. In addition to not requiring previous section for sample preparation, this test reduces the incidence of cohesive failures[24]. In spite of the choice of the test performed, samples of the light-cured resin cement were excluded as a result of early fractures observed in cement cylinders prior to the test.

The production process can influence the final features of the milled lithium disilicate ceramic, which can interact with the resin cement to promote adhesion. Furthermore, the chemical composition of resin cements can also influence this bond. Depending on the ceramic manufacturing technique

and the type of cement used, there are differences in the effectiveness of bonding of different resin cements to CAD/CAM-created lithium disilicate ceramics after thermocycling. This knowledge can be clinically relevant for cementation of milled lithium disilicate ceramic prostheses. One of the limitations of this *in vitro* study is the isolated use of resin cement, which does not reproduce clinically observed events. The laboratory tests aimed at simulating oral conditions in order to predict clinical bond performances[9]. Restorations in the oral cavity are susceptible to several challenges, such as complex occlusal forces, immersion in saliva and exposure to food and drinks at different temperatures, chemical compositions and pH values. However, no laboratory test can properly predict the clinical performance of the resin-ceramic bond[9]. Another limitation of this study is the comparison of two cement types (dual- and light-cured) of the same brand. However, these cement types were recommended by the manufacturers of the ceramic used in the present study. It would be interesting to test different resin cement types in future research. Studies with long-term water storage and thermocycling are also needed, to evaluate new materials. Thus, based on results in the current study, the null hypotheses were rejected because different bond strength values were observed for milled lithium disilicate glass ceramic subjected to thermocycling and bonded with different cement types.

CONCLUSIONS

Based on the adopted methodology and materials, and on results observed in the current study, it was concluded that:

1) Thermocycling reduced the values recorded for both cements for the cementation of CAD/CAM-created lithium disilicate ceramic restorations.

2) The microshear bond strength of the dual-cured resin cement was significantly higher than that of the light-cured resin cement.

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DECLARATION OF CONFLICTING INTERESTS

The authors declare no potential conflicts of interest regarding the research, authorship, and/or publication of this article.

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Self-reported periodontitis in cannabis club members in Montevideo, Uruguay. An exploratory study

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ABSTRACT
In 2013, the Oriental Republic of Uruguay enacted a law regulating the cannabis market, and since then, an increasing number of users has been registered. Previous reports based on data from other countries link cannabis smokers to worse periodontal health status. The aim of this study is to describe self-reported gingival and periodontal health status and estimate the prevalence of periodontitis among Uruguayan cannabis club members. A cross-sectional study was conducted on a convenience sample of cannabis club members in Uruguay. A survey was used to gather sociodemographic data and information on oral hygiene, use of cannabis and other drugs, and self-reported gingival and periodontal health status. Eight questions validated in Spanish were used to estimate the prevalence of total periodontitis and severe periodontitis, based on two previously implemented predictive models. The survey was completed by 50 people, of whom 68% were male and 78% were in the 20-40 year age range. Based on the first model, estimated prevalence was 12% for total periodontitis and 10% for severe periodontitis. Based on the second model, estimated prevalence was 36% for total periodontitis and 12% for severe periodontitis. These findings suggest that smoking cannabis could potentially have a detrimental effect on oral health status..
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Keywords: *cannabis - periodontitis - epidemiology - marijuana abuse.*

Periodontitis auto-reportada en miembros de clubes de cannabis en Montevideo, Uruguay. Estudio exploratorio

RESUMEN
En 2013 se aprobó la ley que regula el mercado del cannabis en la República Oriental del Uruguay y desde entonces se ha registrado un creciente número de consumidores. Reportes previos a partir de datos de otros países vinculan un peor estado de salud periodontal en fumadores de cannabis. El objetivo de este estudio es describir el estado gingivo-periodontal auto-reportado y estimar la prevalencia de periodontitis en participantes de clubes cannábicos uruguayos. Se realizó un estudio transversal a partir de una muestra por conveniencia de integrantes de clubes cannábicos en Uruguay. Mediante una encuesta se recolectaron datos socio-demográficos, de higiene oral, de consumo de cannabis y otras drogas, así como de auto-reporte del estado de salud gingivo-periodontal. A partir de 8 preguntas validadas en español se estimó la prevalencia de periodontitis total y severa a través de dos modelos predictivos previamente implementados. Un total de 50 personas completaron la encuesta. El 68% eran hombres y el 78% estaba dentro del rango de 20 a 40 años de edad. Con el primer modelo la prevalencia estimada de periodontitis total fue 12% y de periodontitis severa 10%. Con el segundo modelo fue 36% para periodontitis total y 12% para periodontitis severa. Estos hallazgos sugieren que fumar cannabis podría tener un potencial efecto deletéreo sobre las condiciones de salud bucal.

Palabras clave: *canabis - periodontitis - epidemiología - abuso de marihuana.*

INTRODUCTION

In December 2013, the Oriental Republic of Uruguay enacted law 19.172 establishing a regulatory framework for the cannabis market. The Institute for Regulation and Control of Cannabis (*Instituto de Regulación y Control del Cannabis*, IRCCA) was created to regulate plantation, cultivation, harvest, production, processing, stocking, distribution and sale of cannabis, and to promote actions to reduce the risks and damage associated to the problematic use of cannabis[1]. In June 2018, 35.246 people were

registered for regulated access to marijuana, of whom 2.339 figured as members of 91 cannabis clubs[2]. Data for frequency of marijuana consumption in Uruguay according to age range show that approximately one out of every five persons between the ages of 26 and 35 years uses it daily, with this age range having the highest consumption. In addition, it was found that out of every 100 persons who had tried marijuana, 7 showed problematic use of the substance[3].

Xerostomia is one of the adverse effects observed in the oral cavity associated to cannabis consumption[4]. This condition is related to difficulties in adequate control of biofilm and to increased gingival inflammation[5]. Moreover, gingivitis and periodontal diseases are among the most prevalent diseases in in the world. World prevalence of total periodontitis has been reported as 5% to 70%, with differences among age ranges[6]. Moreover, it has been reported that approximately 11% of the population has severe periodontitis[7]. Risk factors for periodontitis, such as smoking and diabetes mellitus, have been extensively studied[8], as have environmental and behavioral factors, and certain microbiological aspects.

Self-reporting of different medical conditions such as diabetes, hypertension or myocardial infarction has been used for several years as a basis on which to monitor health status and trends over time in populations. In 2003, in the USA, self-reported evaluation was promoted as an alternative measure for surveillance of periodontal disease, since clinical examination requires great use of resources, both human and logistic, substantially increasing costs[9].

Later, in 2007, results of the Australian National Survey of Adult Oral Health[10] were published. This survey included 11 questions (6 gingivitis and periodontal disease screening questions and 5 conventional risk indicators) in large population surveys, yielding useful levels of validity in predicting moderate/severe to severe periodontitis.

A joint study by the Centers for Disease Control and Prevention (CDC) and the American Academy of Periodontology (AAP)[11] proposed to use eight questions in English and Spanish, which were subsequently assessed for validation in populations in the USA (including Spanish speakers)[12]. The use of this tool was studied further in Brazil, as part of a questionnaire consisting of 18 questions[13], and in France, where an additional four questions were included[14]. Validation in Spanish was performed in Spain, both in Madrid[15] and in Barcelona[16], where a set of 12 questions, including the 8 mentioned above[11], was used.

Verhulst et al[17] applied the questionnaire created by Eke and Genco[10] at the Academic Centre for Dentistry, Amsterdam (ACTA, by its acronym in Dutch), based on which they proposed 3 models for predicting total periodontitis and severe periodontitis. Model 1 uses self-reported answers on periodontal disease, demographic data and oral biomarker values, model 2 uses self-reported answers and demographic data, and model 3 uses only self-reported answers on periodontal disease. The authors found that model 3 provides acceptable values for predicting periodontitis.

A systematic review with meta-analysis published in 2016[18] shows that in general, self-reporting is useful for identifying presence of periodontitis. The sensitivity values for most of the questions were lower than the specificity values, therefore these questions are better at identifying people who do not have periodontitis.

Several epidemiological studies have addressed gingival and periodontal health status in cannabis smokers[19-22], finding a positive association between smoking cannabis and periodontal health status. A recent systematic review[23] of a wide range of studies suggests this association may be dose-dependent, and recommends further studies with longer follow-up times and control for risk factors/indicators such as tobacco, diabetes and age. It also recommends reporting how long the substance has been used, use frequency, and substance composition and quantity. Thus, exploratory studies are still required to further address the issue of association between marijuana consumption and periodontal diseases.

The aims of this exploratory study are therefore to describe self-reported gingival-periodontal status and to estimate the prevalence of periodontitis based on a predictive model among members of cannabis clubs in Montevideo, Uruguay.

MATERIALS AND METHODS

A cross-sectional study was performed from July to October 2018, based on a convenience sample of cannabis club members in Uruguay. This survey was approved by the Ethics Committee at the School of Dentistry, University of the Republic (Uruguay).

In June 2018, there were 109 clubs with membership registered at the IRCCA, representing 2339 persons

over 18 years of age. Fifty percent of these clubs were in Montevideo. Invitations to participate in the survey were extended through the president of the IRCCA, who informed the directors of the cannabis clubs via email, asking them to send club members the invitation to participate in the survey. People who expressed interest in taking part in the study were contacted by the research team to provide further information on the study and arrange a meeting for interviews. When there was no answer, invitations were sent for a second time after two months.

After reading and signing informed consent, each subject completed a voluntary, self-administered survey containing multiple-choice questions. With guidance from two researchers (SP and MM), questions were asked about identity data, sociodemographic characteristics, and profile regarding consumption of cannabis, alcohol and other drugs.

Self-reported gingival-periodontal health status was evaluated based on the Spanish version of a set of questions suggested by Eke and Genco[11] (Q1-Q8). Before beginning this study, the questions were assessed for clarity, ease of understanding and cultural adaptation by testing them on 20 patients at the Periodontics Clinic at the School of Dentistry, University of the Republic. Finally, the data collected in the surveys were entered in spreadsheets for subsequent analysis. Total exposure to tobacco was calculated in "pack years" for current smokers, following Susin et al[24], forming 3 groups: Light (1 to 2734 packs), Moderate (2735 to 7300 packs) and Heavy (more than 7300 packs). Mean age ± standard deviation (SD) was calculated. Prevalence was estimated for total periodontitis (TP) and severe periodontitis (SP), based on two predictive models (models 2 and 3) proposed by Verhulst et al[17]. For total periodontitis, model 2 employs the data from questions Q2, Q3, Q4, Q8 and age, while model 3 includes data from questions Q2, Q3, Q4 and Q8. For severe periodontitis, model 2 employs data from questions Q2, Q3, Q4, Q8, age and sex, while model 3 uses questions Q2, Q3, Q4 and Q8.

RESULTS

A total 56 members of five cannabis clubs agreed to answer the questionnaire. Of these, 50 completed the survey and 6 decided not to participate due to lack of time. Table 1 provides respondent demographics, showing that the proportion of males was higher than females (68% vs. 22%), 60% had university-level studies, and 76% were employed or self-employed. It shows that the population is young, with mean age 32 (±7.8) years, with the age range 20 to 40 years making up 78% of the total.

For toothbrushing frequency, 90% of the respondents reported brushing their teeth twice a day or more, while 10% reported doing so only once a day or less. Regarding tobacco use (Table 2), the number of smokers was equal to the number of former smokers and similar to the number of people who had not smoked tobacco previously. Table 2 also shows the characteristics of tobacco consumption and the number of "pack years"[24] for respondents who

Table 1. Participant demographics. Montevideo, 2018

		n	%
Sex	Female	11	22
	Male	34	68
	Not reported	5	10
Age	20 - 30	20	40
	31 - 40	19	38
	41 - 50	7	14
	Not reported	4	8
Education level	Primary	0	0
	Secondary	15	30
	Tertiary	30	60
	Not reported	5	10
Occupation	Employed (public or private)/ self-employed	38	76
	Student	5	10
	Unemployed	2	4
	Not reported	5	10
Toothbrushing frequency	≤1/ day	5	10
	≥2/ day	45	90

Table 2. Participant tobacco use profile. Montevideo, 2018

		n	%
Smoking habits	Smoker	17	34
	Ex-smoker	17	34
	Never smoked	15	30
	Not reported	1	2
Pack years	Light	10	59
	Moderate	5	29
	Heavy	2	12

agreed to report how long they had been smokers and how much they consumed, of whom 10 were light, 5 moderate and 2 heavy smokers.

Table 3 shows consumption of alcohol and other drugs. Most respondents reported consuming alcohol one to four times a week (62%). In addition, 21 respondents reported using other recreational drugs (e.g., cocaine, designer drugs or others), of whom 12 declined to provide frequency of use.

Table 4 shows time and frequency of cannabis use, showing that 56% had smoked cannabis for more than 10 years and 70% did so daily. Half the sample reported using less than 20g per month, with the other half reporting more than 20 g per month, among whom 6 persons used more than 40 g per month.

Evaluation of the 8 specific questions on self-reported periodontal disease (Table 5) found that

Table 3. Participant non-cannabis drug use profile. Montevideo, 2018

	Frequency of use during the past year	n	%
Alcohol	5 - 7	5	10
	1 - 4	31	62
	<1	9	18
	No use	3	6
	Declines to answer	2	4
Recreational drugs	**Type**		
	Cocaine, designer drugs, others	21	42
	None	10	20
	Declines to answer	19	38
	Frequency		
	Daily	1	5
	<1/ week	8	38
	Declines to answer	12	57

Table 4. Participant cannabis use profile. Montevideo, 2018

		n	%
Years of use	21 - 30	4	8
	16 - 20	10	20
	11 - 15	14	28
	6 - 10	15	30
	1 - 5	7	14
Weekly frequency	7	35	70
	4 - 6	9	18
	1 - 3	6	12
Grams per month	> 41	3	6
	20 - 40	22	44
	1 - 20	25	50
Cannabis use (not smoked)	Yes	22	44
	No	27	54
	Declines to answer	1	22

Table 5. Participant self-reported periodontal disease. Montevideo, 2018

		n	%
Q1 - Do you think you might have gum disease?	Yes	10	20
	No	39	78
	Don't know/ Don't answer	1	2
Q2 - In general, how would you describe the health status of your teeth and gums?	Excellent	4	8
	Very good	9	18
	Good	16	32
	Regular	17	34
	Bad	3	6
	Don't know/ Don't answer	1	2
Q3 - Have you ever had ever gum treatment such as root scaling or planing, sometimes known as "deep cleaning"?	Yes	8	16
	No	41	82
	Don't know/ Don't answer	1	2
Q4 - Have you ever had a tooth that became loose on its own without having had a lesion?	Yes	9	18
	No	40	80
	Don't know/ Don't answer	1	2
Q5 - Has a dental professional ever told you but you have lost bone around your teeth?	Yes	5	10
	No	44	88
	Don't know/ Don't answer	1	2
Q6 – In the last 3 months, have you noticed at tooth that does not seem to look good?	Yes	14	28
	No	35	70
	Don't know/ Don't answer	1	2
Q7 – Apart from toothbrushing, during the last seven days, how many times have you used dental floss or some other tool for cleaning between your teeth? (number of days)	1-2	15	30
	3-4	9	18
	5-6	3	6
	7 +	15	30
	Never	7	14
	Not reported	1	2
Q8 – Apart from toothbrushing, during the last seven days how many times have you used a mouthwash or other treatment for tooth diseases or problems?	1-3	12	24
	4-6	6	12
	7+	10	20
	Never	19	38
	Not reported	3	6

only one person did not answer any of the questions, so that the answer rate was 98% for all questions. Table 6 shows prevalence of total periodontitis (TP) and severe periodontitis (SP). With model 2, prevalence of total periodontitis was 12% (6 cases) and prevalence of severe periodontitis was 10% (5 cases), while with model 3, the values were higher, TP 36% and SP 10%.

Table 6. Prevalence of total periodontitis and severe periodontitis

		n	% (CI:95%)
Model 2	Total periodontitis	6	12,2 (3.0 - 21.4)
	Severe periodontitis	5	10.2 (1.7 - 18.6)
Model 3	Total periodontitis	18	36.7 (23.2 - 50.2)
	Severe periodontitis	5	10.2 (1.7 - 18.6)

DISCUSSION

This study evaluated self-reported periodontitis among recreational cannabis users who were members of cannabis clubs in Montevideo, Uruguay. This study has no parallel in the literature because it was conducted in Uruguay, which regulates recreational cannabis use, allowing distribution and/or sale of cannabis in pharmacies, home cultivation and the so-called cannabis clubs. These clubs are associations that gather cannabis users and are located throughout the country. The clubs are registered at the IRCCA, a public, government-sponsored legal entity. The current study is one of the first to focus on cannabis club members with the aim of learning about situations of disease, and the first to address periodontal health issues. Possibly as a result of this, plus mistrust regarding the use of data, there were difficulties in collecting data.

Given the confidentiality required for the study, the sampling process was administered by the IRCCA, which sent the consent forms and the survey to the coordinators of each club. The coordinators sent the forms to club members, although some of them prevented the process from continuing. The response rate from clubs was very low – less than 5%. Questionnaires were applied to a total 56 Uruguayan cannabis club members. Thus, this study does not intend to claim external validity, but provides a first exploratory study in order to propose future research hypotheses so that, over time and when the use policy matures, other studies may be conducted with higher response rates.

Due to the peculiarity of the sample, it is not currently possible to achieve greater approximations by means of, e.g., clinical examinations or other additional parameters. This study used self-reported results for periodontitis provided by cannabis club members, as well as their sociodemographic characteristics, hygiene habits, and associated addictions, among other factors. Self-reported periodontitis was evaluated through the Spanish language version of the questions developed by Eke and Genco[11].

Six of the subjects declined to respond to the questionnaire, alleging lack of time to answer. Therefore, although there is not a response rate for the total sample, about 10% did not respond effectively, a rate similar to those reported in previous studies such as Eke et al[25].

The subjects who participated in this study were mainly male (68%), mostly in the age range 20 to 40 years (78%), and more than half had reached university-level studies. These situations are in line with the distribution data for recreational cannabis users[3]. In terms of occupation, distribution was similar to that of the general population, so it may be inferred that the study sample is very similar to the universe of cannabis users in Uruguay.

This study considered additional use of other illegal drugs which are often associated with recreational use of cannabis[26]. Prevalence of use of illegal drugs such as cocaine or other synthetic drugs was 42% of the sample. It is worth highlighting that, given the peculiarity of the question, 38% of the participants declined to answer, with an even higher percentage not answering the question about frequency of use of other drugs.

Ninety percent of the sample had consumed alcohol during the past year, which is higher than the percentage recorded in the country in previous years (71.1%)[3]. In turn, 62% of the sample reported consuming alcohol 1 to 4 times a week, which was also higher than previous data for the country[3]. With regard to smoking, one third of the sample were current smokers, which is similar to national data. However, frequency of tobacco consumption was lower than observed in the general population[3].

The pattern of cannabis use shows that most respondents had been using it for over 10 years. This is considered long-term exposure., i.e., they were not occasional users or short-term users, so it may be inferred that at the time of reporting, any damage could already be detected. One of the

important features of this study is that, in contrast to the occasional exposures reported in another study[27], it evaluates frequent and regular cannabis users, since most of them consumed it on a daily basis and in considerable amounts per month, with 50% consuming more than 20 g per month. It is important to highlight that the values recorded for prevalence as well as frequency of use (daily and over the past 12 months) in cannabis users in Uruguay has increased relative to surveys conducted in 2016[3] and 2007[28], with highest prevalence of use among men aged 18 to 34 years, with similar sociodemographic characteristics.

In the current study, the results for estimated prevalence of self-reported periodontitis differed according to the model applied, particularly for total periodontitis, which was 12.2% in model 2 versus 36.7% in model 3. In contrast, both models found the same percentage (10.2%) for severe periodontitis. This may be explained by the characteristics of this convenience sample, where there is predominance of male sex and persons younger than 40 years, which are variables that are employed in model 2 proposed by Verhulst et al[17]. These data are similar to the distribution found for Uruguay's adult population in its 2015 National Oral Health Survey[29], although it is interesting to note the difference in terms of age ranges, because the survey presents data for a population aged 35 to 44 years. It is therefore possible that exposure to cannabis could have a slight influence on the onset of periodontitis at an earlier age.

The main limitations of this study were the difficulty to access a larger sample, and the calculation of the response rate. However, among the advantages of this peculiarity is the fact that the sample consisted of recreational cannabis users who were officially registered at clubs. The fact that periodontitis was evaluated through self-reporting may also be considered a limitation, even though self-reporting is currently widely validated, despite the possibility of memory bias[30].

To conclude, this study showed that among young people who are members of cannabis clubs in Uruguay, the prevalence of self-reported periodontitis can be considered high compared to available data for the general population with similar sociodemographic and behavioral conditions. This suggests that smoking cannabis could have a potential detrimental effect on oral health status. This exploratory study provides a basis for further research using larger samples with the aim of evaluating how cannabis smoking affects the prevalence and progression (by means of longitudinal studies) of periodontitis in users who are members of cannabis clubs.

DECLARATION OF CONFLICTING INTERESTS
The authors declare no potential conflicts of interest regarding the research, authorship, and/or publication of this article.

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Comparison of cone-beam computed tomography, clinical and surgical analysis for detection of maxillary molar furcation

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ABSTRACT
The aim of this study was to compare the performance of cone-beam computed tomography (CBCT), clinical and surgical probing in assessing maxillary molar furcation involvement (FI). Furcation defects (n= 120) were assessed through CBCT, clinical and intra-surgical evaluation (ISE). Furcation Involvement, vertical and horizontal bone loss were assessed through clinical probing, CBCT and probing during ISE. Three trained radiologists evaluated CBCT images and intra- and interobserver agreement were calculated by Kappa test and Intraclass Correlation Coefficient (ICC). McNemar and Wilcoxon tests were used to compare clinical probing, ISE and CBCT. Accuracy, sensitivity, specificity, positive and negative predictive values were calculated to detect FI. Clinical findings showed 28 sites with Degree I, 25 sites with Degree II, and 8 sites with Degree III. Good intra- (k=1.00) and interobserver agreement (k=0.773) were observed. Intraobserver and interobserver agreement for horizontal bone loss were moderate, k=0.485 and k=0.549, respectively. Intra-surgical findings showed Degree I at 21 sites, and Degree II and Degree III FI at fifteen sites each. Clinical evaluation showed 75% agreement with ISE and 78% with CBCT. Accuracy for clinical detection of FI was 75%, while for CBCT evaluation ranged from 72.5% to 77.5%, considering the 3 observers. Significant differences were found at distal sites using CBCT (p<0.05).
Clinical evaluation and CBCT showed similar results for the presence or absence of FI. Concerning horizontal and vertical bone loss, CBCT was not considered a precise examination method for incipient bone defects.
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Keywords: *cone-beam computed tomography - furcation defect - periodontal disease.*

Comparação da avaliação clínica, cirúrgica e por tomografia computadorizada de feixe cônico na detecção de lesões de furca em molares superiores

RESUMO
Este estudo teve como objetivo comparar o desempenho da tomografia computadorizada de feixe cônico (TCFC), sondagem clínica e cirúrgica na avaliação do envolvimento da furca de molares superiores (EF). Defeitos de furca (n= 120) foram avaliados por meio de TCFC, avaliação clínica e intra-cirúrgica (IC). O envolvimento da furca, perda óssea vertical e horizontal foram avaliados através de sondagem clínica, TCFC e sondagem durante IC. Três radiologistas treinados avaliaram as imagens de TCFC e a concordância intra e interobservador foi calculada pelo teste Kappa e Coeficiente de Correlação Intraclasse (ICC). Para comparação da sondagem clínica, IC e CBCT foram utilizados os testes de McNemar e Wilcoxon. A precisão, sensibilidade, especificidade, valores preditivos positivos e negativos foram calculados para a detecção de EF. Os achados clínicos mostraram 61 sítios com EF, sendo 28 Grau I, 25 locais de Grau II e 8 locais de Grau III. Observou-se boa concordância intra- (k = 1,00) e interobservador (k = 0,773). A concordância intraobservador e interobservador para perda óssea horizontal foi moderada, k = 0,485 e k = 0,549, respectivamente. Os achados intra-cirúrgicos mostraram EF grau I em 21 sítios e grau II e grau III em quinze sítios cada. A avaliação clínica mostrou 75% de concordância com IC e 78% com CBCT. A acurácia para detecção clínica de EF foi de 75%, enquanto para avaliação de CBCT variou de 72,5% a 77,5%, considerando os 3 observadores. Diferenças significativas foram encontradas em sítios distais em CBCT (p <0,05).
A avaliação clínica e a TCFC mostraram resultados semelhantes para a presença ou ausência de EF. Em relação à perda óssea horizontal e vertical, a TCFC não foi considerada um exame preciso para defeitos ósseos incipientes.

Palavras-chave: *tomografia computadorizada de feixe cônico - defeito de furca - doença periodontal.*

INTRODUCTION

Early detection of furcation involvement (FI) favors treatment success, preventing the progression of alveolar bone loss and loosening of teeth[1,2]. Periodontal diagnosis of furcation involvement is based on clinical, radiographic and intra-surgical probing of the furcation entrance. Although intra-surgical measurement is invasive, it has been considered the gold standard for classifying the extent of furcation involvement, especially in upper molars[3]. Despite its accuracy for FI diagnosis, intra-surgical measurement should only be used in specific cases, while the conservative approach (clinical and radiographic examinations) can be routinely performed[4].

Periapical, interproximal and panoramic radiographs have been widely used as complementary examinations for periodontal disease diagnosis, primarily due to their easy acquisition, low cost and satisfactory resolution[5,6]. Even though intraoral radiographs are still the most frequently used technique for diagnosing FI[7], they are often not precise, because two-dimensional radiographs may show overlapping roots, making it challenging to evaluate furcation lesions correctly[8,9].

Due to the limitations of conventional radiographs, cone-beam computed tomography (CBCT) has been indicated for the diagnosis of FI, since it provides detailed information about periodontal tissue support and interradicular bone, which are fundamental components for treatment planning[10,11]. It is worth using CBCT in FI diagnosis in upper molars when surgical treatment is indicated[2]. CBCT is highly accurate for detecting furcation involvement, and has been extensively used for challenging diagnoses, such as Degree I or II[12,13].

In vivo studies[3,5,10,14,15] have shown the potential of 3D images compared to intra-surgical assessments for diagnosing FI in maxillary molars, but results are controversial regarding CBCT accuracy for this purpose. The aim of this study was therefore to compare CBCT images with clinical and intra-surgical analyses to diagnose maxillary molar furcation involvement.

MATERIALS AND METHODS

This was a prospective cross-sectional study, performed in full accordance with the World Medical Association Declaration of Helsinki and approved by the Institutional Review Board of Federal University of Goiás, Brazil (#67419517.4.0000.5083) and University Center of Anápolis (#2.126.098). It was also registered by ReBEC (Brazillian Register for Clinical Trials - #RBR-33mj49). This study followed the CONSORT guidelines for clinical trials.

To calculate the sample size, 83% mean agreement was considered, as previously reported[3,5], performed at a significance level of 95%, for a test power of 80%, two-tailed. A number of 41 sites per analyzed group was determined. The following inclusion criteria were established: patients older than 18 years, with periodontitis in the upper molar indicated for surgical treatment. Exclusion criteria were teeth with caries, cervical resorption, fused roots, metallic crowns, amalgam restorations close to the alveolar bone crest and endodontic treatment; pregnant and/or lactating women. The study group was comprised of 12 patients (8 men and 4 women), aged 40 to 55 years and diagnosed with periodontitis. A total 40 teeth, 120 sites, with or without FI, were included.

Patients received prior periodontal therapy, including oral hygiene instructions, scaling and root planing, and occlusal adjustment when necessary. The same periodontist performed examination and treatment for all patients.

Clinical measurements

The sites were classified according to the presence or absence of FI, and horizontal and vertical bone loss were measured. Horizontal bone loss was assessed with Nabers probe (PQ2N, Hu-Friedy, Chicago, IL, USA) on the distal, buccal and mesial sites, according to the classification system of Hamp *et al.* (1975)[16]. Vertical bone loss was evaluated with the North Carolina probe (PCPUNC-15, Hu-Friedy, Chicago, IL, USA), measuring the distance from cementoenamel junction (CEJ) and the bottom of the periodontal pocket at the center of the buccal surface[17]. The examiner had no access to the CBCT images.

Intra-surgical measurements

Intra-surgical evaluation (ISE) was considered the gold standard and was performed using the same parameters described previously (Fig 1). During ISE, the periodontist had no access to the previous clinical measurements or the CBCT images.

Image acquisition and analysis

All CBCT images were acquired with an i-CAT Cone Beam 3D Imaging System-Next Generation (Imaging

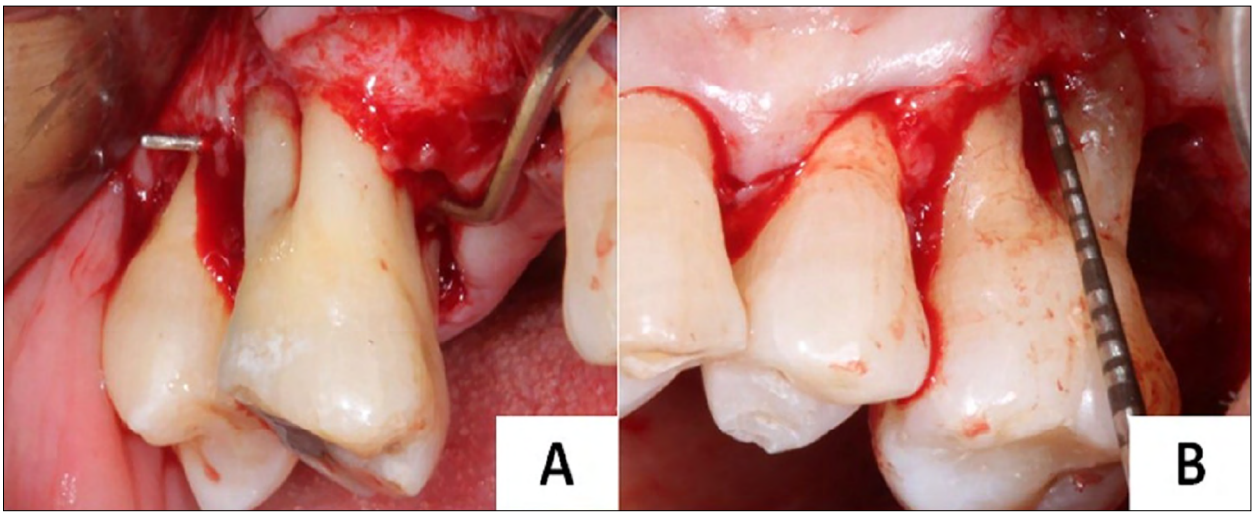

Fig.1: Image of measurements made during intra-surgical assessment (gold standard). A) Horizontal assessment of the furcation involvement B) Vertical assessment in millimeters of the furcation lesion.

Sciences International, PA, USA) device using the same parameters: voxel size 0.125 mm^3, tube setting 120 kV (voltage) and 5 mA (current), acquisition time 26.9s, and an 8 x 8 cm field of view (FOV). InVivo Dental Application software (Anatomage 5.3.2, USA) was used for CBCT image analysis.

Three previously calibrated radiologists performed the tomographic measurements. All the images were independently analyzed twice by each observer, with an interval of 2 weeks between the analyses. The observers had no access to the clinical and intra-surgical evaluations. Horizontal and vertical bone loss were measured in the slice that showed the greatest extension of FI (Fig 2). Vertical measurements were performed in the sagittal plane by positioning the cursor at the beginning of the FI and extending it to the defect's maximum point (Fig 2D).

Statistical analysis

Cohen's Kappa test (κ) was used to establish presence

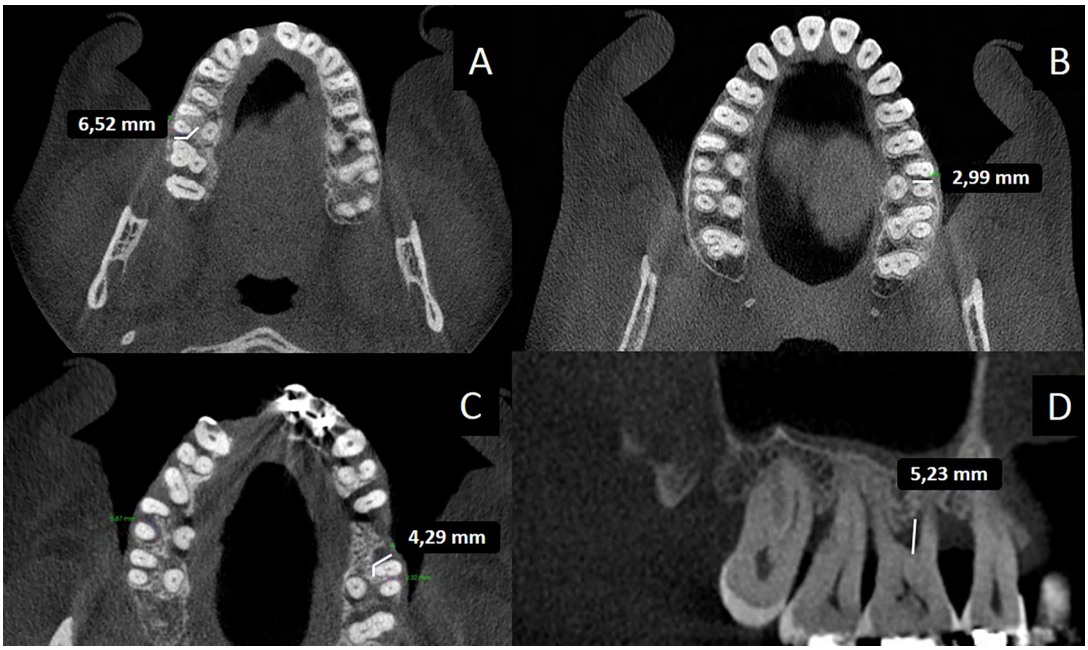

Fig. 2: Image of measurements made on CBCT images on software InVivo Dental Application. A) Horizontal measurement at distal site; B) Horizontal measurement at buccal site; C) Horizontal measurement at mesial site; D) Vertical measurement on buccal face.

or absence of FI and horizontal bone loss. An Intraclass Correlation Coefficient (ICC) was used to analyze the intra- and inter-observer agreement in the tomographic measurements for vertical bone loss. For the Kappa test, the following interpretation was considered: k ≤ 0.20, poor; k = 0.21-0.40, fair; k = 0.41-0.60, moderate; k = 0.61 - 0.80, good and k = 0.81 - 1.00, very good[18]. For the ICC interpretation, Bland and Altman's reference[19] was used: ICC < 0.4, small; 0.4 ≤ ICC < 0.75, moderate and ICC ≥ 0.75, excellent.

Clinical and CBCT measurements (horizontal and vertical) were compared using the Wilcoxon test, which was also used to compare them to the gold standard (intra-surgical measurements). McNemar's test was used to compare the groups regarding the presence or absence of furcation involvement. Accuracy, sensitivity, specificity, positive and negative predictive values were calculated for the detection of furcation lesions.

Table 1. Absolute frequencies and percentage (%) of horizontal bone loss (Hamp et al. 1975) at the evaluated sites, according to different methods of assessment

		Assessment	Clinical	Intra Surgical	CBCT		
		Observer	---	---	1	2	3
Distal n = 40	Hamp	0	12	15	25	28	30
			10%	12.5%	20.8%	23.3%	25%
		I	8	9	1	0	1
			6.7%	7.5%	0.8%	0%	0.8%
		II	16	10	9	6	3
			13.3%	8.3%	7.5%	5%	2.5%
		III	4	6	5	6	6
			3.3%	5%	4.2%	5%	5%
Buccal n = 40	Hamp	0	18	21	26	27	29
			15%	17.5%	21.7%	22.5%	24,2%
		I	14	10	5	2	2
			11.7%	8.3%	4.2%	1.7%	1.7%
		II	6	4	5	5	4
			5%	3.3%	4.2%	4.2%	3.3%
		III	2	5	4	6	5
			1.7%	4.2%	3.3%	5%	4.2%
Mesial n = 40	Hamp	0	29	33	35	35	33
			24.2%	27.5%	29.2%	29.2%	27.5%
		I	6	2	0	0	0
			5%	1.7%	0%	0%	0%
		II	3	1	2	1	5
			2.5%	0.8%	1.7%	0.8%	4.2%
		III	2	4	3	4	2
			1.7%	3.3%	2.5%	3.3%	1.7%
Total n = 120	Hamp	0	59	69	86	90	92
			49.2%	57.5%	71.7%	75%	76.7%
		I	28	21	6	2	3
			23.3%	17.5%	5%	1.7%	2.5%
		II	25	15	16	12	12
			20.8%	12.5%	13.3%	10%	10%
		III	8	15	12	16	13
			6.7%	12.5%	10%	13.3%	10.8%

RESULTS

Distribution of FI

The horizontal FI recorded in the clinical, CBCT and intra-surgical evaluations are shown in Table 1. The intra-surgical findings revealed Degree I (21 sites), II (15 sites) and III (15 sites), while 69 sites showed no FI (57.5%). The clinical findings presented 28 Degree I, 25 Degree II, and 8 Degree III lesions and 59 sites without FI (49.2%). The CBCT findings showed 6 Degree I, 16 Degree II, and 12 Degree III lesions and 86 sites showed no FI (71.7%).

Diagnostic tests for clinical and CBCT assessment

Diagnostic tests for the detection of FI are shown in Table 2. Accuracy for clinical detection was 75%, while for CBCT evaluation it ranged from 72.5% to 77.5%, considering the 3 observers.

Sensitivity was higher for CBCT (92.7%) than for clinical evaluation (72%), while specificity was lower for CBCT (45.1-56.9%) than for clinical detection of FI (78.8%).

Intra- and inter-observer agreement on CBCT images

Intra- and inter-observer agreement for the presence or absence of FI and horizontal bone loss are shown in Table 3 and those for the vertical bone loss in Table 4.

Table 2. Accuracy, sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) for the identification of furcation lesion

	Observer(s)	Accuracy	Sensitivity	Specificity	PPV	NPV
Clinical	-	75	72	78.8	81.7	68.3
CBCT	1	77.5	92.7	56.9	74.4	85.3
	2	74.2	92.7	49	71.1	83.3
	3	72.5	92.7	45.1	69.6	82.1

Table 3. Intra- and interobserver agreement for the presence or absence of furcation lesion and horizontal bone loss

Agreement	Observer(s)	Assessment	Kappa	P value	Agreement
Intra-observer	**1**	Presence or absence	1	<0.001*	Very good
		Horizontal bone loss	1	<0.001*	Very good
	2	Presence or absence	0.895	<0.001*	Very good
		Horizontal bone loss	0.901	<0.001*	Very good
	3	Presence or absence	1	<0.001*	Very good
		Horizontal bone loss	0.485	<0.001*	Moderate
Inter-observer	**1 x 2**	Presence or absence	0.702	<0.001*	Good
		Horizontal bone loss	0.590	<0.001*	Moderate
	1 x 3	Presence or absence	0.697	<0.001*	Good
		Horizontal bone loss	0.549	<0.001*	Moderate
	2 x 3	Presence or absence	0.773	<0.001*	Good
		Horizontal bone loss	0.625	<0.001*	Good

*Statistical significance for Kappa test

Table 4. Intra- and interobserver agreement for vertical bone loss

Agreement	Observer(s)	ICC	P value	Agreement
Intra-observer	1	0.916	0.155	--------
	2	0.947	0.124	--------
	3	0.896	0.145	--------
Interobserver	1x2	0.942	<0.001*	Excellent
	1x3	0.922	<0.001*	Excellent
	2x3	0.928	<0.001*	Excellent

ICC – Intraclass Correlation Coefficient
*Statistical significance for ICC

For vertical bone loss, both intra-observer agreement (ICC=0.916) and inter-observer (ICC=0.965) agreement were considered excellent. Regarding the presence or absence of FI, intra-observer agreement was very good (k=1.000) and inter-observer agreement was good (k=0.773). Horizontal bone loss showed moderate (k=0.485) to very good (k=1.000) intra-observer agreement and moderate (k=0.549) to good (k=0.625) inter-observer agreement.

Comparison and agreement of clinical, CBCT and Intra-surgical assessment

Table 5 shows statistically significant results for the comparison and agreement tests between the clinical and CBCT data measurements and intra-surgical evaluations for all variables: presence or absence of FI and horizontal and vertical bone loss.

Comparing clinical and ISE, there was a significant difference between presence and absence of FI with moderate (k=0.501) and poor (k=0.442) agreement, respectively, for horizontal bone loss and very good (k=0.940) agreement for vertical bone loss ($p<0.05$). Comparing CBCT to ISE, there was a significant difference for the presence or absence of FI on CBCT to distal sites with moderate agreement for all assessments ($p<0.05$). Horizontal bone loss showed poor agreement with CBCT assessment ($p<0.05$).

Table 5. P values for comparisons and agreement between clinical and CBCT with intra-surgical measurements (gold-standard) at the evaluated site

Measure	Assessment	Observer	Distal	Buccal	Mesial	All sites	Kappa or ICC	p-value	Classification
Presence or absence of furcation lesion	Clinical	---	0.508	0.549	0.344	0.100	0.501	<0.001***	Moderate
	CBCT	1	0.006*	0.227	0.625	0.002*	0.519	<0.001***	Moderate
		2	0.002*	0.109	0.625	<0.001*	0.441	<0.001***	Moderate
		3	<0.001*	0.039*	1	<0.001*	0.402	<0.001***	Moderate
Horizontal bone loss	Clinical	---	0.297	0.837	0.658	0.442	0.364	<0.001***	Poor
	CBCT	1	0.050	0.195	0.608	0.028**	0.364	<0.001***	Poor
		2	0.005**	0.782	0.467	0.030**	0.367	<0.001***	Poor
		3	0.003**	0.128	0.914	0.004**	0.297	<0.001***	Poor
Vertical bone loss	Clinical	---	---	0.907	---	---	0.940	<0.001****	Very good
	CBCT	1	---	0.084	---	---	0.487	<0.001****	Moderate
		2	---	0.116	---	---	0.740	<0.001****	Moderate
		3	---	0.028**	---	---	0.540	<0.001****	Moderate

* Statistical significance, McNemar test
** Statistical significance, Wilcoxon test
***Statistical significance, Kappa test
**** ICC: Statistical significance, ICC test

DISCUSSION

Our results showed that neither clinical evaluation nor CBCT presented high agreement with the gold standard (intra surgical evaluation - ISE). Accuracy values for FI detection were below 80%, which indicates that caution is required when prescribing and interpreting CBCT exams for this purpose. Other studies have reported high precision for CBCT for detection of FI[2,20]. Our study's accuracy results may be related to a sample of incipient furcation lesions, which are difficult to diagnose, even in imaging exams. In a previous study, Yusof et al.[14] found no difference between clinical and intra-surgical evaluation; however, they evaluated molars (both upper and lower) with extensive bone losses. In another study[15], CBCT measurements showed high agreement with ISE in evaluating incipient furcation defects, as in the present study, but assessment was made by a single observer, and a larger FOV was employed. It is essential to mention that these variations in the agreement (greater or lesser) between CBCT and ISE in these studies may occur due to changes in the parameters of the CBCT devices (such as FOV, voxel size, voltage), as has been shown in a recent study by Rinne et al.[21].

In the present study, the descriptive analysis concerning presence of FI showed a 14.2% underestimation by CBCT. Similar results were found in other clinical studies[3,5,10]. Clinical assessment overestimated presence of FI in 11.96% of the cases, showing superior results compared to CBCT. Our results differed from Darby et al.[1], who found in clinical assessments an overestimation of 58% of FI and an underestimation of 20% of FI compared to CBCT.

The findings of the intra-observer agreement for the presence and absence of FI and horizontal bone loss were considered very good. These results were better than those reported in a previous *in vitro* study by Kolsuz et al.[22], who found good agreement. It is important to emphasize that *in vivo* studies present difficulties related to soft tissue and anatomical variations[7]. This makes our results surprisingly better than those of *in vitro* studies in which these limitations are not present[8,23].

For horizontal bone loss, the good and moderate interobserver agreement supports the notion that observers have difficulty in classifying FI compared to detecting presence or absence of the defect. Other clinical studies[2,20,24] did not report the results of the interobserver agreement on CBCT, so we could not compare our findings.

There was no statistically significant difference regarding intra-observer agreement for vertical bone loss; however, inter-observer agreement was excellent. These results show the validity and reproducibility of CBCT in monitoring the height of the bone defect at the buccal surface of teeth with vertical bone loss.

As observed in this study, the distal site was diagnosed better ($p<0.05$) than the buccal and mesial sites. These results corroborate Walter et al.[2], who found a greater diagnostic precision at the distal site. Similar results were also described by Qiao et al.[20], who reported significant agreement in the diagnosis of FI by ISE. Moreover, the study by Zhang et al.[25], CBCT showed more significant agreement in the detection of distal sites by clinical evaluation, while in the recent study by Komšić, et al.[15], the buccal site was better diagnosed than the distal and mesial sites.

In the present study, the general agreement of CBCT with intra-surgical assessment for the FI involvement was moderate (k: 0.519) and poor (k: 0.367) for horizontal bone loss. These results show that for the classification of bone loss, the statistical error of the method could have been relatively high. On the other hand, better results were found in the clinical studies by Qiao et al.[20] and Walter et al.[2], with very good agreement between CBCT and intra-surgical assessment, while Yusof et al.[14] reported excellent agreement. Remarkably, these studies had samples with extensive FI, especially Degrees II and III, while in the present study, much of the sample consisted of incipient defects. In the early stages of FI, the cavities are small and have irregular borders, making them difficult to diagnose by CBCT[26].

Moderate agreement was found upon comparing CBCT and ISE for vertical bone loss for all observers ($p <0.05$). Qiao et al.[20] reported only the difference of 0.36 mm between the measurements, but not the agreement result. Padmanabhan et al.[8] evaluated 25 furcation defects in lower molars, using a pachymeter for the evaluation of vertical bone loss, and found a difference of only 0.12 mm by CBCT compared to ISE. The differences found in those studies are small compared to the present study, and both showed statistically significant differences[8,20]. However, Yusof et al.[14] did not find statistically significant differences between CBCT

and ISE measurements for vertical bone loss.

In our study, accuracy results for FI identification were similar for clinical assessment and CBCT evaluation by all three observers. Interestingly, despite the similar results, a statistical difference was found upon comparing clinical assessment and CBCT by McNemar's test. This can be clearly understood by the sensitivity and specificity findings individually. In the clinical evaluation, all diagnostic test results presented a balanced ratio between sensitivity (72%) and specificity (78.8%), which resulted in a 75% accuracy value. Contrarily, CBCT accuracy for all observers was defined by high sensitivity compensation combined with low specificity. This implies that CBCT showed a high performance in detecting the presence of FI (92.7% sensitivity) but was not as useful to exclude false positive cases. These findings should be taken into consideration when indicating and interpreting a CBCT exam for this purpose.

According to the meta-analysis published by Haas et al.[27], although there is moderate scientific evidence supporting the use of CBCT to assess furcation lesions and FI, it should not be considered the first choice. Other systematic reviews[28,29] corroborate this analysis, stating that CBCT should be indicated for selected cases, considering a risk-benefit balance. These researchers agree with Yang et al.[30] on that, depending on the types of periodontal bone defects, CBCT is not necessary since clinical assessment is sufficient for detecting and classifying these lesions. Another critical issue to be highlighted is that different CBCT settings and devices may influence image accuracy for FI detection[21,31,32], and a small field of view should be preferred[28].

In summary, clinical, CBCT and intra-surgical measurements showed similar results in assessing maxillary molar furcation involvement. Regarding horizontal and vertical bone loss analyses, CBCT was not found to be an accurate method for incipient lesions. For the diagnosis of incipient furcation lesions, clinical evaluation remains fundamental. The indication of CBCT should be made considering the radiation dose evaluation and the real benefits for diagnosis of the furcation involvement.

DECLARATION OF CONFLICTING INTERESTS

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Prevalence of oral mucosal lesions in an adult population from eight communities in Santo Domingo, Dominican Republic

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ABSTRACT

The purpose of the study was to evaluate the prevalence of Oral Mucosal Lesions (OMLs) in an adult population from Santo Domingo, Dominican Republic. 751 subjects from eight communities from Santo Domingo accepted the invitation to participate in an oral screening from October 2016 to January 2017. 248 subjects were evaluated and clinically examined, age range 18-86 years. A validated instrument was designed to record demographic factors, age group, gender, anatomical location, presence or absence of OMLs, risk factors such as tobacco consumption and its frequency, and different forms of tobacco and alcohol use. A systematic oral clinical examination was conducted by a specialist. The presence or absence, and anatomic location of OMLs were recorded. The sample consisted of 44.4% males and 55.6 % females. 228 subjects had 1 or more lesions (91.9%), the median was 3 lesions per patient. In relation to risk factors, tobacco use in general was reported by 26.2 % of the subjects, with cigarette smoking reported by 75.4%, followed by other forms as "hookah" 9.2 %, marihuana 9.2%, cigars ("puros")4.6% and pipe smoking 1.5%. Among the oral lesions detected by screening, the non-pathological group was prevalent, and included physiologic melanin pigmentation as the most frequent (25.0%) followed by palatal/mandibular tori (20.2%), Fordyce granules (7.9%), and Exostosis (5.6%). Potentially malignant disorders (Oral Leukoplakia, Oral Lichen Planus and Actinic Cheilitis) corresponded to 2.2%, 0.3 %, and 0.3%, respectively. No malignancy was observed clinically. This study contributes to determining the prevalence of OMLs in Dominican Republic and to identifying risk factors. This is the first study reporting the prevalence of oral mucosal lesions among the Dominican adult population. This information is vital for establishing a public health program targeting the high-risk group to improve the oral health status in this population.

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Keywords: *mouth diseases- oral mucosa - Dominican Republic.*

Prevalencia de lesiones de mucosa oral en una población adulta de ocho comunidades en Santo Domingo, República Dominicana

RESUMEN

El objetivo del presente estudio fue evaluar la prevalencia de lesiones de la mucosa oral (LMO) en una población adulta proveniente de Santo Domingo, República Dominicana. 751 individuos procedentes de ocho comunidades de la provincia de Santo Domingo, respondieron a la invitación para participar en el examen bucal, desde Octubre 2016 a Enero 2017. 248 sujetos con un rango de edad de 18-86 años, fueron evaluados y examinados clínicamente. Se diseñó y validó un instrumento para obtener datos de factores demográficos, grupos de edad, género, localización anatómica, presencia o ausencia de lesiones de la mucosa oral, factores de riesgo tales como: consumo de tabaco, frecuencia, diferentes formas de uso de tabaco y alcohol. Un especialista en el área, realizó un examen clínico bucal sistematizado en el cual se evaluó y registró la presencia o ausencia de lesiones y su localización anatómica. De acuerdo a la distribución por género, 44.4% correspondió a masculino y 55.6 % femenino. 228/248 sujetos presentaron 1 o más lesiones (91.9%), siendo la media de 3 lesiones por paciente. En relación a los factores de riesgo, el tabaco se reportó en 26.2%, siendo el fumar cigarrillos el 75.4%, seguido de otras formas como "hookah" 9.2%, marihuana 9.2%, cigarros ("puros") 4.6% y pipa fumada 1.5 %. En cuanto a las lesiones bucales detectadas en el examen, el grupo de condiciones no patológicas fue el más frecuente e incluía a pigmentaciones fisiológicas melánicas (25.0%), seguida de torus palatino/mandibulares (20.2 %), gránulos de Fordyce (7.9%) y exostosis (5.6%),respectivamente. Las lesiones potencialmente malignas detectadas (Leucoplasia oral, Liquen plano oral y Queilitis actínica) correspondieron al 2.2%, 0.3 % y 0.3%, respectivamente. Clínicamente, no se observó malignidad. Este estudio contribuye a determinar la prevalencia de LMO en República Dominicana e identificar factores de riesgo. Los hallazgos representan el primer estudio que muestra la prevalencia de las lesiones de mucosa oral en la población adulta dominicana. Se recomienda la creación de un programa de salud pública orientado a grupos de alto riesgo para mejorar el estatus de salud oral en esta población.

Palabras clave: *lesiones bucales - mucosa oral - República Dominicana.*

INTRODUCTION

Numerous professionals have focused on the importance of identifying Oral mucosal lesions (OMLs), during routine dental treatments. Therefore, epidemiological studies designed to understand the prevalence and incidence of OMLs have been undertaken, which contribute to identifying risk factors in different populations. Ali *et al*.[1] conducted a study to determine the number, types and location of OMLs in patients attending the Admission Clinic at Kuwait University Dental Center, designed to identify risk factors for oral lesions. Oral lesions were divided into six major groups: white, red, pigmented, ulcerative, exophytic and miscellaneous. A total 530 subjects were screened, of whom 308 presented one or more lesions, mainly in the age group of 40 years, and more often associated to smokers than non-smokers. Pentenero *et al.*[2] carried out a retrospective study on 4,098 subjects in an adult population from Turin (Italy), analyzing the association between OMLs and tobacco, alcohol consumption and removable denture wearing. The results showed that tobacco and alcohol was linked with higher prevalence of OMLs, in particular candidiasis, traumatic and frictional lesions. Mehrotra *et al.*[3] determined the prevalence of oral soft tissue lesions in 3,030 subjects from a semi-urban district in Vidisha (India). They explored not only the prevalence, but also attempted to correlate numerous risk factors. Carrard *et al.*[4] conducted a cross-sectional study in an urban population in southern Brazil to assess the prevalence of OMLs based on a multivariable risk assessment of demographic, socioeconomic, behavior and oral risk indicators, concluding that this population needed OMLs prevention and treatment. Their findings on potentially malignant oral lesions were related to smoking, alcohol and socioeconomic disparities. Amadori *et al*.[5] analyzed OMLs in adolescents in a retrospective cross-sectional study. A total 1,544 cases were registered with 36 different OMLs types, and included healthy and systemic disease. Rivera *et al*.[6] also documented a retrospective study to evaluate the frequency of OMLs in an elderly Chilean population. They used the WHO epidemiological guide for oral disease, finding and classifying 277 lesions. Prinyanka *et al*.[7] documented that the prevalence of mucosal lesions among alcohol-dependent subjects was 31.5%, which was higher than in the controls (25%). Ottapura *et al*.[8] reported the prevalence of OMLs in association to tobacco among migrant workers, showing that current use of smoked tobacco, smokeless tobacco and alcohol was 41.8%, 71.7% and 56.6%, respectively. OMLs were seen in 36.3% of participants and 44.6% of the smokeless tobacco users presented lesions. Additionally, the lesions were more common among current alcohol users (42.8%) than non-users (12.3%). To the best of our knowledge, there are no previous published data on the epidemiological evaluation of OMLs in adult subjects from Dominican Republic. The purpose of this study was to evaluate the prevalence of OMLs in an adult population from eight communities in Santo Domingo, Dominican Republic. This study represents the first screening-based research conducted in the country, and it will contribute to understanding and preventing OMLs.

MATERIALS AND METHODS

This study was conducted in accordance with the Declaration of Helsinki (1975), as revised in 2013, and reviewed and approved by the National Committee of Bioethics (CONABIOS) (Protocol # 042-2016), Santo Domingo, Dominican Republic.

Participants

The total population consisted of 751 subjects from eight different communities in Santo Domingo, Dominican Republic. The researchers visited the selected neighborhoods to define an appropriate study setting and distribute invitation flyers to residents. Individuals that accepted the invitation to participate in an oral screening visited the clinical facilities. To participate in the study, subjects had to meet the following inclusion criteria: good general health, 18 years of age or older. 248 individuals met the inclusion criteria and were clinically examined from October 2016 to January 2017. A questionnaire was used to record sociodemographic factors, including occupation, socioeconomic level, level of education, age and gender. Other risk factors such as tobacco consumption (active or current smoker, former smoker or never smoker), as well as the frequency of smoking and other forms of tobacco consumption; alcohol use, independently or in combination, and types of alcohol (rum, wine, whisky, beer, liqueur) as form of alcohol exposure were investigated. Other factors such as denture wearing or prosthesis were also recorded.

Clinical examination

A systematic intraoral clinical examination was conducted by a single examiner who is specialist in the area of Oral Medicine and Pathology. The examination was performed using dental light, mirror, spatulas and gauze. The clinical diagnosis was established and classified according to the epidemiology guide for the diagnosis of oral mucosal diseases (ICD-WHO)[9]. Correlation with risk factors was assessed. The following items were assessed during the clinical examination: appearance of the lesion, anatomical location, extension, dental status, trauma, use of prosthesis and whether the prosthesis was well adapted. In addition, cases requiring further examination or biopsies were referred to the Department of Periodontology, School of Dentistry, Pontificia Universidad Católica Madre y Maestra, Santo Domingo, Dominican Republic for definitive diagnosis.

Statistical analysis

The data was tabulated using Microsoft Office Excel®2016, and processed for analysis using StataIC®14.0. Quantitative variables were expressed as mean and standard deviation (± SD) and qualitative variables were summarized as percentages (%). Proportions were compared using the Chi Square Test (X2) and quantitative values were compared using the Mann Whitney U-Test. All statistical analyses were performed at a level of 95%, considering a p-value of ≤ 0.05 as statistically significant.

RESULTS

Of the total 248 clinically evaluated subjects, 138 (55.6%) were female, and 110 (44.4%) were male. The minimum age was 18 and the maximum was 86 years, with mean value 42.48 years (SD ± 16.55). In relation to risk factors, tobacco use was reported by 26.2 % of the examined subjects, of whom 75.4% were cigarette smokers. Other types of tobacco consumption were "hookah", which is a mixture of tobacco and herbs smoked in a pipe, cigars ("puros") and pipe (Fig. 1). Only 44 subjects answered the question on how long they had been using tobacco, and an average of 21.56 years was recorded (SD ± 15.80).

Alcohol consumption was reported by 63.7% of subjects, beer being the most popular drink, followed by rum, wine, whisky and "aguardiente"; all of them reported drinking at least once a week (Fig. 2).

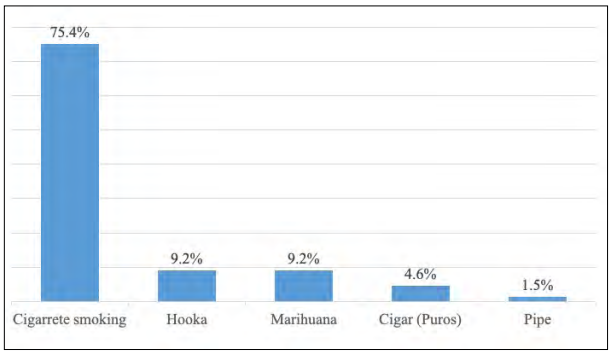

Fig. 1: Distribution of different forms of tobacco consumption.

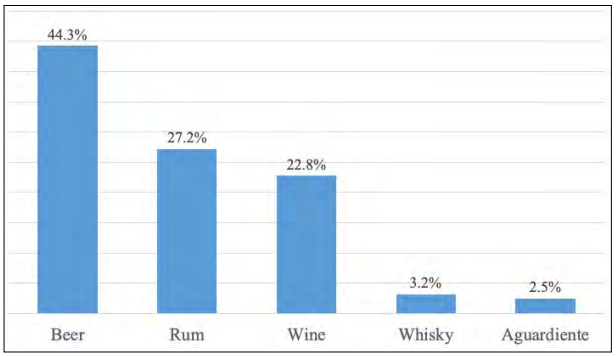

Fig. 2: Distribution of different forms of alcohol consumption.

Use of removable dentures/ prosthesis was reported by 1.3% of the study population.

Table 1 shows the distribution of the evaluated subjects according to the geographical location of the communities within Santo Domingo area. The distribution was homogeneous, so all regions were equally represented.

Table 1. Distribution of evaluated subjects according to geographical location in Santo Domingo metropolitan area.

Santo Domingo Communities	Number of Subjects	Frequency (%)
Mejoramiento Social	41	16.5
San Gerónimo	38	15.3
La Zurza	38	15.3
Miramar	29	11.7
Los Guandules	27	10.9
Arroyo Hondo	27	10.9
Los Ríos	26	10.5
Bella Vista	22	8.9
Total	248	100.0

Source: Santo Domingo communities, Dominican Republic

A total 228 out of the 248 evaluated subjects (91.9%) exhibited 1 or more OMLs, with a range of 1 to 7 lesions per patient and a mean of 3 lesions (Table 2). Physiologic melanin pigmentation was the most frequently found entity, followed by palatal / mandibular tori. Potentially malignant disorders detected were Oral Leukoplakia, Oral Lichen Planus and Actinic Cheilitis, corresponding to 2.2 %, 0.3 % and 0.3 %, respectively, of the total lesions detected (Table 3). Fig. 3 shows examples of clinical features of the diagnosed lesions.

Regarding the anatomical site of the OMLs, mandibular gingiva was the most frequent site, followed by maxillary gingiva, oral mucosa, hard palate and tongue (Table 4). When OMLs clinical

Table 2. Number of lesions per patient.

Number of lesions	Number of subjects	Frequency (%)
0	20	8.1
1	38	15.3
2	70	28.2
3	77	31.0
4	26	10.5
5	12	4.8
6	4	1.6
7	1	0.4
Total	248	100.0

Table 3. Distribution of oral lesions according to clinical type

Clinical diagnosis	n	%*	Clinical diagnosis	n	%*
Physiologic melanin Pigmentation	151	25.0%	Papilloma	2	0.3%
Palatal/mandibular Tori	122	20.2%	Dento alveolar abscess	2	0.3%
Fordyce Granules	48	7.9%	Mucocele	2	0.3%
Exostosis	34	5.6%	Lichen planus	2	0.3%
Denture Stomatitis	17	2.8%	Cheilitis	2	0.3%
Nicotine Stomatitis	15	2.5%	Commissural Pits	2	0.3%
Periodontal Disease	14	2.3%	Multifocal Epithelial Hyperplasia	2	0.3%
Pericoronitis	13	2.2%	Actinic Cheilitis	2	0.3%
Smoker Melanosis	13	2.2%	Peripheral Fibroma	2	0.3%
Leukoedema	13	2.2%	Benign Fibro-osseous lesion	2	0.3%
Lingual Varicosities	13	2.2%	Occlusal line	2	0.3%
Leukoplakia	13	2.2%	Piercing associated lesion	2	0.3%
GeographicTongue(Migratory Glossitis)	11	1.8%	Indented Tongue	2	0.3%
Melanotic Macule	10	1.7%	Recurrent Aphthous Stomatitis	2	0.3%
Frictional keratosis	9	1.5%	Circumvallate hyperplastic papillae	2	0.3%
Traumatic Ulcer	9	1.5%	Sialadenitis (Labial)	1	0.2%
Fissured Tongue	9	1.5%	Papillary inflammatory hiperplasia	1	0.2%
Inflammatory Fibrous Hyperplasia	7	1.2%	Black hairy Tongue	1	0.2%
Morsicatio Buccarum	6	1.0%	Verruga vulgaris	1	0.2%
Traumatic Fibroma	6	1.0%	Mandibular Atrophy	1	0.2%
Frenum Tag	5	0.8%	Hemangioma	1	0.2%
Pseudomembranous Candidiasis	5	0.8%	Desquamative Cheilitis	1	0.2%
Ankyloglossia	5	0.8%	Lichenoid oral reaction	1	0.2%
Coated Tongue	4	0.7%	Tonsillitis	1	0.2%
Commissural Cheilitis	4	0.7%	Nevus	1	0.2%
Pyogenic Granuloma	3	0.5%	Ranula	1	0.2%
Enamel Hypoplasia	3	0.5%	Foliate Papillitis	1	0.2%
Total				604	100.0%

*Based on the total number of lesions observed.

Fig. 3: Oral mucosal lesions in the study population. a) Smoker Melanosis. b) Physiologic Melanin Pigmentation. c) Oral Lichen Planus. d) Oral Leukoplakia. e) Actinic Cheilitis. f) Mandibular Exostosis. g) Inflammatory Papillary Hyperplasia. h) Benign Migratory Glossitis (geographic tongue).

Table 4. Anatomical sites of oral mucosal lesions

Anatomic Location	number of lesions	%*
Mandibular Gingiva	170	28.1%
Maxillary Gingiva	163	27.0%
Buccal Mucosa	80	13.2%
Tongue	43	7.1%
Hard Palate	33	5.5%
Lower Lip	27	4.5%
Upper Lip	24	4.0%
Mandibular Retromolar	21	3.5%
Soft Palate	10	1.7%
Lower vestibule	8	1.3%
Maxillary vestibule	6	1.0%
Lingual frenum	4	0.7%
Floor of the mouth	4	0.7%
Upper labial frenum	3	0.5%
Vermillion	3	0.5%
Lower labial sulcus	1	0.2%
Maxillary retromolar	1	0.2%
Anterior pillar	1	0.2%
Uvula	1	0.2%
Posterior pillar	1	0.2%
Total	604	100.0%

diagnosis was correlated with anatomical sites, Oral Leukoplakia and buccal sulcus, on both upper and lower jaws, were positively associated ($p \leq 0.05$). Additionally, other positive associations were found among subjects in the non-pathological conditions group: physiologic melanin pigmentation associated to maxillary and mandibular gingiva, exostosis associated to maxillary gingiva, Fordyce granules associated to oral mucosa and upper lip, and Palatal Torus associated to hard palate, all showing statistically significant association ($p \leq 0.05$).

DISCUSSION

The current study revealed that tobacco in general was as a risk factor in 26.2% of the study population, with cigarette smoking being the most common form. Interestingly, other forms of tobacco use such as smokeless tobacco, documented in previous reports from the literature and related to oral lesions in other countries, especially in the Asian subcontinent (Mehrota *et al.*[3]), were not recorded in the eight communities examined in Santo Domingo. In addition, the practice of "inverted" cigarette smoking, which is relatively common in some Latin American countries, specifically in South America, Venezuela and Colombia[10], was not observed in this Caribbean population either. The present study results regarding tobacco use as a risk factor are similar to those of previous investigations in Kuwait and India[1,3] and contrary to Ottapura *et al.*[8], who reported high prevalence of smokeless tobacco use. Another risk factor considered in this study was alcohol consumption. Beer was found to be the commonest form used on a weekly basis. Other alcohol forms included rum, wine, whisky and, less frequently, "aguardiente". Prinyanka *et al.*[7] analyzed

the prevalence of OMLs in alcohol-dependent and non-alcohol dependent subjects, finding a 31.5% increased risk of oral lesions in alcohol dependent subjects. Among potentially malignant oral lesions in the Prinyanka study, oral leukoplakia was the most frequently observed, followed by Submucous fibrosis, Eritroplakia and Candidiasis. In the present study, Oral Leukoplakia was also the most frequently observed potentially malignant lesion. Oral lichen planus (OLP) and Actinic Cheilitis were also observed. Regarding the clinical observation of Oral Lichen Planus, only the clinical reticular variant of OLP of bilateral occurrence on the oral mucosa was found.

In our study, the age range of patients with OMLs was 18 to 86 years, with mean age 42.48. Other prevalence studies on OMLs have reported a broader age range, including children and adolescents[1,5]. Rivera *et al.*[6] documented higher occurrence of OMLs in an elderly Chilean population. Mujica and Rivera[11] studied a 60- to 74-year-old group of institutionalized patients, finding OMLs mainly associated to trauma caused by dentures.

Raposo *et al.*[12] documented the prevalence of OMLs at a reference hospital in Temuco, Chile, in 300 patients over 20 years of age, finding frequencies of Fordyce granules 30%; Atrophic candidiasis 14.33%; Melanotic macule 13.67%; Lingual varicosities 7.33%; Physiologic pigmentation 6%; Pigmented nevus 4%; Ephelides 3.33%; Traumatic ulcers 4%; Oral leukoplakia 3% and Angular cheilitis 2.68%. These results agree with the present study, where non-pathological conditions were the most prevalent. These authors also showed a statistically significant association between increasing age and the presence of Atrophic candidiasis, Traumatic ulcers and Lingual varicosities. For gender distribution, our study found a slight female preponderance. This is similar to a previous report by Casnati *et al.*[13] which analyzed an urban adult population of Uruguay. These authors also report a correlation of Oral Leukoplakia with "yerba mate" consumption, a common practice in some South American countries such as Uruguay and Argentina. This specific type of consumption was not observed in the evaluated Dominican population.

In the present study, the predominant location observed was on mandibular gingiva, followed by maxillary gingiva, oral mucosa and hard palate as the commonest sites. This particular finding could be related to the presence of Physiological melanin pigmentation, a relatively common condition observed in the study population due to ethnic factors. In contrast, another study by Mehrota *et al.*[3] reported other anatomical sites such as oral mucosa as being the most frequent location for OMLs.

Non-pathological conditions such as Physiologic melanin pigmentation, palatal/mandibular tori and Fordyce granules were more frequent than pathological lesions. Among the latter, those associated to the use of dentures, as Denture Stomatitis, Smoker's palate (Nicotine Stomatitis) and Inflammatory conditions such as plaque-related gingivitis and pericoronitis were the most frequently found. Among the potentially malignant disorders, Oral Leukoplakia was the most frequent lesion, followed by Oral Lichen planus and Actinic cheilitis. It is worth noting that neither Oral Squamous Cell Carcinoma, the most common form of oral cancer, nor other forms of malignancies were detected in this investigation. A low frequency of oral cancer was also detected in other populations. Kansky *et al.*[14] documented only 9 cases of Oral Cancer in a sample of 2395 people in Slovenia. The fact that no evidence of malignancy was observed in this oral screening in the Dominican Republic deserves further studies, including evaluation of numerous risk factors as well as genetic and nutritional conditions.

When the OMLs were correlated with different anatomical sites, Oral Leukoplakia was positively associated to oral mucosa or sulcus ($p \leq 0.05$). Additionally, other significant positive associations were observed in the category of non-pathological conditions, such as between Benign migratory glossitis and tongue, physiological melanin pigmentation and maxillary and mandibular gingiva, exostosis and maxillary gingiva, Fordyce granules and oral mucosa and upper lip, Palatal Torus and hard palate. These results agree with those previously reported by Bhatnagar *et al.*[15] on the frequency of non-pathological alterations.

The use of dental prostheses is associated with an increase in OMLs. In our study, the proportion of patients with prostheses was low (1.3%), nevertheless, some reactive inflammatory lesions such as Fibrous Hyperplasia, Denture Stomatitis and Inflammatory Papillary Hyperplasia were detected. Our findings agree with previous studies by Yin *et al.*[16] in an oral survey from the Sichuan Province, China, where the incidence of dental prosthesis

use was 51.75%, with high prevalence of recurrent aphthous ulcers, oral lichen planus, Inflammatory Papillary Hyperplasia and Fibrous Hyperplasia.

The limitations of the present study should be acknowledged: it did not analyze socioeconomic level, which includes defined criteria of classification and not only income of the participant population, as well as nutritional factors, including validation of a food frequency questionnaire and anthropometric evaluation. Further studies on oral cancer screening and early detection should be implemented in this population. Because no data on oral cancer frequency have been reported previously in this population, no comparison could be made.

To the best of our knowledge, there are no preliminary published data on the epidemiological evaluation of OMLs in adults from Dominican Republic. This is the first screening–based research contributing to the understanding of the prevalence and severity of OMLs in the Dominican Republic and identifying risk factors in this population. The present study also provides baseline data for future studies for improving oral health in the country. Further screening studies in this population should include diet, nutrition, and socioeconomic factors that may influence the presence of OMLs.

Another advantage of this type of study is that it provides an exceptional opportunity for dentists to educate patients on the link between smoking and oral potentially malignant lesions.

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Paget's disease of the jaws: Histopathological features of a series of 31 cases

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ABSTRACT

The aim of the present study was to analyze the histopathological features of Paget's disease of the jaws observed in a series comprising 31 cases. The study comprised all cases of Paget's disease of the jaws filed in the archives of the Surgical Pathology Laboratory of the Oral Pathology Department, School of Dentistry, University of Buenos Aires, between 1960 and 2018. Their microscopic features were evaluated, and available clinical data and radiographic studies were analyzed. Paget's disease of the jaws accounted for 0.05% of retrieved oral-maxillofacial pathologies. Microscopically, all cases showed lamellar bone trabeculae with the characteristic mosaic pattern. Twenty cases (64%) showed osteoblastic-osteoclastic activity, and all showed areas of necrosis. Cemento-osseous trabeculae were observed in 15 cases (48%), and cementicles were observed in 13 (42%). Osteomyelitis was seen in 11 cases (35%), all of which showed cemento-osseous trabeculae with a mosaic structure, sclerosis and necrosis, and chronic inflammation with abscess formation. Mean age was 61 years (44-85 years); 19 cases were women. Localization was the maxilla in 13 cases (42%), and the disease involved other skeletal bones in five cases. To our knowledge, this is the largest series of Paget's disease of the jaws reported to date. Paget's disease is infrequent in the jaws and has distinct histopathological features that not only differ from those observed at other skeletal sites but also require differential diagnosis from other pathologies affecting the jaws exclusively.

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Keywords: *Paget's disease - diseases of bone - diseases of jaws.*

Enfermedad de Paget en los maxilares. Características histopatológicas de una serie de 31 casos

RESUMEN

El objetivo del presente trabajo fue analizar las características histopatológicas de la enfermedad de Paget en los maxilares en una serie de 31 casos. El estudio comprendió todos los casos de enfermedad de Paget de los maxilares provenientes del Laboratorio de Patología Quirúrgica de la Cátedra de Anatomía Patológica de la Facultad de Odontología de la Universidad de Buenos Aires, entre 1960 y 2018. Se evaluaron las características microscópicas y se analizaron los datos clínicos y estudios radiográficos disponibles. La enfermedad de Paget en los maxilares representó el 0,05% de las patologías buco-maxilofaciales. Microscópicamente, todos los casos mostraron trabéculas óseas laminares con el característico patrón en mosaico. Veinte casos (64%) mostraron actividad osteoblástica-clástica y todos los casos mostraron necrosis focal. En 15 casos (48%) las trabéculas presentaron aspecto cemento-óseo y en 13 casos (42%) se observaron cementículos. Once casos (35%) presentaron cuadros osteomielíticos y todos ellos mostraron trabéculas cemento-óseas con estructura en mosaico, esclerosis y necrosis, e infiltrado inflamatorio crónico con formación de abscesos. La media de edad fue 61 años (44-85 años), y 19 fueron mujeres. Trece casos (42%) se localizaron en maxilar superior y 5 casos presentaron compromiso de otros huesos. A nuestro entender, esta es la serie más grande de enfermedad de Paget en los maxilares reportada hasta la fecha. La enfermedad de Paget es poco frecuente en los maxilares y presenta características histopatológicas que además de ser diferentes a las observadas en otros sitios del esqueleto plantean el diagnóstico diferencial con otras entidades que se presentan exclusivamente en los maxilares.

Palabras clave: *enfermedad de Paget - lesiones óseas - lesiones de los maxilares.*

In memory of our Professors and Mentors Rómulo Luis Cabrini and Eduardo H. Santini-Araujo.

INTRODUCTION

Paget's disease of bone (PDB) is a chronic disease characterized by abnormal bone turnover affecting one or more bones of the skeleton[1-3]. It was first described in 1877 by Sir James Paget who named it osteoitis deformans[4]. It occurs in patients over the age of 55 years and is more prevalent in men (M:F ratio: 2:1)[5,6].

PDB has an unusual geographic distribution, with an increased incidence in the United Kingdom, Germany, France, New Zealand, and Australia[6,7], and being less common in China, Japan, India, Southeast Asia and America[8-13]. The incidence and severity of Paget's disease has decreased over the last 35 years[14-17].

Although the etiology of the disease remains unclear, association with different underlying conditions has been suggested. The familial occurrence of PDB has prompted genetic studies, which have reported a mutation in the gene SQSTM1[18,19]. In turn, the decrease in the incidence of the disease has brought about the search for environmental factors[14,18-21]. A possible viral etiology has also been suggested based on evidence of measles virus nucleocapsid protein in pagetic osteoclasts[22,23].

Paget's disease is asymptomatic in the early stages of the disease, in which osteoclastic activity predominates, subsequently causing an increase in osteoblastic activity that leads to sclerosis and bone deformity. Signs and symptoms vary with progression of the disease according to the affected skeletal site. The most common presenting features are pain, deafness, bone deformities, osteoarthritis, pathological fracture, and, though very rarely, malignant transformation[6,24-28].

When the disease involves the jaws, it occurs predominantly in the maxilla, which undergoes progressive enlargement causing displacement of the teeth and malocclusion[29,30]. With time, the disproportion in the size between the maxilla and the mandible gives rise to the inverted triangle type of facial contour[3,30].

Regardless of the affected skeletal site, the microscopic features of PDB vary throughout the course of the disease. In the early phase, active bone resorption predominates, showing abnormally large osteoclasts containing multiple nuclei. The haversian canals enlarge and coalesce, and the bone marrow shows highly vascularized loose fibrous tissue[6,30]. The intermediate stages of the disease are characterized by dysregulated bone remodeling in which active osteoblastic activity coexists with osteoclastic activity, leading to apposition of new bone on the surfaces of the previously resorbed trabeculae. Uncoordinated bone resorption and apposition results an anarchic trabecular structure and altered mechanical properties. Finally, the last phase of Paget's disease is characterized by sclerotic lamellar bone trabeculae with multiple compartments separated by irregularly arranged incremental and reversal lines, resulting in the characteristic microscopic appearance of PDB with the typical mosaic or jigsaw puzzle pattern[2,6,31].

In addition to the histologic characteristics described above, Paget's disease of the jaws shows specific features. In the osteolytic phase, varying degrees of tooth mobility and ultimately tooth loss may occur[3,29-31]. In the intermediate phase, hypercementosis with ankylosis of teeth to the adjacent bone and the presence of spherical structures similar to psammoma bodies can be observed. In the later stages of the disease, these spherical structures fuse and a fibrillar structure can be seen on their surface[29-31].

Radiographic features vary widely with the stage of the disease and osteoclastic/osteoblastic activity. A radiodense image with a cotton wool appearance is typically seen in the intermediate and late phases[29,31,32-36].

Diagnosis is mainly based on radiographic studies, bone scintigraphy, and evaluation of serum bone turnover markers. A biopsy is nevertheless necessary not only to confirm diagnosis, especially in controversial cases where differential diagnosis from other diseases must be established, but also for a better understanding of the biological behavior of the disease.

The main differential diagnoses of Paget's disease of the jaws include cemento-osseous dysplasia[31,32], chronic sclerosing osteomyelitis[31] and medication-related osteonecrosis of the jaws[36]. In view of the increasing incidence of the latter, a complex and emerging pathological condition, it is highly relevant to gain further knowledge about pathological entities with similar morphologic characteristics, as is the case of Paget's disease of the jaws [37,38].

The aim of the present study was to perform a detailed analysis of the histopathological features of Paget's disease of the jaws observed in a series comprising 31 cases. To our knowledge, this is the

largest series of Paget's disease of the jaws reported to date.

MATERIALS AND METHODS

The study projet was approved by the Ethics Committee of the School of Dentistry of the University of Buenos Aires (# 20020170200308BA). The study comprised all cases of Paget's disease of the jaws (n=31) filed in the archives of the Surgical Pathology Laboratory of the Oral Pathology Department, School of Dentistry, University of Buenos Aires, between 1960 and 2018. The microscopic features of the hematoxylin-eosin-stained histological sections were analyzed and recorded, and the clinical and radiographic data available on the filed records were examined.

RESULTS

The 31 retrieved cases of Paget's disease of the jaws accounted for 0.05% of all oral-maxillofacial pathologies diagnosed at our laboratory in the studied period (58 years).

Mean age was 61 years, age range 44 to 85 years, and frequency peaked in the sixth decade of life. Nineteen cases (61%) were women. The most frequently affected site was the maxilla (13 cases). No information regarding mandibular or maxillary location was available in seven cases, and the disease involved other skeletal bones (polyostotic form) in five cases.

Microscopically, all cases showed the typical trabecular structure: lamellar trabeculae with a mosaic pattern containing multiple compartments. The observed compartments were mostly demarcated by resorption lines (Fig. 1A). Trabeculae were markedly thicker in 24 cases (77%). Twenty cases (64%) showed osteoblastic and osteoclastic activity (Fig. 1B) and 11 cases (35%) showed no cellular activity (Fig.1C). All cases exhibited areas of necrosis evidenced by the absence of osteocytes inside lacunae. The medullary spaces were occupied by fibrous tissue, which was mostly loose and well vascularized. The observed microscopic features were consistent with Paget's disease of bone. The

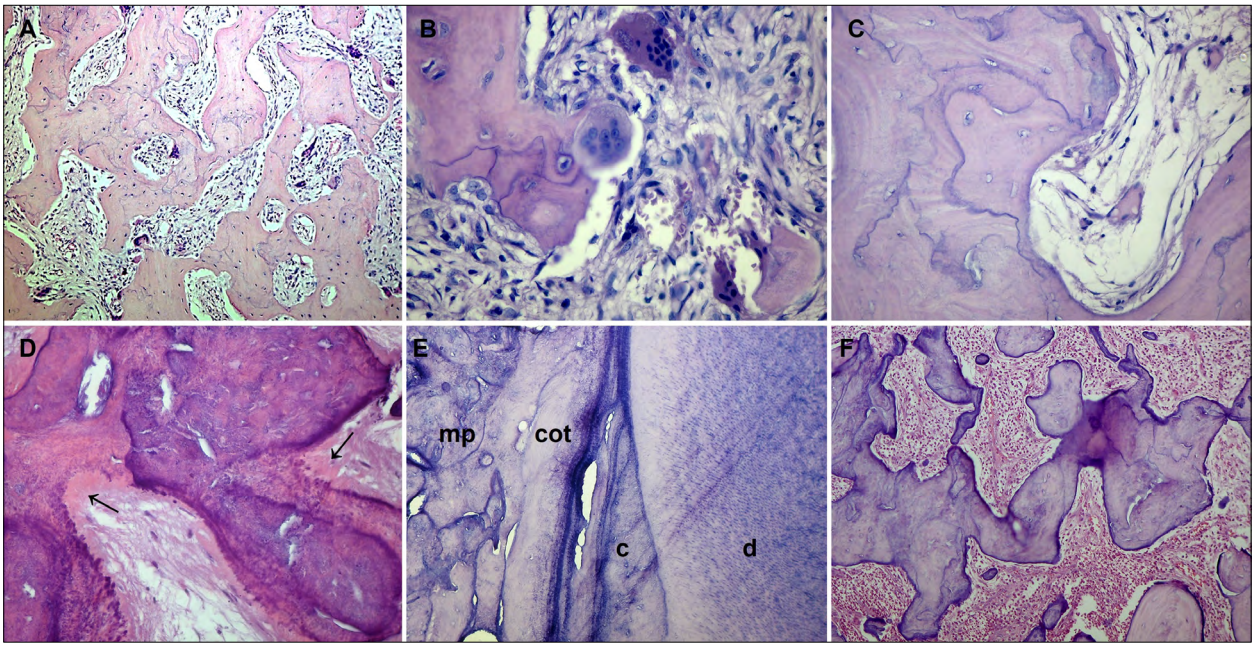

Fig. 1: Photomicrographs of Paget's disease of the jaws.
A. Lamellar bone trabeculae showing a mosaic pattern, and fibrous bone marrow. H-E Orig. Mag. X100.
B. Osteoblasts and osteoclasts on the surface of the bone trabecula. H-E Orig. Mag. X400.
C. Absence of cells on the surface of the trabecula. H-E Orig. Mag. X400.
D. Cemento-osseous trabeculae with a fibrillar surface (↑). H-E Orig. Mag. X400.
E. Tooth root exhibiting hypercementosis and ankylosis. d: dentine ; c: cement; cot: cemento-osseous trabecula; mp: Mosaic pattern. H-E Orig. Mag. X400
F. Necrotic cemento-osseous trabeculae with multiple compartments and medullary spaces occupied by an inflammatory process. H-E Orig. Mag. X100.

occurrence of cemento-osseous trabeculae in 15 cases (48%) was a remarkable finding. The latter trabeculae were mineralized structures arranged in the typical mosaic pattern but had rounded and scalloped surfaces with less cellularity, resembling cementum. Some sectors of the surface exhibited fibrillar structures as those described by Cooke[30] (Fig. 1D). Cementicles were observed in 13 cases (42%). The involved tooth was submitted for histopathological examination in two cases, and both submitted teeth were found to exhibit cementum apposition resulting in hypercementosis and ankylosis with the typical mosaic pattern (Fig. 1E). Osteomyelitis was observed in 11 cases (35%), which were clinically and radiographically diagnosed as osteomyelitis associated with Paget's disease of the jaws. All the cases exhibited thick cemento-osseous trabeculae with multiple compartments and necrosis. An acute/chronic inflammatory process and microbial colonies were observed in the bone marrow (Fig.1F).

DISCUSSION

Few case series of Paget's disease of the jaws have been reported in the literature to date; all comprised a small number of cases and were mostly published in the 80's[29,33-35]. In 2012, Werner de Castro *et al.* published a series of 134 cases of Paget's disease of bone, which included only five cases located in the jaws[36].

There is no available information on the incidence and frequency of Paget's disease of the jaws in Argentina. We herein report 31 cases of Paget's disease of the jaws diagnosed at a single referral center within a period of 58 years, and that accounted for 0.05% of all oral maxillofacial pathologies filed in the laboratory archives. To our knowledge, this is the largest series of cases of this disease reported to date. Sixty-five of the cases in this series were diagnosed before 1980, confirming the decreasing trend in the incidence of the disease worldwide.

In addition to the typical histopathological characteristics of Paget's disease involving other bones of the skeleton, Paget's disease of the jaws shows formation of cemento-osseous trabeculae and cementicles. The latter histopathological feature could be misleading, and diagnosis can be confused with other dysplastic and neoplastic diseases affecting the jaws, such as cemento-osseous dysplasia, cementoblastoma, and ossifying cemento-fibroma. These pathologies affect the jaws exclusively; the former is dysplastic, the latter two are tumor lesions of odontogenic origin, and all threes show formation of cemento-osseous trabeculae with a paget-like pattern.

Paget's disease of bone is infrequent in the jaws. Most studies on this pathological entity reported the clinical and radiographic features, but few analyzed the specific histopathology and possible etiopathogenesis of the disease in this location[29-31].

Rushton (1938) was the first author to report hypercementosis of the teeth in Paget's disease of the jaws[29]. Lucas (1955) pointed out that the observed hypercementosis could be differentiated from hypercementosis related with other causes by the absence of periodontal ligament and lamina dura; the author also found that hypercementosis becomes evident in the later stages of the disease[30]. Cook (1956) analyzed five teeth with root hypercementosis from patients with Paget's disease of the jaws, and observed marked cellularity in the newly-formed cementum, which in turn showed signs of remodeling in a mosaic pattern similar to that described in bone tissue. The author also observed nodules of bone fused together by woven bone that had a fibrillar structure on its surface, at right angles to the central nodules[29].

In our case series, 15 cases (48%) exhibited cemento-osseous trabeculae with marked signs of remodeling in a mosaic pattern, and in line with the aforementioned authors, showed a fibrillar pattern on the surface of the trabeculae. Thirteen cases (42%) showed cementicles, and the two cases submitted with the involved teeth for histopathological evaluation exhibited hypercementosis with tooth ankylosis and the typical mosaic pattern.

It is known that formation of Paget-like trabeculae increases risk of infection. Eleven cases of Paget's disease of the jaws in our series (36%) showed osteomyelitis, and their microscopic features were similar to those reported in bisphosphonate-related osteonecrosis of the jaws. Our research group found the Paget-like trabecular structure containing multiple compartments to be a distinctive microscopic feature of bisphosphonate-related osteonecrosis[37-38]. Based on this observation, we have posited the existence of an etiopathogenic mechanism that might also explain the necrosis and inflammation seen in Paget's disease of the jaws. Bisphosphonates induce new bone formation and

are therefore used in oncological treatment to limit metastasis-related bone destruction and to treat different bone metabolism alterations. Similarly, a mechanism that remains unknown to date would cause the chaotic bone architecture found in Paget's disease, favoring necrosis and the subsequent inflammation and invasion of oral microorganisms into the bone.

Of note, we observed a subtle difference between the mosaic pattern of trabeculae in Paget's disease of the jaws and the Paget-like structure of trabeculae in bisphosphonate-related osteonecrosis of the jaws: in the latter condition the cement lines are regular and linear, following the laminar arrangement of trabeculae, whereas in Paget's disease of the jaws, they show a scalloped appearance indicating reabsorption. Although diagnosis of both pathological entities is defined based on clinical and biochemical data, in-depth analysis of their microscopic features could contribute to differential diagnosis and provide a better understanding of the biological behavior and pathogenesis of these complex diseases.

All the cases of Paget's disease of the jaws presenting osteomyelitis showed cemento-osseous trabeculae, which were thicker and contained multiple compartments. Formation of cemento-osseous trabeculae is likely reactive to inflammation, and these trabeculae probably have little or no repair capacity.

It must be pointed out that when bone pathologies are in the jaws, they usually have distinct characteristics that are different from those observed when they are located in other bones of the skeleton. This is especially evident, for example, in osteosarcoma of the jaws and medication-related osteonecrosis of the jaws, as well as in Paget's disease of the jaws. These differences could be attributed to the greater remodeling activity of the jaws due to stimuli of the masticatory forces and to the persistence of and/or coexistence with tissues that have the potential to form cementum-like and dentin-like structures.

DECLARATION OF CONFLICTING INTERESTS

The authors declare no potential conflicts of interest regarding the research, authorship, and/or publication of this article

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Trigeminal nerve injuries. Four years' experience at a single Argentine referral center and a literature review

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ABSTRACT
The aim of this retrospective study was to describe the etiology and characteristics of trigeminal nerve injuries referred to a specialized center in Buenos Aires, Argentina. A retrospective analysis was performed of patients referred from February 2016 to January 2020. Age, sex, intervention performed, nerve affected, time elapsed from injury, diagnosis, location, and whether patient had signed informed consent were recorded. A descriptive analysis of the data was made, and 95% confidence intervals were calculated for prevalence. The study sample consisted of 30 subjects (31 nerve injuries), 19 female and 11 male, average age (±SD) 40 ± 17 years. The inferior alveolar nerve was the most frequently injured nerve (74%,) while the lingual nerve accounted for 26%. The most common etiologies were inferior molar extractions (47%), dental implants (20%), and local anesthesia (13%). Other etiologies were autologous mandibular bone grafts for dental implants, removal of cysts associated with the inferior third molar, and endodontic treatment. Dental Institutions at which treatment was provided were found to be significantly associated with patients being warned and asked to sign informed consent ($p<0.05$), while dentists working at private offices requested fewer consents. The most frequent symptom was paresthesia, and 5 patients suffered spontaneous or evoked pain. Only 2 patients intended to file legal claims. Dentists should be aware of the debilitating effects resulting from trigeminal injuries, the complexity of their resolution and the importance of carefully planning dental procedures to prevent them.

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Keywords: *trigeminal nerve injuries - trigeminal nerve - mandibular nerve - inferior alveolar nerve - lingual nerve.*

Lesiones del nervio trigémino. Cuatro años de experiencia de un servicio quirúrgico odontológico en Argentina y revisión bibliográfica

RESUMEN
El objetivo de este estudio fue describir la etiología y características de las lesiones del nervio trigémino remitidas a un servicio de referencia especializado en Buenos Aires, Argentina. Se realizó un análisis retrospectivo de los pacientes remitidos desde febrero de 2016 a enero de 2020. Se registraron edad, género, intervención recibida, nervio afectado, tiempo transcurrido desde la lesión, diagnóstico, ubicación y firma del consentimiento informado previo a la intervención Se realizó un análisis descriptivo de los datos y se calcularon intervalos de confianza del 95%. La muestra del estudio consistió en 30 sujetos (31 lesiones nerviosas), 19 mujeres y 11 hombres, con una edad promedio (± DE) de 40 ± 17 años. Aproximadamente 3 de cada 4 lesiones correspondieron al nervio alveolar inferior, representando el resto al nervio lingual. Las etiologías más frecuentes fueron la extracción dentaria (47%), los implantes dentales (20%) y la aplicación anestesia local (13%). Otras etiologías fueron la regeneración ósea para la colocación de implantes mandibulares, la extirpación de quistes asociados al tercer molar inferior y el tratamiento endodóntico. Se encontró que el tipo de establecimiento donde se realizó el procedimiento odontológico que generó la lesión, se asoció significativamente con los pacientes a los que se les advirtió y se les pidió que firmen el consentimiento informado ($p<0.05$); los odontólogos que trabajan en consultorios privados obtienen una menor proporción de consentimientos que los de las instituciones. El síntoma más frecuente fue la parestesia y 5 pacientes sufrieron dolor espontáneo o evocado. Solo 2 pacientes tenían intención de iniciar acciones legales. Teniendo en cuenta que son lesiones potencialmente permanentes, y de resolución compleja, la comunidad odontológica debe realizar especiales esfuerzos para disminuir esta complicación.

Palabras clave: *lesión nerviosa - nervio trigémino - nervio mandibular - nervio alveolar inferior - nervio lingual.*

INTRODUCTION

Sensory deficiencies of the trigeminal nerve manifest as numbness, lack of sensation, increased sensitivity, or even pain, with dental treatment being the most frequent etiology. Altered sensation and pain may interfere with speaking, eating, kissing, shaving, applying makeup, tooth brushing and drinking. Cases of depression, suicidal thoughts, self-biting, dribbling, and retention of food on the chin in public have also been observed[1,2]. Trigeminal nerve injuries can be caused by extraction of inferior third molars, dental implant surgery, ostectomy, incision, orthognathic surgery, maxillofacial trauma, surgery for oral pathology, edema and post-surgical infection, and even anesthetic injection[3-6]. The highest prevalence of trigeminal nerve injuries in oral surgery are the terminal branches of the mandibular nerve, the inferior alveolar nerve (IAN) and its mental branch, and the lingual nerve (LN)[7]. LN injury generates the most severe symptoms[8] and may affect the sense of taste[2]. In addition to the patient suffering, the professional becomes emotionally and legally involved. Medical-legal implications are another concern that has increased in recent years, probably due to an increase in dental implant surgery and endodontic therapy[9].

The aims of this study were to analyze the etiology and characteristics of trigeminal nerve injuries that were referred to an Oral and Maxillofacial Surgery Department in Buenos Aires, Argentina and compare them to reports in the literature.

MATERIALS AND METHODS

A retrospective analysis was conducted of patients referred to the Oral and Maxillofacial Surgery Department of the School of Dentistry of Buenos Aires University, Argentina, from February 2016 to January 2020. A total 30 patients with trigeminal nerve injuries were referred during this period. Referrals came mainly from dental and medical healthcare providers in Buenos Aires City and surroundings. Patients provided informed consent authorizing use of information without personal identification. The project was approved by the Ethics Committee of the School of Dentistry of Buenos Aires University (CETICA.FOUBA 002/2021). Clinical and image evaluations were performed. Age, sex, reason for consultation, nerve affected, time elapsed from injury to consultation, type of dental care facility (private office or dental institution where the dental procedure that caused the lesion was performed), description of the intervention, and medication provided were analyzed. Patients were asked whether they had been warned in advance regarding possible complications in the procedure, and whether they had been asked to sign informed consent. Pinprick testing and mapping of the affected zone, thermal stimulation, directional discrimination, two-point discrimination and subjective evaluation by the patient (including pain) were recorded.

Immediate treatment consisted of removal of the etiological agent[10] (1 case); medication only for the first week after injury with corticosteroids (dexamethasone 8 mg per day for 3 days, 4mg per day for 3 days, 1mg per day for 3 days), NSAIDs (ibuprofen 400mg per 8 hours for 21 days)[11-13] (10 cases). Other treatments consisted of low-frequency laser sessions when symptoms did not improve[14] (8 cases), or administration of carbamazepine when symptoms interfered with everyday activities (2 cases). In addition, neurological and psychological consultations were suggested.

The following numerical variables were described: mean, standard deviation (SD), median, minimum (Min), maximum (Max), first quartile (Q1) and third quartile (Q3). Categorical data were described using absolute frequencies (AF) and percentages with 95% confidence intervals (95% CI). The CI95 were estimated using the score method (Newcombe & Merino Soto, 2006). For the comparison of frequencies, Chi-square or Fisher's exact test were used, with a significance level of 5%. The following software was used: Calc, from Apache OpenOfficeTM v. 4.1.6 (Apache Software Foundation, 2018), Infostat v. 2020 (Di Rienzo et al., 2020) and MedCalc v. 19.2.6 (MedCalc Software bvba, 2020).

For a review of the literature, Medline/PubMed was searched using the terms "trigeminal nerve injury" till August 2021. Articles reporting series of trigeminal nerve injury caused by dental procedures were selected.

RESULTS

Thirty patients were evaluated, 19 female (63%) and 11 male (37%) (ratio 1.7 to 1), average age 40 ± 17 years (median = 37, Q1-Q3 = 26-54). Time from injury to consultation ranged from immediately after the surgery to 3 years (median = 14 days, Q1-Q3 = 6-60; mean ± SD = 115 ± 282).

Injuries were generated at two types of dental care facilities: dental institutions and private offices. There were 15 cases from each (50%; 95% CI: 33% to 67%). Nineteen patients had been warned of the possibility of injury (63%; 95% CI: 46% to 78%), while 11 patients had not been warned (37%; 95% CI: 22% to 54%). A significant association was found between type of dental care facility and warning to the patient (Chi-square = 17; df = 1; $p<0.05$): while only 4 of 15 patients (27%) had been warned at the private offices, all 15 patients (100%) had been warned at the dental institutions. Regarding informed consent, 21 patients had been asked to sign prior to the intervention (70%; 95% CI: 52% to 83%), while 9 patients had not been asked sign (30%; 95% CI: 17% to 48%). A significant association was found between type of dental care facility and being asked to sign informed consent (Fisher's exact test: $p<0.05$): while 6 of 15 patients had been asked to sign at the private offices (40%), all 15 patients (100%) had been asked to sign at the dental institutions.

All injuries corresponded to the mandibular branch of the trigeminal nerve. IAN was affected in 22 cases and LN in 7 cases. One patient presented injury of both nerves after the extraction of an impacted mandibular third molar. IAN involvement was found to be significantly more frequent (Chi-square = 7.26; df = 1; $p<0.05$).

The most frequent cause of nerve injury was tooth extraction, which accounted for almost half the cases (Table 1). All these injuries corresponded to lower molars (11 third molar, 2 second molar, and 1 first molar). In one of the cases of extraction of the lower second molar, a conjunction with another complication occurred: a bisphosphonate-related osteonecrosis of the jaws (zoledronic acid). All LN injuries after extraction (6 cases) corresponded to lower third molar. Fig. 1 shows an example of a case of inferior alveolar nerve injury during extraction of an impacted lower third molar. The second most frequent etiology was mandibular dental implant placement. Four dental implant injuries were by direct trauma (Fig. 2) (1 first molar delayed placement, 1 second premolar delayed placement, 1 first premolar delayed placement, and 1 first premolar immediate placement); and 2 were by indirect trauma (Fig. 3) (1 first molar immediate placement, and 1 second molar delayed placement). One of the cases of nerve injury due to the injection of local anesthesia was caused by a buccal mandibular infiltration in the area of the mental foramen, while the other 3 were caused by the application of the inferior alveolar nerve block technique (Halstead approach) in the

Table 1. Distribution of cases according to etiology

Cause	AF	%	CI95
Tooth extraction	14	47	30 to 64
Dental Implant	6	20	10 to 37
Local Anesthesia	4	13	5 to 30
Mandibular bone graft	3	10	3 to 26
Cystic Pathology	2	7	2 to 21
Endodontics	1	3	1 to 17
Total	30	100	

Chi-square = 22.4; df = 5; $p < 0.05$
AF: absolute frequencies; CI95: 95% confidence interval

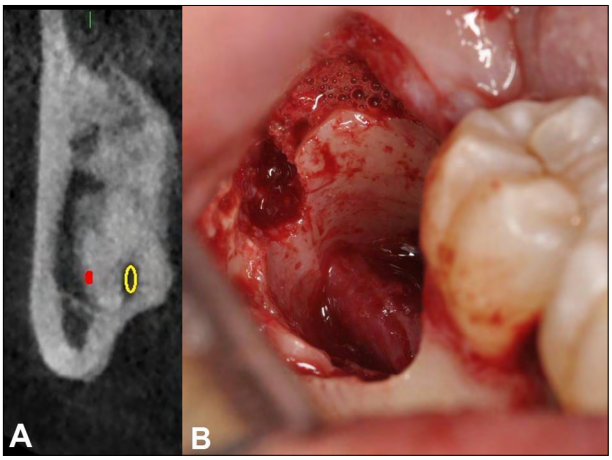

Fig. 1: Female patient, 45 years old, with inferior alveolar nerve injury due to extraction of lower third molar. A. CAT scan prior to extraction (yellow circle marks the lower alveolar canal). B. Clinical photograph showing inferior alveolar nerve.

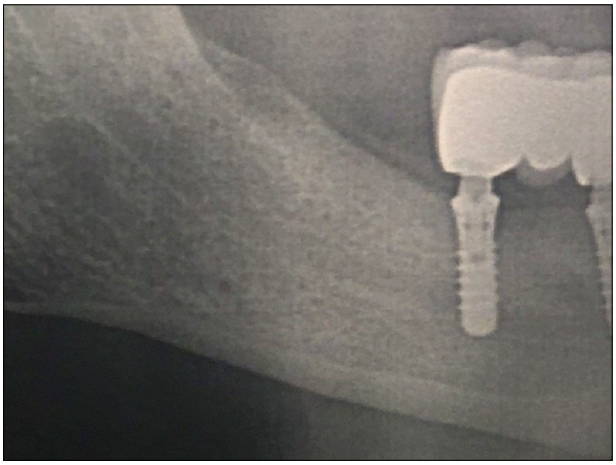

Fig. 2: Male patient, 65 years old, with inferior alveolar nerve injury. Radiograph showing direct trauma generated by the implant (lower first molar) in the inferior alveolar canal.

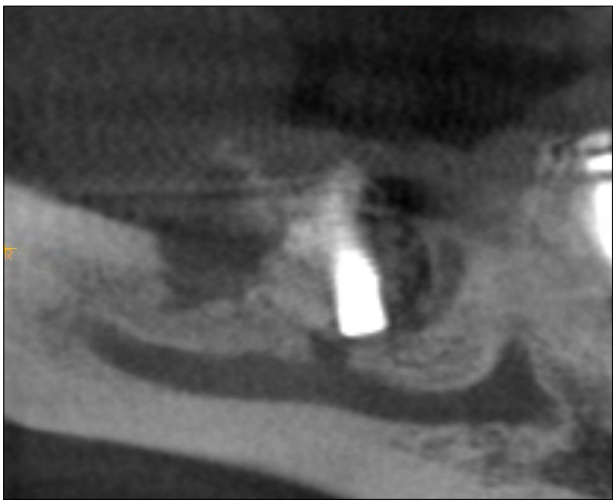

Fig 3: Female patient, 38 years old, with inferior alveolar nerve injury. CAT scan showing the indirect trauma generated by the implant (lower first molar) as a hypodense zone above the inferior alveolar canal.

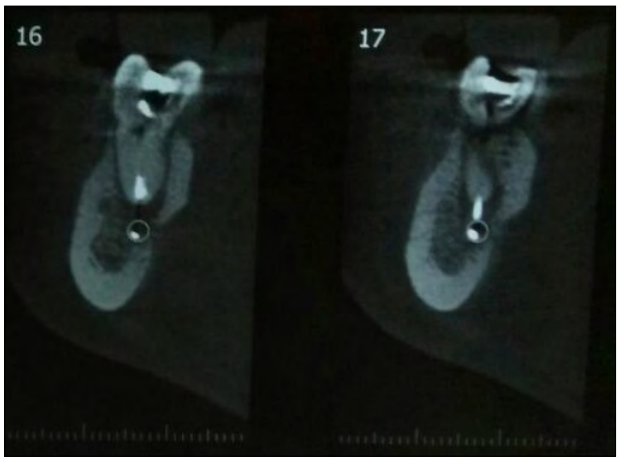

Fig. 4: Female patient, 56 years old, with inferior alveolar nerve injury. CAT scan taken after endodontic treatment, showing presence of filling material within the inferior alveolar canal (distal root of lower first molar).

IAN (1 case) and LN (2 cases). In addition, 3 nerve injuries after inlay autogenous posterior mandible block graft harvested from the mandibular ramus were recorded, and 2 lesions after the removal of cysts associated with the inferior third molar. There was one injury due to an overextended endodontic treatment in the distal root of an inferior first molar, with a paste containing zinc oxide and iodoform (Klepp Licon D®, Argentina) (Fig. 4).

Regarding sensory and gustatory disturbances, the most frequent injury was paresthesia (50%). The second and third most frequent symptoms were anesthesia and dysesthesia, each with 17% of the cases. One LN anesthesia case also referred ageusia. One of dysesthesia cases also presented allodynia of the LN. Hyperesthesia accounted for 13% of the cases, and there was one case of hypoesthesia of the IAN (Table 2).

The evolution of nerve injuries was recorded in 25 patients, because 5 patients did not attend the follow-up visits. Twenty-eight percent of the lesions completely reversed in terms of size and sensitivity; while of the remaining 72% of the lesions, 67% improved in sensitivity, and 44% in size. In 3 patients, an unfavorable evolution of sensitivity was observed, with worsening of the symptoms (Table 3). In the group of 10 patients treated with dexamethasone and ibuprofen during the first week after injury, 3 cases achieved total reversal, and 7 had improved symptoms. Of the 15 patients who were prescribed medication, 4 reversed, 5 improved, 3 remained unchanged, and 3 worsened ($p>0.05$). Only 2 of the 30 patients (female patients, 21 and 26 years old) said they intended to take legal actions

Table 2. Distribution according to sensory and gustatory lesions

Diagnosis	Inferior alveolar nerve			Lingual nerve		
	AF	%	CI95	AF	%	CI95
Paresthesia	13	43	27 to 61	2	7	2 to 21
Anesthesia	3	10	3 to 26	1	3	1 to 17
Hyperesthesia	3	10	3 to 26	1	3	1 to 17
Dysesthesia	2	7	2 to 21	2	7	2 to 21
Dysesthesia + LN Allodynia	1	3	1 to 17	0	0	0 to 11
Hypoesthesia	1	3	1 to 17	0	0	0 to 11
Anesthesia + Ageusia	0	0	0 to 11	1	3	1 to 17
Total	23	77	59 to 88	7	23	12 to 41

AF: absolute frequencies; CI95: 95% confidence interval

Table 3. Distribution according to the evolution of the lesion in relation to size and sensitivity

Outcome	Size			Sensitivity		
	AF	%	CI95	AF	%	CI95
Improved	8	32	17 to 52	12	48	30 to 67
Equal	8	32	17 to 52	2	8	2 to 25
Reversed	7	28	14 to 48	7	28	14 to 48
Does not need treatment	2	8	2 to 25	1	4	1 to 20
Worsened	0	0	0 to 13	3	12	4 to 30
Total	25	100	----------	25	100	----------

AF: absolute frequencies; CI95: 95% confidence interval

for the injury suffered. They both had LN injuries (anesthesia and hyperesthesia) due to inferior third molar extraction (one performed at a private office and the other at a dental institution) and considered their personal and professional lives to be affected by the difficulties generated by the injury, mainly in their speech.

The literature review found 8 series reporting nerve injuries caused by dental procedures, including 42 to 1331 cases. The largest of these (1331 cases) is a recent multi-site study conducted in the UK and Belgium[15]; and the second largest is another European series (Denmark) with 449 cases[8]. Other European studies present 42 to 56 patients[7,16,17]; and studies from USA and Canada report on 73 to 165 cases[2,18,19]. No report was found from Latin America.

DISCUSSION

Trigeminal nerve injuries have low prevalence and there are few reports on them. The current study reports the first series from Latin America. Most injuries involved the inferior alveolar nerve and the lingual nerve, with reports of buccal, infraorbital or palatine nerves being rare[2,8,16]. Some studies have reported higher prevalence of IAN injuries than LN injuries[2,15-17], while others have observed more injuries of the LN[8,19]. In our series, three out of four injuries were IAN.

There is broad consensus that inferior third molar surgery is the most prevalent cause of trigeminal nerve injuries in dentistry[2,8,9,15,16]. In two of the series reported, the IAN Is the nerve most often affected during third molar extraction[2,16], while another series reports more LN injuries[8]. In our series, the distribution was even (*50% for each nerve, one patient with both nerves affected*). Correct planning of flap design and careful dissection of soft tissues are essential to reduce LN injuries[8,20]. Ostectomy has been statistically associated with greater permanent lingual nerve damage than has tooth sectioning[20]. Factors associated with IAN injury include difficult surgery, surgeon's experience, and proximity to the IAN canal[21,22]. If close proximity is confirmed, the risks of temporary and permanent IAN injury are 20% and 1–4% respectively[23]. The patient should be clearly warned of this complication prior to surgery. If the tooth is non-vital, or pathology is associated, careful ostectomy, root sectioning, and tooth removal are recommended. If the tooth is vital and the patient is not compromised, coronectomy of the tooth should be an alternative[24,25].

Reported incidences of implant-related injuries range from 4% to 17% of the total nerve injuries[2,8,10]. There has been an increase in IAN injuries parallel to the increase in implant surgery[17]. In our series, one out of five injuries occurred during implant placements. Lin et al.[26] reported occurrence of short-term (10 days after implant placement) and long-term incidence (1 year after implant placement) of implant-related IAN injuries of 13% and 3%, respectively, although other authors have reported long-term injuries below 1%[27,28]. Nerve injuries associated to dental implant surgery are almost exclusively related to the inferior alveolar nerve, with few reported cases of lingual nerve. When the professional is not sure of placing the implants with a safety zone (≥ 2 mm) to the mandibular canal, a cone beam computed tomography (CBCT) scan is required to take proper bone measurements. Intra-operative radiographs during implant bed preparation are useful to evaluate the distance to the mandibular canal[29]. Special attention and diagnosis should be ensured for immediate implants in the premolar area because the mental foramen often exits next to the apex of both premolars[11]. Dental implant injuries could be caused by direct (mechanical or chemical), or indirect (hemorrhage or scarring) trauma; professionals should pay special attention to bone density near the inferior alveolar canal[28]. It is important to note that intraoperative pain under mandibular infiltration technique is not an indicator of proximity to the canal. Pain under this technique is associated with surgical time and presence of adjacent teeth[30]. A sudden 'give' (feeling of sudden emptiness when drilling the bone tissue) during preparation may be indicative of protrusion through the lingual or buccal plate but may also be associated with perforation of the IAN canal roof. If the professional is not sure of not having intruded into the canal, it is recommended not to place the implant. Moreover, if there is persistent numbness after local anesthesia has worn off, it is recommended to remove the implant before 24-36 hours[10].

Few patients with inferior IAN injury resulting from major maxillofacial surgery or orthognathic surgery present significant complaints. This may be due to the clear pre-surgical consent and information, along with the significant perceived benefits of the surgery[9]. Two studies[7,8]

have reported 4 and 15% of this type of injury resulting from orthognathic surgery. In our series, no injury was reported as a result of this procedure.

Any tooth requiring endodontic therapy in close proximity to the IAN canal requires special attention. If the root canal is overprepared and the apex opened, chemical and physical nerve injuries are possible. Current knowledge of endodontic-related nerve injuries relies primarily on single case reports. It has been reported that tooth locations (mandibular molars), types of extruded materials (most report paraformaldehyde-containing sealer) and obturation technique are factors that may affect nerve injury prognosis[12]. Reported IAN injuries after endodontic treatment, account for 1.4 to 6% of total trigeminal nerve injuries[2,8,16]; we found 3% in our series. Early surgical removal of the excess of endodontic material in contact with the nerve provides the best recovery prognosis (before 72 hours)[31]. Apex and/or tooth removal within 24 hours could also be considered[9].

Nerve injuries caused by local anesthetic injection occur in approximately one in every 25,000 to 175,000 inferior alveolar nerve blocks[19]. It is the second reason for nerve injuries in many studies, accounting for 12.3%-17%[2,8,16]. The nerve that is usually damaged during IAN block injections is the LN, which accounts for 70-78%[2,8]. In our series, 13% of total injuries were caused by anesthesia injections, mainly IAN nerve blocks, also affecting more LN (66%) than IAN (33%). We observed a rare case of IAN injury when buccal infiltrative anesthesia was applied next to the mental foramen to place a clamp for rubber dam isolation during a composite restoration. We observed a rare case of IAN injury when buccal infiltrative anesthesia was applied next to the mental foramen to place a clamp for rubber dam isolation during a composite restoration. Recovery from lesions caused by anesthetic injections has been reported to take place at 8 weeks in 85–94% of cases[32]. Reports of 'electric shock' type sensation during IAN block application is not a specific sign of nerve injury[9]. Low injection pressures, and needles with short bevel angles (45°) may produce less damage than long bevel needles (14°)[33]. Higher incidences have been reported when multiple injections, high concentration local anesthetics, and articaine over lidocaine were applied[9,20], though it is not yet conclusive whether articaine is more likely to induce permanent nerve injury[34].

Unexplainably, several studies on nerve injuries in dentistry report a higher incidence in females, with ratios of 3.3 to 1[19], 2.8 to 1[8], 2.1 to 1[16], 1.9 to 1[1], 1.8 to 1[17], 1.5 to 1[2], and 1.4 to 1[15]. Our study also found predominance in females, with a ratio of 1.7 to 1. This is probably only due to the fact that more women than men seek dental care[8,12].

There is a wide range of nerve injury clinical symptoms, spanning from minimal anesthesia in a small area to devastating effects on the patient's quality of life, such as spontaneous pain. Paresthesia is the main symptom in trigeminal injuries, accounting for approximately half of the cases[1,8], as observed in our study. The prevalence of other symptoms is variable, with the most prevalent being anesthesia, hyperesthesia, dysesthesia, and allodynia[1,8]. In our series, 5 patients suffered spontaneous or evoked pain and 4 patients had unpleasant increased sensitivity.

In a series of trigeminal nerve injuries from USA, 40% of the patients had filed legal claims against the professionals[19], and another study reported that 20% of patients filed lawsuits[18].

Other reports have observed that more than half the lawsuits are associated to the lack of pre-operative informed consent for implant surgery, and most are associated to premolar implants[35]. In our series of 30 patients, only 2 patients intended to sue the acting professionals. Ten patients who were not warned of the possibility of injury did not show intentions of making legal claims. This difference may be due to the observed populations having different idiosyncrasies. Professionals should not underestimate the possibility of generating nerve injuries, and should inform the patient of the risks and benefits of the clinical practice. Informed consent prior to any implant surgery is a legal agreement that

cannot be ignored[36]. In a series of 30 patients who suffered nerve alterations after implant placement in England, only 46% had been asked to sign the consent and 27% said that they had not been warned of this complication[1]. In our study, a significant difference was observed regarding informed consent depending on the type of dental care facility: professionals in their private offices requested fewer informed consents and warned patients less than those who worked at dental institutions.

Trigeminal nerve injury treatment is still controversial, probably due to its low prevalence and the difficulty in designing comparative studies. When nerve injury is confirmed, early removal of the etiological factor, and early administration of oral corticosteroids and NSAIDs are recommended[10-13]. The latter recommendation could not be confirmed in the current study, but further studies using larger samples may be able to clarify this point. Low-level laser therapy and microsurgery have been proposed, although results are controversial[12,14]. Peripheral neuropathy due to diabetes mellitus, alcohol use, hypothyroidism or nutritional deficiency may affect recovery[33]. In a systematic review of the literature on treatment of nerve disorders, it was concluded that clinical trials are still needed to investigate the effectiveness of surgical, pharmacological and physiological treatments[37]. Early referral and treatment appear to be the key factors for better prognosis.

Dentists should be aware of the debilitating effects resulting from trigeminal nerve injuries, the complexity of their resolution and the importance of carefully planning dental procedures to prevent them.

DECLARATION OF CONFLICTING INTERESTS
The authors declare no potential conflicts of interest regarding the research, authorship, and/or publication of this article

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Histomorphometric evaluation of human extraction sockets treated with autologous fibrin, sticky bone or biphasic calcium phosphate

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ABSTRACT
It is essential to maintain the alveolar bone ridge to ensure the success of implant therapy. Platelet-rich fibrin (PRF) may benefit bone repair, but its quantitative microscopic results are still inconclusive. The aim of this study was to histomorphometrically analyze human dental alveoli after extraction treated with autologous fibrin, biphasic calcium phosphate or sticky bone. The sample consisted of healthy adult volunteer patients, with clinical and tomographic indication for single post-extraction graft of upper premolars for maintenance of the alveolar ridge and subsequent implantation. The 10 remaining patients in the study were divided into three groups according to the type of filling used in the dental socket: autologous PRF plug covered by a PRF membrane (G1), PRF associated with an alloplastic graft based on hydroxyapatite with beta tricalcium phosphate covered by a collagen membrane (G2) or alloplastic graft based on beta tricalcium phosphate covered by collagen membrane (control). After 8 months, bone biopsies were performed at the grafted sites and the patients underwent implant-prosthetic rehabilitation. Paraffin-embedded tissue blocks were routinely processed and sectionsfrom different depths were mounted in 3 slides and stained with HE. The histomorphometric evaluation analyzed 30 photomicrographs per block, quantifying the percentage presence of newly formed bone, connective tissue and remaining biomaterial using the ImageJ software. Parametric data enabled intergroup comparisons using ANOVA and Tukey's post-hoc test for multiple comparison with statistical significance of 5% ($p<0.05$), with normality of the 3 groups by the Jarque-Bera test ($p>0.05$). There was a higher mean of newly formed bone in G1 (68.83%) compared to G2 (35.69%) and control (16.28%). There was greater presence of connective tissue in the control (61.56%). Remaining biomaterial was higher in G2 (15.75%), but did not differ statistically from the control. Bone regeneration obtained with PRF alone or sticky bone suggests the efficacy of these therapies, encouraging the clinical use of this blood concentrate in dental procedures.

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Keywords: *platelet-rich fibrin - biocompatible materials - bone regeneration.*

Avaliação histomorfométrica de alvéolos dentários humanos pós-extração tratados com fibrina autóloga, aglutinado ósseo ou fosfato de cálcio bifásico

RESUMO
A manutenção do rebordo ósseo alveolar é prerrogativa para o sucesso da terapia com implantes. A fibrina rica em plaquetas (PRF) poderia beneficiar o reparo ósseo, mas seus resultados microscópicos quantitativos são ainda inconclusivos. O objetivo deste trabalho foi analisar histomorfometricamente alvéolos dentários humanos pós-extração tratados com fibrina autóloga, fosfato de cálcio bifásico ou sua associação. A amostra consistiu de pacientes adultos voluntários saudáveis, com indicação clínica e tomográfica de enxerto unitário pós-exodontia de pré-molares superiores para manutenção de rebordo alveolar e posterior implante. Os 10 pacientes remanescentes no estudo foram divididos em 3 grupos de acordo com o tipo de preenchimento usado no alvéolo dentário: plug de PRF autóloga recoberto por membrana de PRF (G1), PRF associada a enxerto aloplástico de hidroxiapatita com beta fosfato tricálcio recoberto por membrana de colágeno (G2) ou enxerto aloplástico de beta fosfato tricálcio recoberto por membrana de colágeno (controle). Após 8 meses, foram realizadas biópsias ósseas nos locais enxertados e os pacientes seguiram a reabilitação implanto-protético. Blocos histológicos incluídos em parafina foram microtomizados para gerar 3 lâminas de secções em diferentes profundidades, que foram coradas em HE. A avaliação histomorfométrica analisou 30 fotomicrografias por bloco, quantificando a presença percentual de osso neoformado, tecido conjuntivo e biomaterial remanescente pelo programa ImageJ. Os dados paramétricos permitiram comparações intergrupos usando ANOVA e pós-teste de comparações múltiplas de Tukey com significância estatística de 5% ($p<0,05$), haven-

do normalidade dos 3 grupos pelo teste Jarque-Bera (p>0,05). Houve maior média de osso neoformado em G1 (68,83%) em comparação a G2 (35,69%) e controle (16,28%). Houve maior presença de tecido conjuntivo no controle (61,56 %). Biomaterial remanescente foi maior em G2 (15,75%), mas não diferiu estatisticamente para o controle. A regeneração óssea obtida com PRF isolada ou em associação sugere a eficácia destas terapias, encorajando o uso clínico deste concentrado sanguíneo em procedimentos odontológicos.

Palavras-chave: *fibrina rica em plaquetas- materiais biocompatíveis- regeneração óssea.*

INTRODUCTION

Tooth loss causes sequential morphological changes in hard and soft tissues of the alveolar ridge, especially during the first 8 weeks after tooth extraction. Without intervention, there is great loss of volume due to bone resorption[1,2]. Conservative guided bone regeneration (GBR) procedures seek to maintain the alveolar bone structure for subsequent rehabilitation with dental implants[1]. GBR techniques have evolved based on the use of biomaterials, making procedures less traumatic, safer, and cost-effective, re-establishing function and aesthetics in a stable, satisfactory manner[2,3].

Platelet-rich fibrin (PRF), which is a platelet concentrate of leukocytes, platelets and fibrin, was developed in the search for new GBR alternatives[4]. The cellular elements interspersed in a fibrin mesh potentiate the formation of new blood vessels, minimize immunological action because they are from an autologous biological source, promote antibacterial action and secretion of cytokines by leukocytes, and activate mechanisms of tissue repair through of multiple growth factors released gradually[4,5]. PRF has been used since the 2000s and, especially in recent years, experimental protocols and clinical research reports have increased exponentially[5]. The use of PRF has been documented for alveolar preservation, sinus lift, and treatment of intrabony defects or furcation defects[4]. The combination of PRF, cells and different bone substitutes could be a promising alternative in bone regeneration proposals[6]. In addition to its biological benefits, the easy acquisition and affordability of PRF make it attractive for use in GBR[3-5].

Although natural bone grafts of autogenous, allogeneic or xenogeneic origin are good biological alternatives in dentistry[3,7,8], alloplastic materials have gained greater acceptance among professionals and patients, whether due to the fact that there is less morbidity at the surgical site or due to absence of moral and religious issues such as stigmatization of the cadaveric or animal source[3,9]. Biphasic calcium phosphate (BCP) is a synthetic ceramic consisting of hydroxyapatite (HA) and beta tricalcium phosphate (β-TCP) which has been used for decades in GBR, demonstrating biocompatibility, biodegradability and great clinical interest[9]. Few papers have evaluated bone formation at the histological level of BCP compared to other classes of biomaterials[3,9]. However, bone regeneration in human fresh alveolar sites with PRF is as yet incipient, as the literature offers in situ descriptive analyses and lacks more robust quantitative results, which could impair its therapeutic predictability[10]. The same applies to its association with BCP, which has not yet been described in the literature[11-13]. The proposal of this study therefore has scientific originality.

The aim of this study was to perform a histomorphometric evaluation of human extraction sockets treated with either autologous PRF, an association between PRF and BCP, or only alloplastic BCP.

MATERIALS AND METHODS

This research adopted the ethical principles of respect for people, non-maleficence, beneficence, and justice described in the Belmont Report (1978) and the Brazilian guidelines of the Resolution of the National Health Council No. 466 (2012), which establishes the basic requirements for research involving human beings in the country. This research was assessed and approved by the local Ethics Committee of the Universidade Estadual Vale do Acarau via Plataforma Brasil (register CAAE #91602218.0.0000.5053 and approval protocol #2.806.761) before data collection started.

Participants' consent was registered through a Free and Informed Consent Form (FICF), which provided information in appropriate language about the purpose of the research, as well as ensuring the confidentiality of identities.

Type of study

This was a clinical study with quasi-experimental design and a quantitative, structured approach. The

target population consisted of patients recruited in a public university dental care service.

Sample

This study was conducted at a public university dental care service in the city of Sobral, Ceara, Brazil. For a good clinical experimental planning, sample size was calculated using the statistical formula for finite population: $N.\hat{p}.\hat{q}.(Z\alpha/2)^2=n.(\hat{p}.\hat{q}.(Z\alpha/2)^2+(N-1).E^2)$, where N: requested population; n: representative sample; $\hat{p}$: % inside of research subject; $\hat{q}$: % outside of research subject; $Z\alpha/2$: degree of confidence; E: margin of error[14].

Clinical evaluation is an important step in the calculation of the probabilistic sample, considering the profile of the public served between August 2018 and April 2019. Inclusion criteria were systemically healthy patients, of both sexes, young adults in the age group 20 to 45 years, with indication of elective single extractions, unilateral or bilateral, of upper premolars, without active periodontal problems, non-smokers, without the presence of known psychological disorders, good oral hygiene status and without any functions that could compromise occlusion. Exclusion criteria were patients who did not consent to participate in the study, did not agree to undergo laboratory and imaging exams (periapical X-ray, panoramic and tomography) prior to the surgeries for appropriate planning of the cases, or did not sign the FICF. The minimum probabilistic sample calculated for the study was 10 patients.

At first, a higher number of patients was included than the proposed goal, with 15 patients eligible for the study, 6 male and 9 female, totalling 16 surgical beds, with 1 patient indicated for bilateral surgery. After the first surgeries, 5 patients dropped out, which maintained the minimum number of 10 patients and 11 surgical beds for this clinical research. For all groups, a single post-extraction region per quadrant in the upper dental arch was always considered for each type of treatment proposed.

Human socket grafting

Surgical planning involved two general steps for all groups: tooth removal followed by grafting (time 0), and bone biopsy and dental implant (8 months after the initial procedure). Preoperative care included pre-emptive medication (2 tablets of dexamethasone 4 mg 1h before) and mouthwash with Periogard™ (Colgate, Brazil), 0.12% chlorhexidine for 1 minute. Infiltrative anaesthesia was performed with Articaine 100™ (DFL, Brazil), 4% articaine and 1:100,000 epinephrine. Relaxing incision without much extension to the bottom of the vestibule facilitated detachment of the mucoperiosteal flap, surgical access, dislocation and removal of the premolar, biomaterial grafting in the dental socket and suturing the region with 4.0 nylon thread (Tecnew, Brazil).

A trained, qualified team (an oral surgeon, a dental assistant, and a nursing assistant) collected PRF in the dental office. The protocol for obtaining an autologous PRF was based on Resolution No. 158/2015 of the Federal Council of Dentistry in Brazil, which regulates the use of platelet aggregates in dental procedures in the country. The method of obtaining PRF enables acquisition of 4 types of conformation of the biomaterial: PRF clot, PRF plug, PRF membrane and Liquid PRF, where sticky bone represents an association of PRF clot and liquid PRF, as previously described[4]. For this reason, there were two blood samples per venepuncture in patients in the PRF group: before (8 tubes) and after (2 tubes) tooth extraction. The centrifugation step adopted the protocol described by Oliveira et al.[5], using Spin Plus™ equipment (Spinlab, China), with an angle of 45 degrees, 78 mm radius, time 10 minutes and 1500 rpm, resulting in a centrifugal force of 196g to obtain PRF clot and its derivates, plugs or membranes according to manual processing of the surgeon. To obtain liquid fibrin, immersion solution of PRF clot to generate the mixture of i-PRF, centrifugation without additives was performed for 3 minutes at 2700 rpm[15].

The experimental groups and final number of patients were divided into: PRF plug covered with PRF membrane (G1, n=4 patients, or 5 surgical beds); sticky bone or PRF associated with Boneceramic™ (Straumann, Switzerland), a BCP constituted of 60% HA and 40% β-TCP, covered by collagen membrane Lumina coat® (Critéria, Brazil) (G2, n=3 patients, or surgical beds); and Boneceramic™ graft covered by Lumina coat® (Control, n=3 patients, or surgical beds).

Postoperative care included prescription of analgesic medication and use of mouthwashes. Clinical evaluations were performed at seven and thirty days, following the presence of some area of contamination, inflammation or dehiscence of the wound or loss of graft material. Visits after 90 days were scheduled for further evaluation of the procedures. Figs. 1, 2 and 3 summarize the sequence

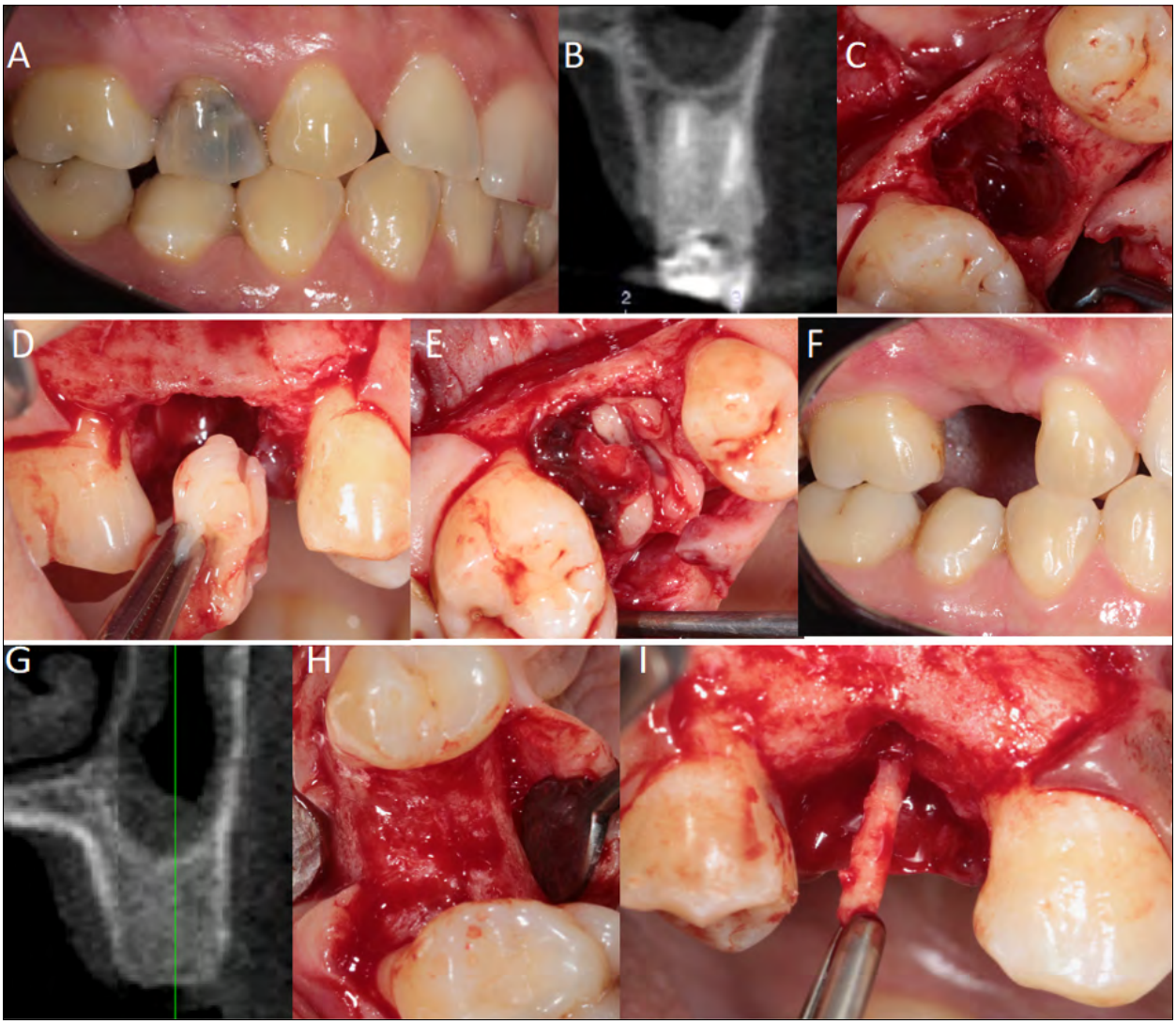

Fig. 1: Pre-, trans- and post-surgical sequence in G1 (PRF). (A) Tooth for removal, (B) initial tomography, (C) fresh socket, (D) insertion of PRF, (E) filling of the socket, (F) repair after 8 months, (G) final tomography, (H) repaired bone and (I) bone biopsy.

of clinical, tomographic, and surgical evaluations and protocols conducted in the different groups.

Data collection

The second surgical access was performed after 8 months. Bone biopsies were performed with a 2mm diameter trephine drill (Maximus, Minas Gerais, Brazil) at a depth of 5mm. Then the metal implants were positioned as indicated by the manufacturer and suture performed on the mucosa. All patients continued to undergo clinical-prosthetic procedures in the following months to manufacture single implant-supported dental prostheses, which were not the subject of this study.

The bone biopsies collected were fixed in 10% formalin v/v (Allkimia, Brazil) buffered with phosphate buffer at pH 7.2 for 48h, washed in running water for 1h, decalcified in acid rapid bone demineralization solution (Allkimia, Brazil) for 36h, washed in running water for 1h, dehydrated in concentrations of 70%, 80%, 90% and 100% ethanol (Dinâmica, Brazil) for 1h each, clarified in 3 xylol baths (Dinâmica, Brazil) for 1h each, embedded in 3 baths of liquid paraffin at 60°C (Synth, Brazil) for 1h each. Blocks were prepared preserving the alveolar longitudinal topography or axial vertical axis of each biological sample. Paraffin blocks were sectioned in a rotating microtome (Leica RM2125 RTS, Germany), obtaining 3 histological slides per block. Sections had an individual thickness of 5µm and a 50µm distance between them to avoid overlapping counts. Slides were Haematoxylin-

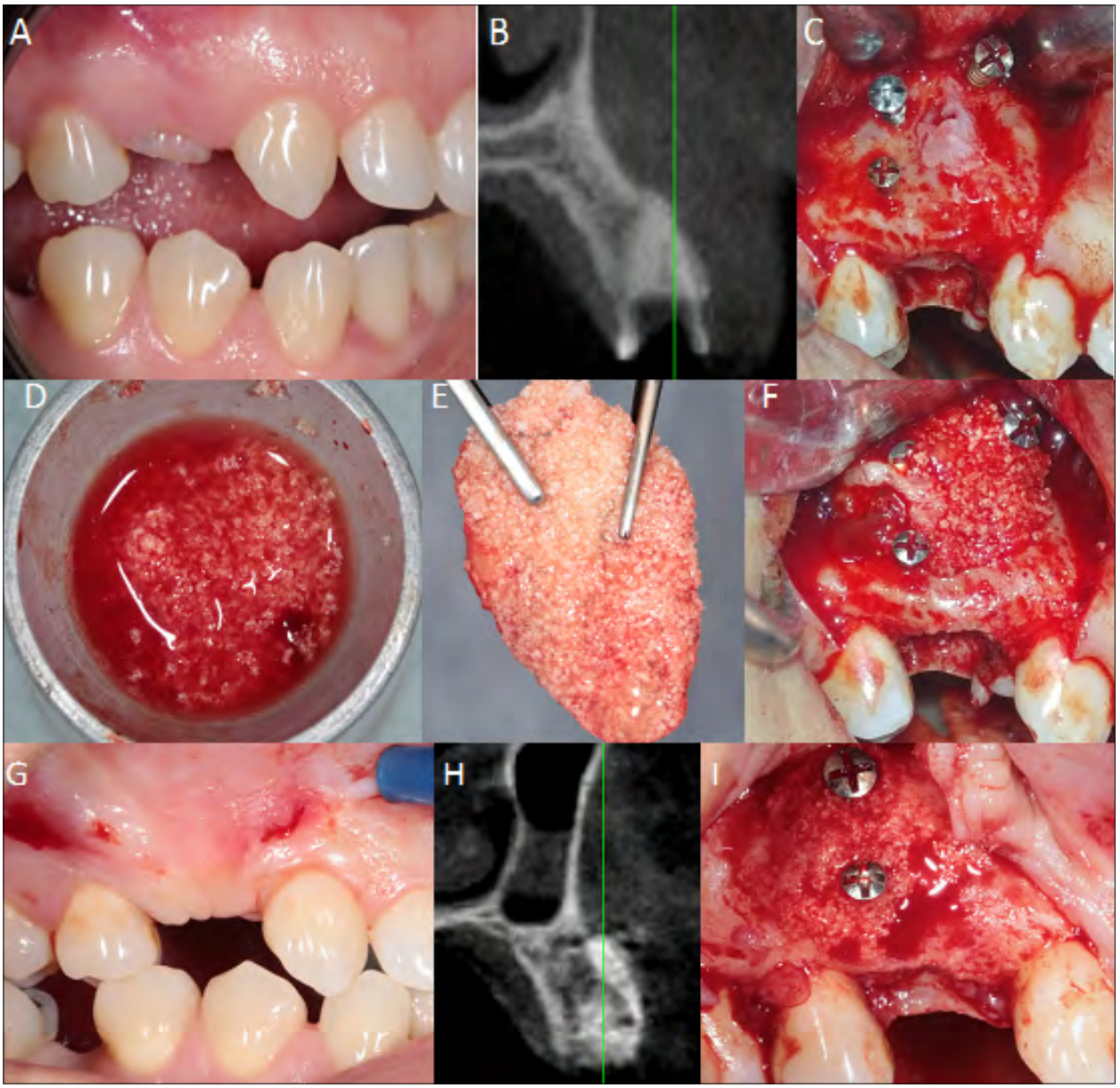

Fig. 2: Pre-, trans- and post-surgical sequence in G2 (sticky bone). (A) Tooth for removal, (B) initial tomography, (C) fresh socket and preparation of the surgical bed, (D) mixture of PRF and bioceramics, (E) sticky bone, (F) filling in the socket, (G) appearance after 8 months, (H) final tomography and (I) repaired bone.

Eosin (HE) stained. Photomicrographs were taken using an optical microscope (FWL-1000; Feldman Wild Leitz, Manaus, Brazil). All images were qualitatively and quantitatively analyzed by an experienced pathologist and a previously trained researcher.

Histological and histomorphometric analysis

All histological slides were evaluated qualitatively for the presence of new bone, connective tissue, and remaining biomaterial in the different groups. The extension of the histological sections was evaluated, and images representative of the aforementioned biological criteria were selected.

For the histomorphometric analysis, 10 fields per section were evaluated in contiguous non-overlapping fields, at 800x magnification. Considering the biological samples collected from the 11 surgical beds of the patients and 3 stained slides for each paraffin block, a total 330 photomicrographs were taken and evaluated.

Images were segmented using the ImageJ 1.8.0

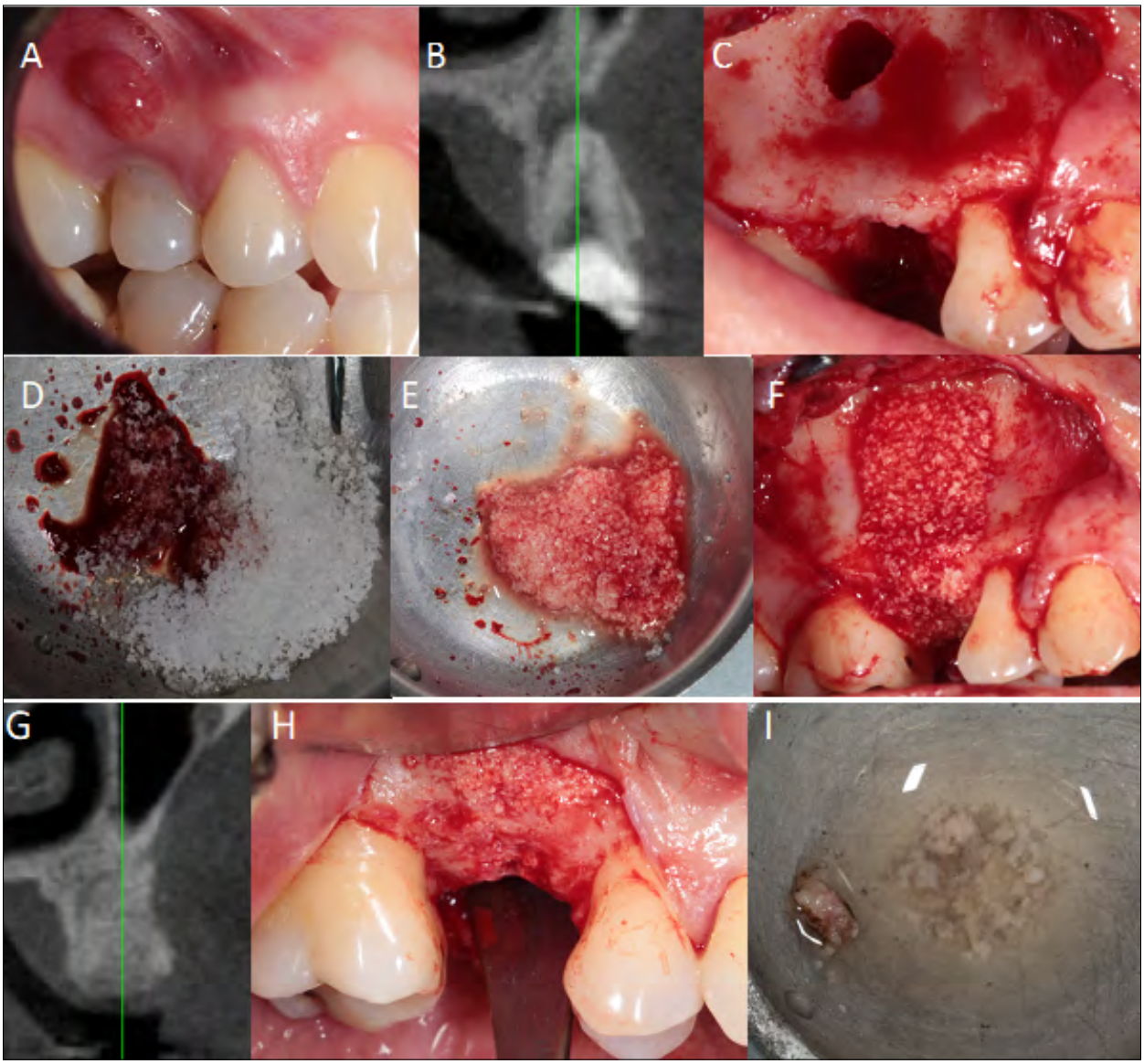

Fig. 3: Pre-, trans- and post-surgical sequence in control (BCP). (A) Tooth for removal, (B) initial tomography, (C) fresh alveolus with periapical fenestration, (D) bioceramics with blood, (E) ready mix, (F) insertion of bioceramics, (G) final tomography, (H) repaired bone after 8 months and (I) bone biopsy.

software (National Institutes of Health, USA), standardized for counting with an automatic grid of 140 points digitally superimposed on each photomicrograph, calibrated in micrometres/pixel. The parameters evaluated by quantitative analysis were new bone (NB), connective tissue (CT), biomaterial (BCP) and other structures (OS) (Fig. 4). From the number of absolute points, the percentage of volume density of each parameter was determined using Equation: %vd = (p/P)*100, where %vd represents the percentage of volume density; p the number of points of the parameter; and P the total number of points.

The data were tabulated and expressed through the mean ± standard deviation for each treatment. The results were displayed in graphs using the Graphpad Prism 7.0 software (Graphpad Software, USA), for comparing the intergroup biological response.

The parametric data of the mean density for each parameter were subjected to a one-way analysis of variance (ANOVA), and for the intergroup analyses, the Tukey post-test was applied comparing the difference between the means of the experimental groups considering significant differences with values of $P<0.05$. The Jarque-Bera test verified the normality of the research data of the 3 groups ($P>0.05$).

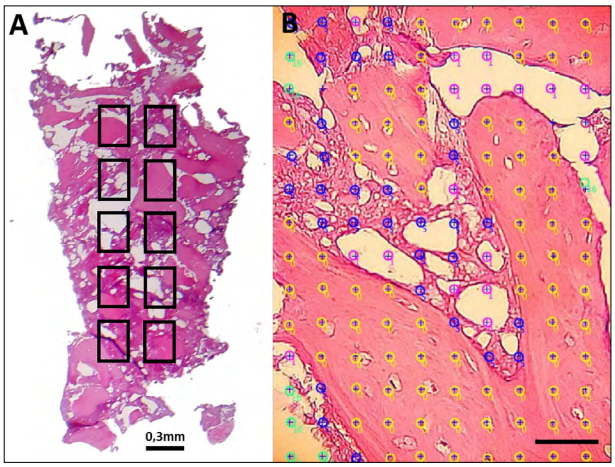

Fig. 4: Diagram of (A) stained slide of bone biopsy of G2 treatment and (B) histomorphometric counting of morphological criteria: new bone (yellow), connective tissue (dark blue), biomaterial (purple) and other unrelated structures (green), scale bar: 50µm.

RESULTS

Fig. 5 shows the microscopic images of the experimental groups. In G1, there were large areas of new trabecular bone with osteocytes, and sometimes lamellar bone with Harversian canals, interspersed with connective tissue, without inflammatory infiltrate, fibrous capsule, or sign of the remaining PRF. In G2, bioceramic granules were present in varying sizes and in aggregates, surrounded by loose connective tissue or in close contact with new bone with osteocytes, proving their osteoconduction. In the control, the presence of connective tissue was greater, with little presence of new bone with osteocytes and some areas containing bioceramic granules, slightly more dispersed than in G2, which could be explained by the lack of agglutination due to absence of PRF.

Histomorphometric analysis showed a higher percentage of new bone in G1 (68.83%±4.95) than the control (16.28%±13.90) and G2 (35.69%±7.86). There are no differences between G2 and control. Connective tissue in the control group (61.56%±11.85) was greater than in G1 (13.95%±3.48) but without statistical differences for G2 (32.67%±13.69). The percentage of remaining biomaterial was higher in G2 (15.75%±11.63) than in G1 (undetectable PRF) but did not differ statistically from the control (8.43%±3.10). There were no statistically significant differences between groups regarding the presence of other structures (G1: 17.21%±5.53, G2: 13.73%±4.78 and control: 15.88%±7.06) (Fig. 6).

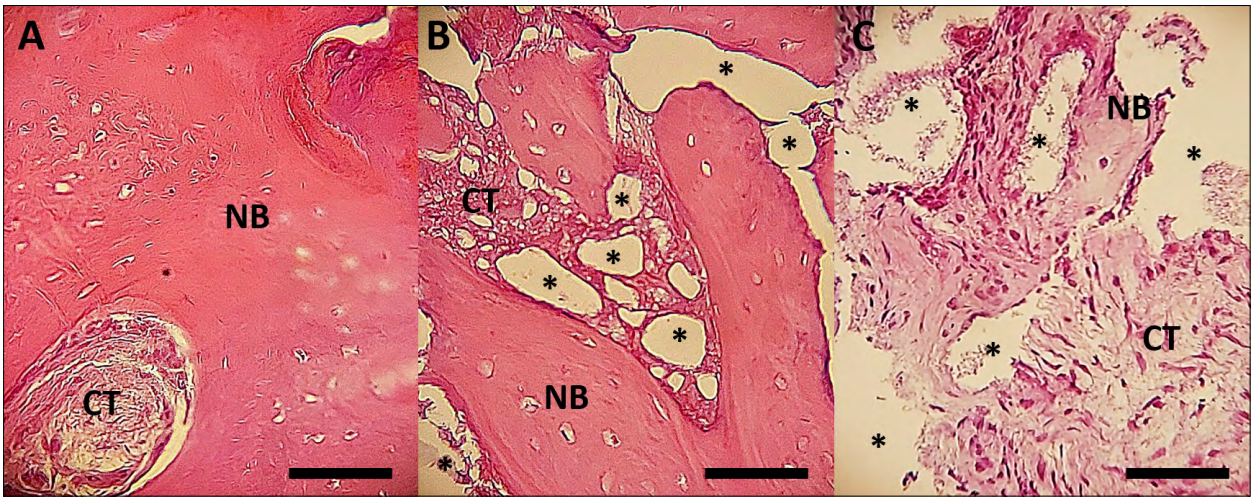

Fig. 5: Histological appearance of the experimental groups. (A) G1 (PRF), (B) G2 (sticky bone) and (C) Control (BCP). New bone (NB), connective tissue (CT) and biomaterial (*). Scale bar: 50 µm.

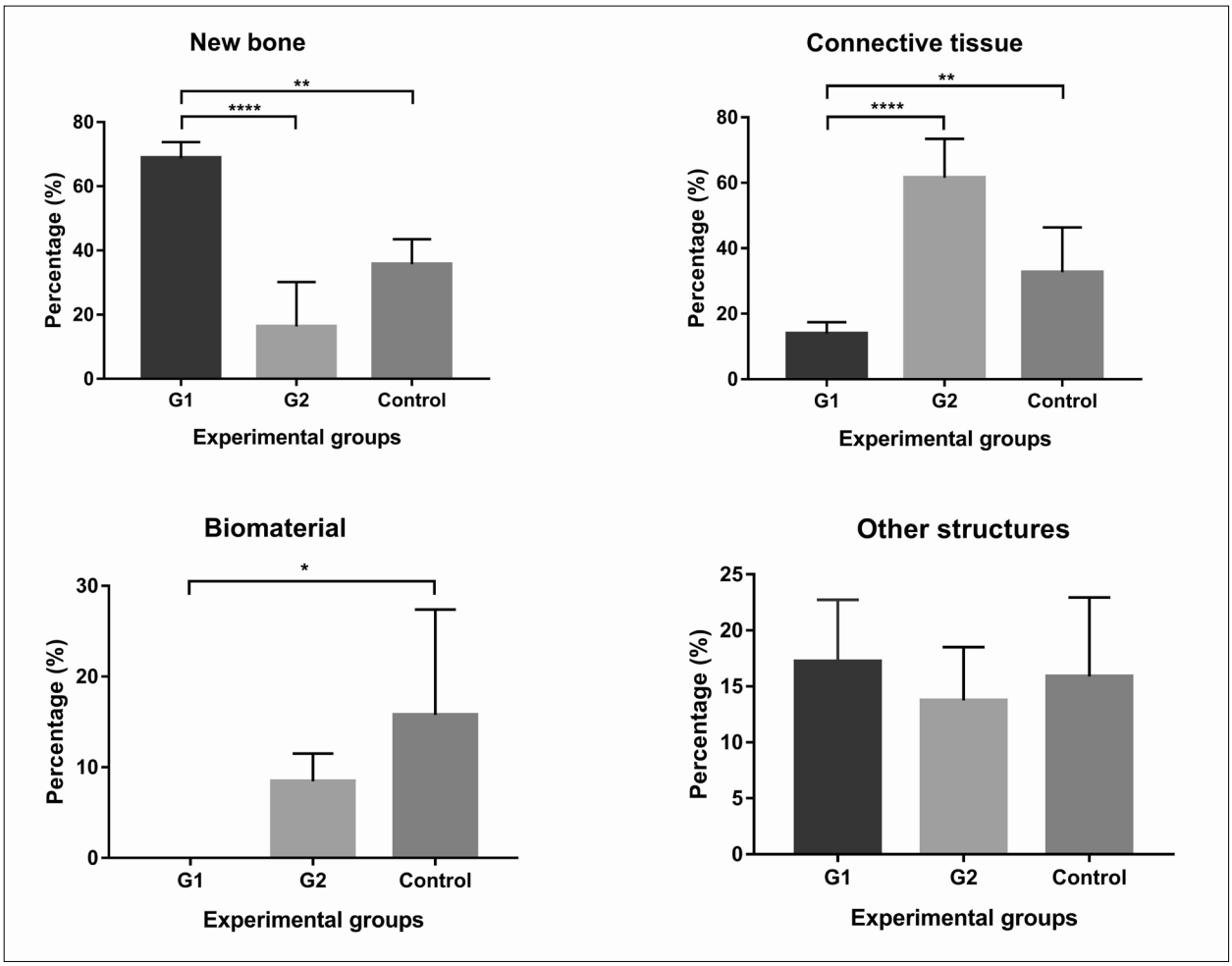

Fig. 6: Percentage of (A) new bone, (B) connective tissue, (C) graft remaining and (D) other unrelated structures in G1, G2 and control after 8-month follow up. Means (± standard deviation) analyzed by the parametric ANOVA test and Tukey post-test. Significant differences between groups for new bone: P <0.01 (**) and P <0.0001 (****); connective tissue: P <0.01 (**) and P <0.0001 (****); and implanted biomaterial: P <0.05 (*).

DISCUSSION

For maintaining and potentially repairing alveolar bone structure, minimally invasive surgery is recommended that could provide a total flap to preserve periosteal vascularization and avoid reduction in the volume of the alveolus, the height of the bone crest and the keratinized gingival margin[16-18]. However, in the present study, a mucoperiosteal flap was performed for better surgical access in the use of PRF, and no negative influence on the clinical parameters was observed after 8 months. A study by Mendoza-Azpur et al. corroborated this option, reporting that dental alveoli treated with PRF and mucous covering after total flap and two diagonal relaxants presented the entire surgical site hermetically protected, promoting healing[19]. In addition, i-PRF generates more elasticity and better adaptation to defects, accelerating bone healing in the dental socket, preventing fibrogenesis and isolating the socket from the oral biofilm[19,20].

Centrifugation can interfere with the quality of blood concentrates and generate heterogeneous results in bone healing[21]. Our protocol for obtaining PRF clot used a greater angle, radius and rotations per minute and shorter time, approaching half the angular speed of 408g used by Canellas et al.[21], with centrifuge angled at 33 degrees, radius of 50 mm, 2700 rpm and action of 12 min. Choukroun et al.[22] evaluated the reduction in g-force in three protocols and concluded that low-speed centrifugation selectively precipitates leukocytes, platelets and growth factors, enriching the biological properties of PRF in tissue repair[22,23]. Centrifugation at 1400 rpm for 8 min could promote higher platelet concentration

and antimicrobial activity and at 3500 rpm a denser fibrin network in PRF membranes in healthy adult patients[24]. Other studies also obtained larger PRF membranes using long spin time but reported no significant differences in PRF membrane sizes between patients aged 21-80 years[25] or dual presence of loose and dense fibrin networks in patients aged 21-60 years[26], which confirms the methodology and sample used in this study.

The time for bone access and collection for histology corroborated the literature. Tooth removal promotes a process of intense bone remodelling in the dental socket from 3 months to 6 months after surgery[20,27]. Several surgical models for GBR have been proposed and the surgical reopening times in general ranged from 3 to 4 months, with few cases with 6, 8 or 9 months[10-12,19,21,28]. The option for the 8-month timing was also determined by the time to resorption of the alloplastic biomaterial, since studies report the presence of biomaterial at less than 8 months[13]. These data contribute significantly to the information available, which is scarce in long-term histomorphometric observations.

The percentage of new bone varies according to the time of analysis and type of treatment in a dental socket. Without grafting, there is low variation after 3 months: 37%[17], 39.69%±11.13[21], 45.4%±7.98[29] to 47.9%±18.1[28]. With grafting, in 3 months it varied from 20% with BioOss™ to 41% with carbonated HA[17], reaching 51.6%±12.34 with Boneceramic™[29]. In 4 months, β-TCP graft reached 26.14%±7.49[19]. In 6 months, the association of 30%HA:70%β-TCP to a collagen membrane reached 40.3%±7.8[30]. In 9 months, β-TCP/collagen composite reached 42.4%±14.6 alone and 45.3%±14.5 associated with membrane[11]. PRF graft alone reached an average of 50.7%±13.3[28] and 55.96%±11.97[21] in 3 months and 77.33%±9.8 in 4 months[19], a percentage close to that found in the current study.

The lower percentage of connective tissue found with sticky bone than BCP alone could be explained by the intrinsic properties of the PRF. Fibrin promotes angiogenesis, increasing oxygenation and nutrition for cell proliferation and formation of extracellular matrix, favouring repair, and reducing inflammatory cell activity[10].

The presence of biomaterials implanted in dental sockets is highly variable. According to different studies, after 3 months, PRF fragments were undetectable[19,21,28], while carbonated HA had 1% remnants and BioOss™ was present in 22% of the area[17]. In 4 months, β-TCP reached 14.85%±6.4[19]. In 6 months, the association of 30%HA:70%β-TCP with collagen membrane reached 6%±4[30]. A composite of β-TCP/collagen remained at 9.7%±7.3 in 9 months and when associated with the membrane, increased its permanence to 12.5%±6.6[11]. These studies using HA[17], β-TCP[11,19] or HA: β-TCP[30] corroborate the average percentages of BCP in fresh sockets from our findings. According to Mendoza et al.[19], remaining granules may promote an inflammatory response, fibrogenesis or delay in alveolus osteogenesis. This reinforces the advantage of using BCP such as Boneceramic™, which kept few residual fragments, balancing the rate of resorption with tissue turnover.

The promising potential of PRF in relation to bone neoformation is supported by scientific evidence[10,19,21,28,31]. The angiogenesis provided by PRF could explain the availability of key elements for the osteogenic microenvironment, including minerals, growth factors (e.g., VEGF, PDGF and TGFβ1) and osteogenic cells[32,33]. Furthermore, miRNA released from platelet microparticles could play a central role in regulating osteogenic phenotype in vitro[34]. Despite the use of PRF to improve soft tissue healing and reduce pain, bleeding and osteitis, there is no definitive evidence for the impact of using PRF alone on bone regeneration in post-extraction sockets[6,35], making the present results unprecedented and relevant for future studies on this topic. It is important to note that many studies on fibrin in alveoli after extraction do not report quantitative results regarding bone formation and the presence of connective tissue[16,18,20,27,31,36], which makes histomorphometry a more accurate analysis tool and strengthens scientific evidence. Use of fibrin associated with β-TCP in sinus lift, a different site from the one used in this study, showed bone neoformation with a 6-month percentage of 33.40%±10.43 for β-TCP and 32.03%±6.34 for the β-TCP associated with fibrin, thus confirming that the association of β-TCP with fibrin would not differ substantially from the alloplastic biomaterial alone[37], as in the present study.

The BCP used in the research shows biocompatibility and osteoconductive potential, favouring the adhesion, proliferation and differentiation of bone cells and the production of mineralized matrix[29]. However, the higher HA content may explain the low bioactivity and slow biodegradation[38]. Still,

BCP present in irregular shapes collaborates with an inflammatory response that acts in the negative immunomodulation for osteoconduction[17]. Thus, the appropriate selection of a biomaterial must consider topography, particle size, crystallinity, composition, and porosity, among other physicochemical characteristics that affect tissue repair[39].

The present study demonstrated that the autologous PRF is promising for GBR procedures in post-extraction socket. The association of PRF with a BCP proved to be beneficial, but less effective than the isolated form. Future studies with greater clinical samples and an emphasis on morphological quantifications are recommended.

DECLARATION OF CONFLICTING INTERESTS
The authors declare no potential conflicts of interest regarding the research, authorship, and/or publication of this article

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Shaping ability of reciprocating and rotary systems in oval-shaped root canals: a microcomputed tomography study

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ABSTRACT
This study compared the shaping ability of single-file reciprocating (WaveOne Gold) and multifile rotary (Mtwo) systems on mandibular oval-shaped canine root canals, using microcomputed tomography (micro-CT). Thirty mandibular canines were scanned by micro-CT and assigned to one of two groups (n=15) according to the system used for root canal preparation: WaveOne Gold or Mtwo. After preparation, the teeth were rescanned, and the percentage of untouched canal area, apical transportation and centering ability were analyzed. The data was evaluated using Kruskal and Mann-Whitney tests (p<0.05). No difference was found in percentage of unprepared canal area between groups in the entire root canal or the apical third, or in centering ability (p>0.05). WaveOne gold had less canal transportation than MTwo at the 5 mm section (p<0.05). WOG and Mtwo systems presented similar shaping ability and centering ability in oval-shaped canals. However, WOG presented less transportation than Mtwo at 5 mm from the apex.
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Keywords: *anatomy - root canal preparation - tooth - x-ray microtomography.*

Capacidade de modelagem dos sistemas reciprocante e rotatório em canais radiculares ovais: um estudo de microtomografia computadorizada

RESUMO
Este estudo comparou a capacidade de modelagem dos sistemas reciprocante de lima única (WaveOne Gold) e rotatórios com múltiplas limas (Mtwo) em caninos ovais inferiores, usando microtomografia computadorizada (micro-CT). Trinta caninos inferiores foram escaneados por micro-CT e divididos em dois grupos (n = 15) de acordo com o sistema usado durante o preparo do canal radicular: WaveOne Gold (WOG) e Mtwo. Os dentes foram reeescaneados e a porcentagem de área do canal não preparada, transporte apical e capacidade de centralização foram analisados. Os dados foram avaliados pelos testes de Kruskal Wallis e Mann-Whitney (p<0,05). Nenhuma diferença foi encontrada na porcentagem de área não preparada entre os grupos em todo o canal radicular e no terço apical e na capacidade de centralização (p>0,05). O sistema WOG promoveu menor transporte do canal do que o sistema Mtwo na região de 5mm aquém do ápice (p<0,05). Os sistemas WOG e Mtwo apresentaram capacidade de modelagem e capacidade de centralização semelhantes em canais ovais. No entanto, WOG promoveu menor transporte do que Mtwo a 5 mm do ápice.

Palavras-chave: *anatomia - preparo do canal radicular - dente - microtomografia por raios X.*

INTRODUCTION

Intracanal microbial reduction is the primary goal of root canal treatment, and is accomplished through irrigation, chemical debridement, and mechanical action of instruments[1], allowing periradicular tissue healing. However, these steps can be difficult to complete due to the complexity of root canal anatomy[2].

The internal canal configuration of mandibular canines has a high incidence of oval-shaped root canals[3]. Several rotary and reciprocating

systems are used to promote complete cleaning of oval-shaped canals[4], but leave unprepared areas after root canal instrumentation[4-6]. Furthermore, anatomical complexities can also make it difficult to control infection during instrumentation, allowing accumulation of hard tissue debris, with microorganisms remaining in areas that instruments are unable to reach[4-6]. Remaining microorganisms might have the potential to perpetuate periapical inflammation and compromise the success of endodontic treatment[7]. Therefore, endodontic instruments with different kinematics and heat treatments have been developed to deal with root canals with complex anatomy, such as oval-shaped root canals[8].

The WaveOne Gold system (Dentsply-Sirona, Ballaigues, Switzerland) is a reciprocating single-file made of a heat-treated gold metal alloy (M-wire)[9,10]. It has a triangular convex cross-sectional design with two cutting edges, resulting in one or two points of contact between the cutting edges and the dentin walls[9], which can increase the flexibility and improve cyclic fatigue resistance when compared to conventional NiTi alloys[11,12].

Mtwo is a well-known NiTi superelastic (SE) rotary system (VDW, Munich, Germany), with an "S"-shaped cross-sectional design, a positive rake angle with 2 cutting edges, and low radial contact to increase flexibility and improve performance during root canal preparation[13,14]. Its shape enables dentin to be cut effectively and greater root canal residue removal[15].

Therefore, the aim of this *ex vivo* study was to evaluate the shaping ability of single-file reciprocating (WaveOne Gold) and multifile rotary (Mtwo) systems on mandibular oval-shaped canine root canals, using microcomputed tomography (micro-CT). The null hypothesis tested was that there would be no difference between WaveOne Gold and Mtwo in (i) shaping ability or in (ii) apical transportation and centering ability of mandibular oval-shaped canine root canals.

MATERIAL AND METHODS

This study was approved by the Iguaçu University Ethics Committee, Rio de Janeiro, Brazil (n.2.435.836).

Sample size calculation

A power calculation was performed based on data from a previous study[16], with G*Power 3.1 software (Heinrich Heine University, Dusseldorf, Germany) using a power $\beta = 95\%$ and $\alpha = 5\%$ as inputs into an independent samples test from the t tests family. The ideal sample size for each group was a minimum of 10 teeth. Five additional specimens per group were added to compensate for possible sample loss.

Specimen selection

Thirty mandibular canines with moderately curved mesial roots (10° to 20°)[17] were selected from a pool of 300 teeth from the Bank of Human Permanent Teeth of Iguaçu University. Teeth had been extracted for reasons unrelated to this study, Consent was secured prior to tooth donation. The teeth evaluated in this study were from patients of the metropolitan region of Rio de Janeiro city.

The remaining attached tissue was removed, and the teeth were stored in distilled water until the time they were to be used. All samples were scanned by micro-CT (SkyScan 1173, Bruker, Kontich, Belgium) operated at 50 kV and 160 mA, with a 1-mm-thick aluminum filter, 320-millisecond exposure time, 12.1 µm pixel size, 0.8 rotation step, and 360º rotation along the vertical axis. The files were then reconstructed into a three-dimensional dataset with the software NRecon v1.6.1.0 (Bruker micro-CT). Reconstruction parameters included a 50% beam hardening correction, ring artifact correction of 10, and fixed contrast limits (0 – 0.05) for all image stacks. The volume of interest extended from the cementoenamel junction to the apex of the root, resulting in the acquisition of 600 to 700 axial cross sections per sample.

Then, CTAn (v.1.14.4, Bruker Micro-CT) and CTVol (v.2.2.1, Bruker Micro-CT) software were used to evaluate root canal morphological and 3D configuration. After that, the teeth were matched according to anatomical similarities of preoperative canal volume, canal surface area, and 3D configuration and randomly assigned to one of two groups (n-15) according to the instrument to be used during root canal preparation: Mtwo (VDW GmbH, Munich, German) or WaveOne Gold (Dentsply-Sirona, Ballaigues, Switzerland).

Root canal procedures

Endodontic accesses were performed with high-speed diamond (1014 HL; KG Sorensen, São Paulo, Brazil) and Endo Z burs (Dentsply-Sirona,

Ballaigues, Switzerland). A 10 K file (Dentsply-Sirona, Ballaigues, Switzerland) was used to determine apical patency, and the working length (WL) was considered 1 mm short of the apical foramen. A glide path was accomplished with a 15 K file (Dentsply Sirona) up to the WL.
The WaveOne Gold (Dentsply-Sirona) and Mtwo rotary (VDW GmbH) systems were activated with a VDW Silver motor (VDW GmbH, Munich, Germany), according to manufacturer's instructions.

WaveOne Gold system
The WaveOne (WOG) primary (25/.07) was used in a reciprocating movement with an in-and-out pecking motion and an amplitude of 3 mm with light apical pressure until the WL was reached. After three movements, the instrument was removed from the canal and cleaned with a wet sterile gaze.

Mtwo system
The root canals were prepared using the sequence 10/.04, 15/.05, 20/.06, 25/.06 at 250 rpm with pecking motion, and small brushing movement with light apical pressure until the WL was reached.
An irrigation protocol was used for both groups. Root canal irrigation was performed with 2 mL of 2.5% sodium hypochlorite (NaOCl) with a 30-G Endo-Eze needle (Ultradent Products Inc; South Jordan, UT, USA) inserted until it was 2 mm from the WL. Final irrigation was performed with 2 mL of 2.5% NaOCl, 2 mL of 17% EDTA (Mil Fórmulas, Rio de Janeiro, RJ, Brazil) for 1 min and 2 mL of 2.5% NaOCl. The root canals were dried with paper points, after which the teeth were scanned for a second time using the same parameters as mentioned above. A single experienced operator performed all procedures.

Micro-CT Evaluation
The teeth were submitted to a second micro-CT scan and reconstructed (NRecon) using the same parameters as described previously. The postoperative stacks of the root canals after preparation were registered with their respective preoperative stacks with an affine algorithm of the 3D Slicer software. The software ImageJ 1.50d (National Institutes of Health, Bethesda, MD, USA) was used to evaluate the initial and final volume (mm^3), surface area (mm^2), percentage of unprepared area, canal transportation and centering ability. The unprepared canal area was determined by calculating the number of static voxels (voxels present in the same position on the canal surface before and after instrumentation) divided by the total number of voxels present on the root canal surface[6], according to the following formula:

$$\frac{\textit{number of static voxels} \times 100}{\textit{total number of surface voxels}}$$

Canal transportation and centering ratio were calculated at 3 cross-sectional levels (3-, 5-, and 7-mm distance from the apical foramen) using the following equations[18]:

Degree of canal transportation = $(m^1 - m^2) - (d^1 - d^2)$
Canal centering ratio = $(m^1 - m^2) - (d^1 - d^2)$ or $(d^1 - d^2) - (m^1 - m^2)$,

where m1 is the shortest distance from the mesial of root canal to the mesial of the non-prepared canal, m2 is the shortest distance from the mesial of root canal to the mesial of the prepared canal, d1 is the shortest distance from the distal of root canal to the distal of the non-prepared canal, and d2 is the shortest distance from the distal of root canal to the distal of the prepared canal[18].

Statistical analysis
The degree of homogeneity between the groups at baseline was confirmed through the analysis of initial volume and initial surface area of the root canals ($p>0.05$). Data distribution was verified for normality with the Shapiro–Wilk test. Due to the lack of normality, a Kruskal-Wallis test was used to compare intragroup transportation and centering ability parameters. The Mann-Whitney T test was used to compare canal transportation and centering ability between the same canal sections in different groups. The data were processed with Prism 7.0 (GraphPad Software, Inc., La Jolla, CA, USA) and expressed as the median, minimum and maximum values. The significance level was set at 5%.

RESULTS
The degree of homogeneity of the matched teeth regarding canal volume and surface area before root canal preparation was confirmed ($p>0.05$). No significant difference was found regarding the percentage of unprepared root canal areas between

groups for the entire root canal or in the apical third (p>0.05). There was an increase in volume and surface area after root canal preparation compared to the initial sample in the groups tested. These results are described in Table 1 and Fig. 1.

No significant difference was observed in centering ability between the experimental groups (p>0.05). Canal transportation showed no statistically significant differences in the intragroup comparison at the evaluated sections in either group (p>0.05). When each section was analyzed separately, WaveOne gold had less transportation than the MTwo file only at the 5 mm section (p<0.05). No statistical difference was found in centering ability at any of evaluated levels between groups (p>0.05). The total analyzed values are shown in Table 2.

Table 1. Median, minimum and maximum values of volume, surface area and percentage of unprepared canal area in root canal and apical third, after the different root canal preparations in WaveOne Gold and Mtwo Groups

	WaveOne Gold	Mtwo	WaveOne Gold	Mtwo
Volume (mm³)	**Root canal**		**Apical third**	
Intact canal volume	10.76 (4.20 ± 28.52) ^{aA}	10.83 (5.73 ± 22.22) ^{aA}	0.84 (0.32 ± 2.23) ^{aA}	0.99 (0.48 ± 2.44) ^{aA}
After preparation	12.19(7.06 ± 30.31) ^{bA}	13.45 (6.56 ± 22.89) ^{bA}	0.94 (0.56 ± 3.03) ^{bA}	1.07 (0.49 ± 11.27) ^{bA}
Area (mm²)				
Intact canal area	63.42 (48.09 ± 113.10) ^{aA}	82.14 (56.79 ± 109.50) ^{aB}	10.79 (3.12 ± 18.12) ^{bA}	11.79 (4.05 ± 20.56) ^{aA}
After preparation	70.21 (52.89 ± 118.60) ^{bA}	85.67 (61.12 ± 116.30) ^{bB}	11.33 (3.75 ± 19.44) ^{bA}	11.79 (4.09 ± 21.73) ^{bA}
Unprepared canal area (%)	7.96 (3.00 ± 77.64) ^{A}	10.18 (1.51 ± 71.90) ^{A}	11.33 (3.12 ± 18.12) ^{A}	11.79 (4.05 ± 21.73) ^{A}

Different lowercase letters indicate statistically significant differences between columns and different uppercase letters indicate statistically significant differences between rows (p<0.05).

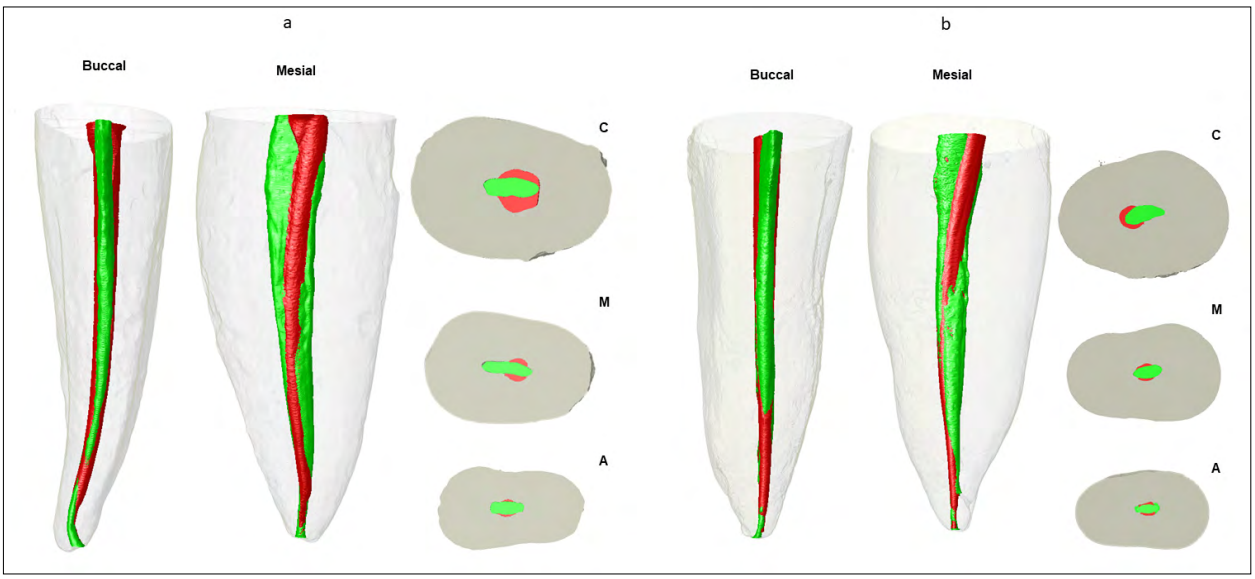

Fig. 1: *Representative 3D micro-CT images before (green) and after (red) root canal preparation of experimental groups: a) WaveOne Gold and b) MTwo. Representative transverse section of canals before (green) and after (red) root preparation at coronal (C), middle (M), and apical (A) thirds.*

Table 2. Canal transportation and centering ability (mm) in the root canals sections after preparation for the two instrumentation systems

		Transportation	Centering ability
Instrumentation system	**Level (mm from the apex)**	**Median (min - max)**	**Median (min - max)**
WaveOne Gold	3mm	0.06 (0.00 - 0.19) aA	0.05 (0.00 – 0.23) aA
	5mm	0.07 (0.00 - 1.09) aB	0.03 (0.00 - 0.12) aA
	7mm	0.04 (0.00 - 0.28) aA	0.03 (0.00 - 0.27) aA
MTwo	3mm	0.52 (0.01 – 1.00) aA	0.52 (0.01 – 1.00) aA
	5mm	0.42 (0.00 - 0.88) aA	0.30 (0.00 - 1.00) aA
	7mm	0.42 (0.00 - 0.87) aA	0.48 (0.01 - 1.00) aA

Different lowercase letters in each column indicate statistically significant differences within the same group between all evaluated sections.
Different uppercase letters in each column indicate statistically significant differences between groups for each evaluated canal section.

DISCUSSION

The development of nickel-titanium (NiTi) rotary systems led to progress in root canal instrumentation[19]. However, failures may occur in oval and flattened canals because the instruments generally provide a rounded cross-section preparation, presenting a challenge to prepare all root canal walls. The instrumentation of these cases is more difficult due to the greater amount of dentin that must be removed to accomplish the ideal root canal shape[3,20]. The unprepared areas may harbor remnants of tissue and bacterial byproducts that could cause persistent infection and affect the success of endodontic treatment[21].

Neither of the systems evaluated in this study was able to completely prepare the root canal, which agrees with previous studies[22-24]. Also, no significant difference was found for unprepared areas between WOG and Mtwo instruments, either in the entire root canal or in the apical third. Thus, the first hypothesis was accepted. These results can be attributed to the standardization of the apical third by the diameter of the instruments tested[25,26].

NiTi instruments have led to significant progress in root canal preparation[27]. Centering ability was evaluated as described by Gambill et al.[18], who defines centering ability as the ability of the endodontic instrument to remain on the central axis of the root canal. In the present study, no significant difference was observed in centering ability between experimental groups, which is in line with other studies[12,28]. Although our study showed similar shaping ability in general results, when each section was analyzed separately, WOG file had less transportation than the MTwo instrument at the 5 mm section from the apex, which partially rejects the second hypothesis. This result can be explained by the fact that WOG is a gold wire heat-treated instrument, while Mtwo is a NiTi SE instrument which does not have controlled memory. The thermally treated NiTi alloys present a higher percentage of martensitic phase, which is more flexible than conventional NiTi files, and may explain why there is less canal transportation of WOG at the 5 mm section from the apex[29]. The present study selected only long oval-shaped canals because they are considered a significant clinical challenge[30]. Moreover, the sample was selected through micro-CT analysis, which provides excellent pairing of teeth, reducing the anatomical bias related to heterogeneity of root canal morphology[4]. The micro-CT technique affords reliable results in the evaluation of data on 2D and 3D parameters of root canal preparation because it is a trustworthy, precise method for this kind of analysis[5].

Based on our results, WaveOne Gold and Mtwo systems presented similar shaping ability and centering ability during oval-shaped root canal preparation. However, WOG presented less transportation than MTwo at the 5 mm section from the apex.

DECLARATION OF CONFLICTING INTERESTS
The authors declare no potential conflicts of interest regarding the research, authorship, and/or publication of this article

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Comparison between indexes for diagnosis and guidance for treatment of dental caries

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ABSTRACT
The objective of this study was to establish the correlation between an index for caries treatment needs and an index for caries lesions detection and evaluation. A cross-sectional study was performed on three samples of children aged 3 (n = 302), 5 (n = 183), and 11-14 years old (n = 60), attending early childhood centers and schools in the cities of Avellaneda, Rio Grande, and Buenos Aires. Dental caries treatment needs were estimated on every child through the Caries Treatment Needs Index (CTNI) for programming resources allocation. Also, a diagnosis of dental caries was made according to ICDAS II criteria. After the diagnostic procedures, a dental care program was developed. The ICDAS II variable was operationalized by grouping the codes into four categories: G1: code 0; G2: code 1-2; G3: code 3; G4: code 4-5-6. Measures of central tendency and dispersion were calculated for both variables, and the correlation was calculated using the Spearman coefficient. Results revealed that in the group of 3-year-old children, a significant correlation was observed in G1 (rho = -0.822); G2 (rho = 0.330); G3 (rho = 0.509) and G4 (rho = 0.710) between both indexes. For the group of 5-year-old children, a significant correlation was observed in G1 (rho = -0.821); G2 (rho = 0.260); G3 (rho = 0.344) and G4 (rho = 0.840). In the group of children 11-14 years of age, a significant correlation was observed in G1 (rho = -0.692); G3 (rho = 0.437) and G4 (rho = 0.764). The indices analyzed in this study (CTNI and ICDAS II) show reasonable equivalence for use in clinical and epidemiological studies based on the statistical analysis.
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Keywords: *dental caries - epidemiology - Argentina - children - validation study.*

Comparación entre índices para el diagnóstico y orientación del tratamiento de caries dental

RESUMEN
El objetivo de este estudio fue establecer la correlación entre un índice de necesidad de tratamiento de caries y un índice para la detección y evaluación de lesiones de caries. Se realizó un estudio de corte transversal en tres muestras de niños de 3 años (n = 302), 5 años (n = 183) y 11-14 años (n = 60), que asistían a centros y escuelas de primera infancia en las ciudades de Avellaneda, Rio Grande y Buenos Aires. Las necesidades de tratamiento de caries dentales se estimaron en cada niño a través del Índice de Necesidad de Tratamiento de Caries (INTC) para la asignación de recursos de programación. Además, se realizó un diagnóstico de caries dental según los criterios de la ICDAS II. Después de los procedimientos de diagnóstico, se desarrolló un programa de atención odontológica. La variable ICDAS II se operacionalizó agrupando los códigos en cuatro categorías: G1: código 0; G2: código 1-2; G3: código 3; G4: código 4-5-6. Se calcularon medidas de tendencia central y dispersión para ambas variables y la correlación se calculó mediante el coeficiente de Spearman. Los resultados revelaron que en el grupo de niños de 3 años se observó una correlación significativa en G1 (rho = -0,822); G2 (rho = 0,330); G3 (rho = 0,509) y G4 (rho = 0,710) entre ambos índices. Para el grupo de niños de 5 años se observó una correlación significativa en G1 (rho = -0,821); G2 (rho = 0,260); G3 (rho = 0,344) y G4 (rho = 0,840). En el grupo de niños de 11 a 14 años se observó una correlación significativa en G1 (rho = -0,692); G3 (rho = 0,437) y G4 (rho = 0,764). Los índices analizados en este estudio (INTC e ICDAS II) muestran una equivalencia razonable para su uso en estudios clínicos y epidemiológicos, basado en el análisis estadístico realizado.

Palabras clave: *caries dental - epidemiología - Argentina - niños - estudio de validación.*

INTRODUCTION

Health is interpreted as the outcome of factors related to ways of life, lifestyles and quality of life, and recognizes the involvement of the following variables: (a) the oral and dental healthcare models in different countries as a result of the characteristics of their respective healthcare systems and (b) the characteristics of knowledge management implemented at graduate and postgraduate levels of healthcare studies[1-3].

Dental caries is the outcome of complex interaction over time between acid-producing bacteria, fermentable carbohydrates, and the host's internal and external factors. The risk of developing caries includes physical, biological, socio-environmental, and behavioral characteristics and factors related to living conditions and lifestyle. The micro-circumstances for caries development include different microorganisms, incompetent salivary flow, insufficient exposure to fluoride, and chemically propitious nutrition variables. The outcome of these processes causes a progressive net loss of minerals in dental tissues, enabling caries lesions to develop[4]. The initial caries lesion is intended to maintain or recover the health of the affected tooth/teeth[5].

Available indicators for addressing dental caries conceptually and operatively must conform to the theoretical framework on which studies are based and enable precise identification of the process of clinical development of the disease from its early stages to its complications. The available indicators may be simple or complex. The transfer of the results expressed by the different values must help to systematize diagnosis and guide proactive, efficacious, effective, and long-lasting interventions. The various indicators that have been developed can be classified according to the different variables that they address[6]: the past history of caries; the risk factors involved[7]; the stages in the development process of dental caries lesions[8-9]; and the integration between the process of caries lesion development in terms of magnitude (severity and extension) and the treatments recommended according to the approach for risk control[10-11]. Also, it is necessary to clarify the differences between caries diagnosis and lesion identification. Diagnosis involves the dental professional's interpretation regarding the sum of available data, while lesion identification involves applying an objective method to determine whether or not the lesion is present, and once it has been identified, the assessment can be established. Pitts et al.[12-14] and Ekstrand et al.[15,16] developed the International Caries Detection and Assessment System (ICDAS II) – a system for caries detection and assessment that integrates three dimensions based on the lesion development process, which synthesizes substantial evidence for making political, sanitary and clinical decisions.

In 1993, the Caries Treatment Needs Index (CTNI) (Fig. 1) was designed and applied. The CTNI is based on the interaction of two axes: one based on the lesion's progression and the other based on the technological resources needed to control the risk of dental caries[17-18]. The progression axis identifies the magnitude of severity and extent. The component referring to the magnitude of severity identifies the process of tissue compromise of the dental caries lesion, going from a clinical threshold recognized as white spot[19], which is distinct from the white spot caused by hypomineralization to subsequent progression in cavitation, including dental tissues. The magnitude of extent in the "mouth unit" is expressed by the number of mouth quadrants with visible lesions. The technological axis includes the risk component and the available technological development component. The risk component results from variables identified and often caused by the previous omission of appropriate actions for controlling the process. Technological development is based on contextualized scientific evidence and expressed as the proper strategies and their application per mouth unit and teeth, according to the magnitude recorded in dental quadrants.

Any index must be validated regarding the reliability and validity of the construct, contents, and criteria[20]. Rigorous application of indices by the examiner requires a calibration process including (a) theoretical knowledge of the indicator and cutoff points between its categories, (b) practical recognition in situations "on paper" and in the clinic, and (c) calibration *per se*, establishing inter-examiner differences between the "gold standard" or "reference examiner" and the new examiner, and intra-examiner differences, i.e., the variations recorded among observations made by one professional.

Dental caries is one of the most prevalent chronic diseases globally, affecting people throughout their lifetime[21]. Today, its distribution and severity vary among different regions, and its onset is strongly

CODE	DIAGNOSTIC	ANALYSIS UNIT	TREATMENT PLAN
00	Sound teeth with history of preventive measures	Mouth	Preventive program: Low or moderate caries risk
01	Sound teeth without history of preventive measures		Preventive program: Low or moderate caries risk
02	Presence of non-cavitated caries lesions (white spots)		Preventive program: High caries risk
03	Presence of cavitated lesions affecting enamel and/or dentine	1 quadrant	Preventive program: High caries risk + Restorative treatment
04		2 quadrants	Preventive program: High caries risk + Restorative treatment
05		3 quadrants	Preventive program: High caries risk + Restorative treatment
06		4 quadrants	Preventive program: High caries risk + Restorative treatment
07	Presence of cavitated lesions affecting enamel and/or dentine and with pulp involvement	1 quadrant	Preventive program: High caries risk + Restorative treatment + Pulp treatment
08		2 quadrants	Preventive program: High caries risk + Restorative treatment + Pulp treatment
09		3 quadrants	Preventive program: High caries risk + Restorative treatment + Pulp treatment
10		4 quadrants	Preventive program: High caries risk + Restorative treatment + Pulp treatment
11	Presence of extense cavitated lesions without possibilities of restorative treatment or presence of abscess or fistula	1 quadrant	Preventive program: High caries risk + Restorative treatment + Surgical treatment and eventual rehabilitation
12		2 quadrants	Preventive program: High caries risk + Restorative treatment + Surgical treatment and eventual rehabilitation
13		3 quadrants	Preventive program: High caries risk + Restorative treatment + Surgical treatment and eventual rehabilitation
14		4 quadrants	Preventive program: High caries risk + Restorative treatment + Surgical treatment and eventual rehabilitation

Fig. 1: Caries Treatment Needs Index (CTNI)

associated with environmental, sociocultural, economic, and behavioral factors[22-24]. The epidemiological profile of dental caries differs significantly between central and developing countries. However, variables that identify complex social issues such as poverty provide a perspective from which to analyze heterogeneity within the homogeneity of countries. In Argentina, Piovano et al.[22] studied the magnitude of dental caries, establishing the treatment needs in a sample of 2917 children, adolescents, and adults. Kassebaum et al.[23]conducted a worldwide systematic review and meta-regression using epidemiological data on untreated caries and subsequent estimates of internally consistent prevalence and incidence. Separate meta-regression was performed for untreated caries in primary and permanent teeth, respectively, using the Global Burden of Disease model[24].

Different authors have compared dental caries indices. Campus et al.[25] compared ICDAS, CAST, NYVAD, and DMF indices. They demonstrated that the most significant difference among methods was shown by the number of sound teeth ($p < 0.01$). In a cross-sectional study, Sarno et al.[26] demonstrated that the mean time taken to apply the DMF was 3.8 min; for ICDAS, it took 8.9 min, and for CAST, 4.7 min. The mean numbers of decayed, missing, and filled teeth were 6.0 according to the DMF, 6.2 according to ICDAS, and 5.9 according to CAST. When the disease extension indicator was used, the following percentages of teeth were affected by caries: DMF 22.12%, ICDAS 49.11%, and CAST 33.2%. The DMF underestimated the

occurrence of caries lesions in individuals but was the fastest method to apply. ICDAS obtained detailed information regarding lesion severity, but it was a time-consuming method and challenging to analyze. A systematic review based on specificity and sensitivity studies of each system[27] revealed that sensitivity and specificity are greater with ICDAS than with the dmft/DMFT index and provide up to 43% more information in identifying non-cavitated lesions. Still, it takes longer to perform and involves more resources because it uses light, compressed air, and prophylaxis before the examination. Banava et al.[28] revealed that the ICDAS provides more accurate information than DMF for the investigators and epidemiologists. Similar findings were reported by Melgar et al., who informed that the DMFT index might underestimate 60% of non-cavitated lesions in children and 16.6% in adults[29].

The objective of this study was to establish the correlation between an index for caries treatment needs and an index for caries lesions detection and evaluation.

MATERIALS AND METHODS

The study project was approved by the Ethics Committee of the School of Dentistry of the University of Buenos Aires. (PAIIO-02 2019-2024).

A cross-sectional study was designed on a non-probabilistic sample of children and early adolescents (n=546) with no previous dental care during the last year (Table 1):

(a) A group 3-year-old children (n= 302) from an early childhood center (Avellaneda, Provincia de Buenos Aires).
(b) A group of 5-year-old children (n= 183) from two kindergartens (Rio Grande, Provincia de Tierra del Fuego, Antártida e Islas del Atlántico Sur).
(c) A group of 11 to 14-year-old early adolescents (n= 61) from a middle school (Ciudad Autónoma de Buenos Aires).

According to existing national criteria, the three institutional settings were classified as belonging to marginal urban level neighborhoods.

Before including children in the study, their legal guardians were asked for informed consent, and each child's formal assent was verified. All participants and their legal guardians were informed of the results of the examinations and diagnoses and included in and/or referred to a dental care program at local institutions.

Table 1: Frequency and percentage distribution per sex according to age groups

Age groups		Sex		Total
		Male	Female	
Up to 3 years	Frequency	155	147	302
	%	51.3%	48.7%	100.0%
5 years	Frequency	88	95	183
	%	48.1%	51.9%	100.0%
11 to 14 years	Frequency	29	32	61
	%	47.5%	52.5%	100.0%
Total	Frequency	272	274	546
	%	49.8%	50.2%	100.0%

No statistical difference observed in the proportion of sexes in age groups.

Clinical diagnoses were performed by one researcher who was calibrated in caries diagnosis according to CTNI (18) and ICDAS II criteria (kappa 0.75). Results of dental examinations were recorded in individual charts and used to design individual treatment plans.

On each patient, the indexes were operationalized as follows: CTNI grouped according to 4 standardized categories: code 00-02 (sound teeth with or without preventive measures or presence of non-cavitated caries lesions; code 03-07 (presence of cavitated lesions affecting enamel and/or dentine in 1 to 4 quadrants); code 07-10 (presence of cavitated lesions affecting enamel and/or dentine and with pulp involvement in 1 to 4 quadrants); and code 11-14 (presence of extense cavitated lesions without possibilities of restorative treatment or presence of abscess or fistula in 1 to 4 quadrants).

ICDAS II were grouped in 7 categories: Code>0; Code ≥3; Code 1-2; Code 3; Code 3-4; Code 5-6 and Codes 4-5-6. In all cases, only active caries lesions were included. Also we grouped for other analysis ICDAS codes in 3 categories: Category ICDAS >0 (number of surfaces with lesions ICDAS code 1 to 6); category ICDAS 3 to 6 (number of surfaces with lesions ICDAS code 3 to 6); and category ICDAS =0 (number of surfaces without lesions ICDAS code =0).

Statistical analysis

Frequencies, percentages, median, and quartiles were calculated for the values recorded using both diagnostic methods. The Jonckheere-Terpstra ordered alternatives test for independent samples was used to compare the distribution of lesions ICDAS = 0, ICDAS>0, and ICDAS 3 or higher among the

4 CTNI groups. For pairwise comparison, they were adjusted using Bonferroni's correction. Correlation between CTNI and ICDAS was established by Spearman's rho coefficient. For comparison between indices, the ROC curve was used with Hanley and McNeil´s approximation method. Area under the curve was calculated with 95% confidence intervals; cutoff variable was established as CTNI 00-02 / 03-14 and CTNI 00-06 / 07-14; and variable dependent on the distribution of affected surfaces as ICDAS > 0, ICDAS 3-6, ICDA 4-6, and ICDAS 5-6.

RESULTS

Analysis of correlation of surfaces diagnosed according to ICDAS II and CTNI

The distribution of lesions ICDAS = 0, ICDAS > 0 and ICDAS 3 or greater with CTNI grouped as: 00-02, 03-06, 07-10 and 11-14 is shown in Fig. 2. Statistically significant differences are observed upon comparing CTNI grouped in 4 categories to the distribution of surfaces according to ICDAS=0, ICDAS>0, ICDAS 3 or greater ($p<0.000$). Pairwise comparison of CTNI according to distribution of ICDAS lesions showed statistically significant difference except between categories 07-10 and 11-14, with non-significant differences in the three comparisons performed. As the complexity of the record of lesions according to CTNI increases, there is an increase in the number of surfaces diagnosed with ICDAS>0 and with ICDAS ≥ 3, and a decrease in caries-free surfaces recorded with ICDAS II., except between categories 07-10 and 11-14, with non-significant differences in the three comparisons performed. As the complexity of the record of lesions according to CTNI increases, there is an increase in the number of surfaces diagnosed with ICDAS>0 and with ICDAS ≥ 3, and a decrease in caries-free surfaces recorded with ICDAS II.

The correlation between CTNI scores and distribution of surfaces for ICDAS>0, ICDAS=3-6, ICDAS=4-6, ICDAS=5-6, ICDAS=3-4, ICDAS=1-2 and for the number of surfaces without lesions ICDAS=0 was calculated for the age groups studied and for the total sample (Table 2; Fig. 3). In all cases, the results of Spearman's correlation were statistically significant, except for the group 11-14 years for distribution of lesions ICDAS=1-2. The correlation between CTNI scores and the distribution of surfaces for ICDAS>0, ICDAS=3-6, ICDAS=4-6, ICDAS=5-6, ICDAS=3-4 for each age group was very high. For distribution of lesions ICDAS=1-2, the correlation was low but statistically significant. The correlation between CTNI score and distribution of lesions ICDAS=0 (number of surfaces without lesions) was very high, and high and inverse (negative) for each age group. Fig. 3 describes the correlation between CTNI and ICDAS>0.

Analysis of the results of the ROC curve for distribution of lesions ICDAS for CTNI 00-02 vs. 03-14 and CTNI 00-06 vs. 07-14.

For CTNI 00-02 / 03-14, area under the ROC curve is very high for ICDAS>0 and ICDAS=3-6, and high for ICDAS=4-6 and ICDAS=5-6 (Table 3; Fig. 4). For CTNI 00-06 / 07-14, area under the ROC curve is very high for ICDAS>0, ICDAS=3-6, ICDAS=4-6 and ICDAS=5-6 (Table 4; Fig. 5).

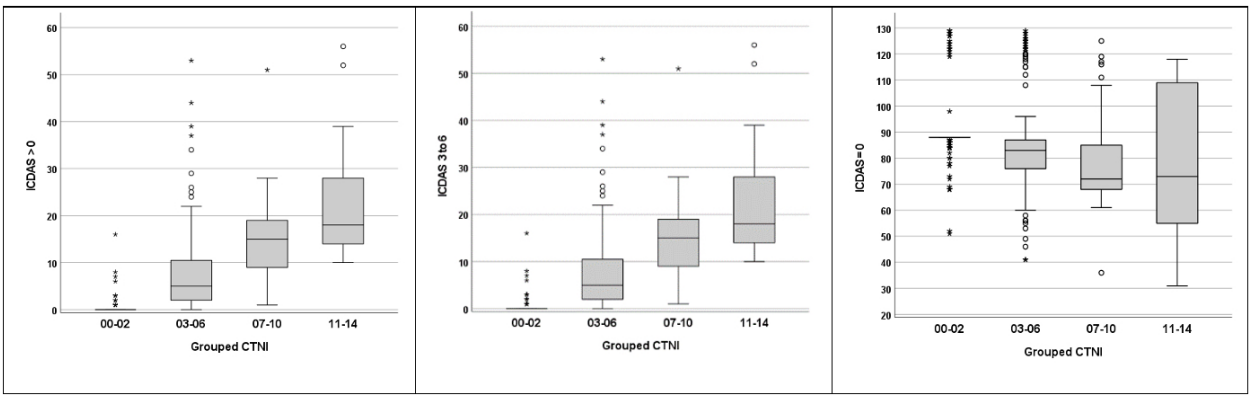

Fig. 2: Distribution of quantity of ICDAS surfaces per grouped CTNI
ICDAS>0: number of surfaces with lesions ICDAS 1 to 6
ICDAS 3to 6: number of surfaces with lesions ICDAS 3 to 6
ICDAS=0: number of surfaces without lesions ICDAS =0

Table 2: Spearman's correlation between CTNI and different groupings of ICDAS lesions for the 3 age groups and for the total

Up to 3 years n=302	ICDAS>0	ICDAS=3-6	ICDAS=4-6	ICDAS=5-6	ICDAS=3-4	ICDAS=1-2	ICDAS=0
Spearman's rho	0.822	0.804	0.710	0.653	0.605	0.319	-0.724
p value	0.000	0.000	0.000	0.000	0.000	0.000	0.000
5 years n=183	**ICDAS>0**	**ICDAS=3-6**	**ICDAS=4-6**	**ICDAS=5-6**	**ICDAS=3-4**	**ICDAS=1-2**	**ICDAS=0**
Spearman's rho	0.821	0.870	0.844	0.839	0.439	0.214	-0.791
p value	0.000	0.000	0.000	0.000	0.000	0.004	0.000
11-14 years n=61	**ICDAS>0**	**ICDAS=3-6**	**ICDAS=4-6**	**ICDAS=5-6**	**ICDAS=3-4**	**ICDAS=1-2**	**ICDAS=0**
Spearman's rho	0.656	0.784	0.660	0.615	0.582	-0.068	-0.619
p value	0.000	0.000	0.000	0.000	0.801	0.603	0.000
Total n=546	**ICDAS>0**	**ICDAS=3-6**	**ICDAS=4-6**	**ICDAS=5-6**	**ICDAS=3-4**	**ICDAS=1-2**	**ICDAS=0**
Spearman's rho	0.897	0.900	0.812	0.777	0.698	0.529	-0.486
p value	0.000	0.000	0.000	0.000	0.000	0.000	0.000

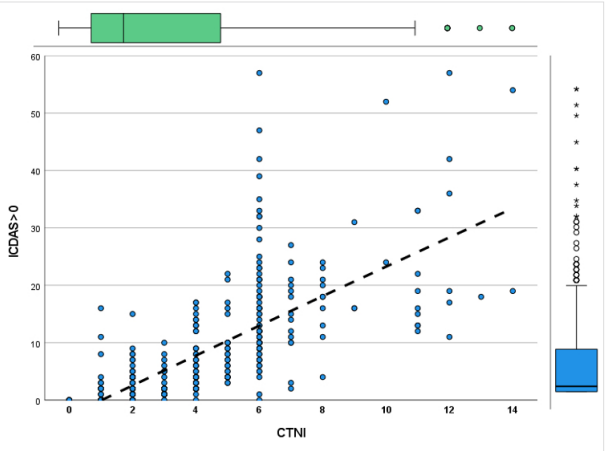

Fig. 3: Spearman's correlation between CTNI and ICDAS lesions > 0 for total sample.

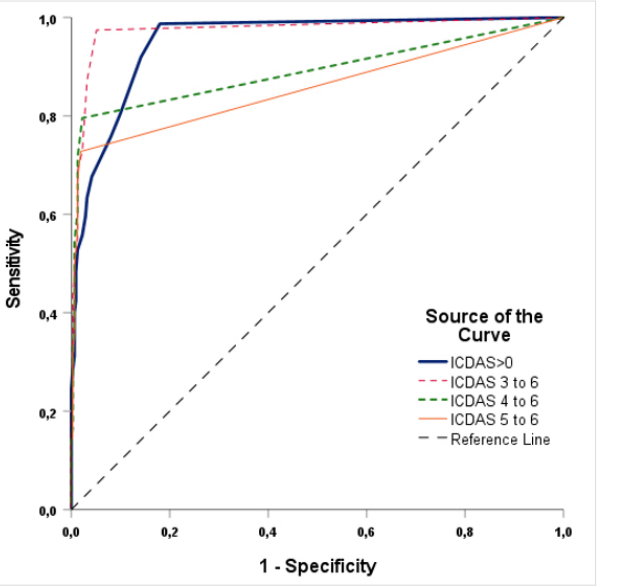

Fig. 4: ROC Curve. Cutoff values CTNI: 00-02 vs. 03-14 for distribution of surfaces ICDAS>0, ICDAS 3 or greater, ICDAS 4 or greater, ICDAS 5 or greater

Table 3: Area under the curve and CI95% for cutoff values CTNI: 00-02 vs. 03-14 for distribution of surfaces ICDAS>0, ICDAS 3 or greater, ICDAS 4 or greater, ICDAS 5 or greater

Area under the curve for CTNI: 00-02 vs. 3-14				
Variables of test result	Area	p value	CI 95%	
			Lower limit	Upper limit
ICDAS>0	0.952	0.000	0.936	0.969
ICDAS 3 -6	0.974	0.000	0.960	0.989
ICDAS 4 or greater	0.890	0.000	0.858	0.922
ICDAS 5 or greater	0.857	0.000	0.821	0.893

Table 4: Area under the curve and CI95% for cut-off values CTNI: 00-06 vs. 07-14 for distribution of surfaces ICDAS>0, ICDAS 3 or greater, ICDAS 4 or greater, ICDAS 5 or greater.

Area under the curve for ICDAS= 7-14				
Variables of test result	Area	p value	CI 95%	
			Lower limit	Upper limit
ICDAS>0	0.927	0.000	0.900	0.955
ICDAS 3 -6	0.933	0.000	0.908	0.959
ICDAS 4 or greater	0.931	0.000	0.907	0.955
ICDAS 5 or greater	0.919	0.000	0.885	0.954

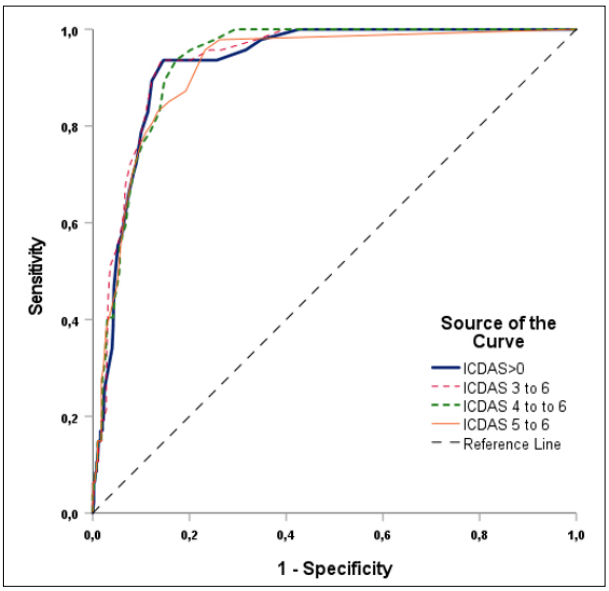

Fig. 5: ROC Curve. Cutoff values CTNI: 00-06 vs. 07-14 for distribution of surfaces ICDAS>0, ICDAS 3 or greater, ICDAS 4 or greater, ICDAS 5 or greater.

DISCUSSION

In general terms, health indicators represent summary measurements capturing relevant information on different attributes and dimensions of health status and healthcare system performance which, viewed jointly, intend to reflect the health situation and can be used for surveillance. Indicators must be easy for analysts to use, interpret, and understand to users, such as decision-makers and managers.

A set of health indicators with quality attributes that are appropriately defined and maintained provide information for preparing an epidemiological profile and other kinds of analysis of the health-disease-attention-care situation. The selection of such a set of indicators –and its levels of disaggregation– can vary according to the availability of information systems, data sources, resources, needs, and specific priorities in each region or country.

It is essential to monitor indicator quality because it conditions users' confidence level in health information, and therefore, the regular use of indicators.

Health indicators also depend on the policy for disseminating them, including the timeliness and frequency they are compiled. Availability of a basic set of indicators provides the raw material for analyzing health. Moreover, it can facilitate the monitoring of health objectives and goals, foster analytical capacities in healthcare teams, and serve as a platform for promoting the development of intercommunicated health information systems.

Valid and reliable health indicators are essential tools required by epidemiology for health management. The indices compared in this study provide the possibility of analyzing the impact on health scenarios. Clinical discrimination among different stages in caries lesion processes, activity, and arrest, supported by various histological and histochemical studies, finds appropriate clinical instruments.

In statistics, a proxy variable is a measure that enables other more valuable variables to be found, whether for designs or for including the results indistinctly in information technology. For such purpose, the proxy variable must have a strong correlation –though not necessarily linear or positive– with the inferred value. Both the indices analyzed in this study meet this requirement. When comparing results obtained by other authors, the INTC takes as criteria for categorizing the development process of dental caries, identifying the first of the visual differences with healthy tissue. In such a sense, it coincides with the ICDAS in all its formulations.

The CTNI index. Based showed a higher correlation than other indicators with ICDAS II. It is worth highlighting the frequent calibrations requiring and verifying the equivalence between the therapeutic criteria recommended in the CTNI and the ICDAS II. The indices analyzed in this study (CTNI and ICDASII) show reasonable equivalence for use in clinical and epidemiological studies based on the statistical analysis. Comparing these indicators to other indicators, including their application in studies on different life stages, would enable their validity and relevance to be considered for global health studies.

DECLARATION OF CONFLICTING INTERESTS
The authors declare no potential conflicts of interest regarding the research, authorship, and/or publication of this article

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IADR
International Association for Dental Research

Latin American Region IADR

SAIO
SOCIEDAD ARGENTINA DE INVESTIGACION ODONTOLOGICA
División Argentina de la International Association for Dental Research

VIII CONGRESO de la REGIÓN LATINOAMERICANA de la IADR

LIV REUNIÓN CIENTÍFICA ANUAL SAIO

11 al 13 noviembre 2021

Buenos Aires / **ARGENTINA**

On November 11, 12 and 13, the 8th IADR Latin American Region Congress and the 54th Annual Meeting of the Argentine Society for Dental Research (SAIO) -the Argentine Division of the IADR- was held virtually. The President of the Meeting, Dr Analía Garrofé, and the Organizing Committee worked remotely from the City of Buenos Aires.

Three hundred and fourty two participants, including researchers, graduate students and alumni attended the meeting. During the three-day meeting, we shared research work presentations, lectures, round tables and group sessions. Virtuality allowed for the presence of Dr Christopher Fox and Dr María del Carmen López Jordi on behalf of International Association for Dental Research (IADR) and Latin American Region (LAR), respectively.

We were honored by the presence and lectures of Doctors Jacques E. Nör, Jaime Castellanos, Pablo Rodríguez, María Elina Itoiz, Ricardo Macchi, Valentim Barão, Vanesa Pereira Prado, Yasmi Crystal, María del Carmen López Jordi, Aldo Squassi, Sylvia Piovesan Suárez, Noemí Bordoni, Rita Villena, Débora Heller, Lilia Bernal-Cepeda, María Fidela de Lima Navarro, Paulo Cesar, and Loreto Abusleme Ramos

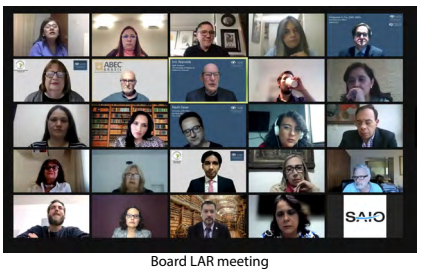
The Annual Meeting began on November 10th, in a pre-session in which the executive meetings of the SAIO groups, the LAR Cariology and Dental Materials groups, the OICAL and Caries-Out groups meetings were held, and as the last activity of the date´s agenda, the executive meeting of the LAR Council was also held.

Proposals for research projects were presented during the meeting of the Research Group related to the subject of the project in order to encourage discussion and closer collaboration among experts and peers in each dental discipline.

Thanks to the 342 registered participants, the "Virtual" 8th IADR Latin American Region Congress and LIV Annual Scientific Meeting of SAIO 2020 have had an excellent scientific level. We are grateful to Doctor Analía Garrofé -President of the Organizing Committee-, and his great work team, for paying attention to all the details for ths scientific meeting to succeed.

We look forward to seeing you at the LV Annual Scientific Meeting 2022 in Buenos Aires City. We wish Doctor Javier Fernandez Solari, President of the upcoming Meeting the greatest success!

Congress virtual platform

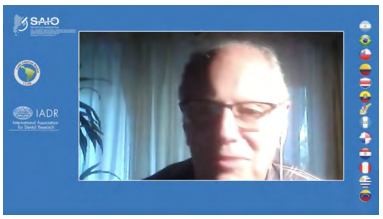


Board LAR meeting

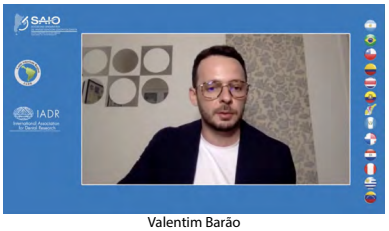
Jacques E. Nör

Jaime Castellanos

Pablo Rodríguez



Ricardo Macchi



Valentim Barão

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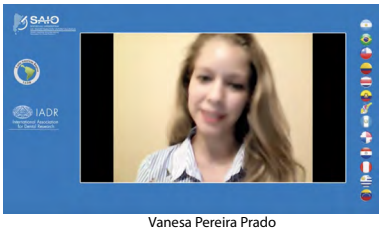

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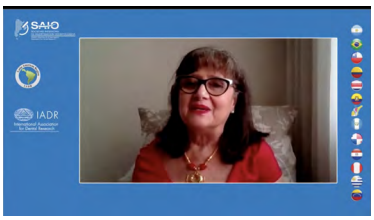

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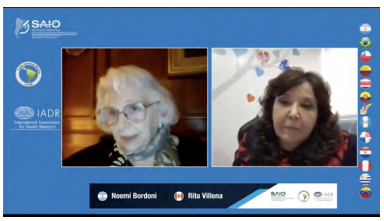

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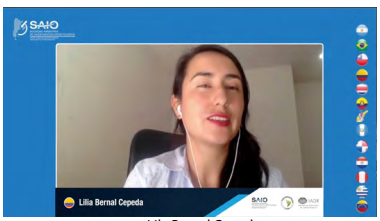

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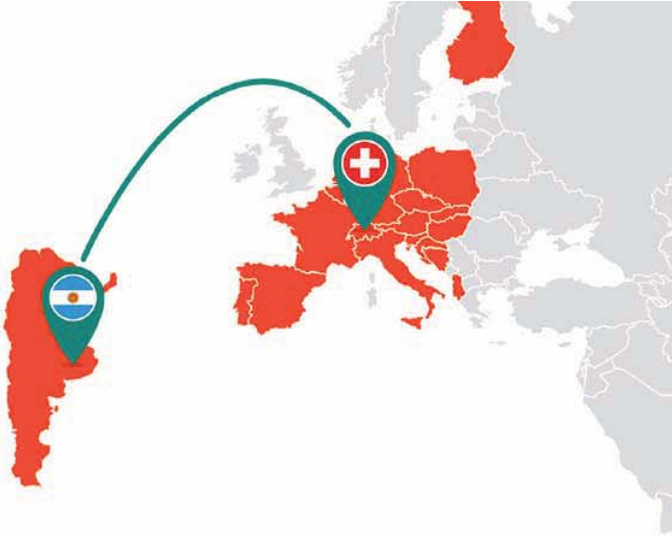

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La principal causa de la biocorrosión dental en la actualidad proviene de la dieta rica en bebidas y alimentos ácidos. En estos casos, el flujo salival cambia y afecta directamente la función remineralizante en el esmalte, haciéndolo más vulnerable. Debido a esto, todos somos susceptibles al daño del esmalte. Por lo tanto, el secreto es protegerlo y fortalecerlo diariamente, evitando la degradación temprana de los dientes y manteniéndolos saludables durante toda la vida.

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