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Precision and accuracy of four current 3D Printers to achieve models for Fixed Dental Prosthesis

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ABSTRACT
The aim of this study was to compare the accuracy and precision of 3D printers used to obtain models of fixed dental prostheses. A fixed dental prosthesis preparation was scanned and reproduced by four 3D printers: RapidShape P40, Asiga MAX, Varseo, and Photon. The impressions were scanned again, and the dataset was compared to the original dataset. Mean discrepancies (µm) were 52.97±20.48 (RapidShape P40), 68.27±43.53 (Asiga MAX), 62.22±56.21 (Varseo), and 80.03±28.67 (Photon). There was no difference (p=0.314) in accuracy; however, the precision differed (p=0.015) among the 3D printers. The printers had distinct precision but did not differ in accuracy.
Received: November 2019; Accepted March 2020.

Keywords: *dental models, dental prosthesis, three-dimensional printing.*

Precisão de quatro impressoras 3D para obtenção de modelos para prótese fixa

RESUMO
O objetivo desse trabalho foi comparar a acurácia e a precisão de impressoras 3D utilizadas para a obtenção de modelos para prótese fixa. Um preparo para prótese fixa foi escaneado e reproduzido por 4 impressoras 3D: RapidShape 3D, Asiga MAX, Varseo e Photon. As impressões foram novamente escaneadas, e o dataset escaneado foi comparado ao original. Os escaneamentos foram sobrepostos digitalmente e determinada a discrepância entre os modelos original e impresso. A discrepância média (µm) entre os modelos foi de foi 52,97±20,48 (RapidShape 3D), 68,27±43,53 (Asiga MAX), 62,22±56,21 (Varseo) e 80,03±28,67 (Photon). Não houve diferença (p=0,314) entre os valores médios, os quais representam a acurácia; entretanto, o desvio padrão dessas foi diferente (0,015), indicando diferença na precisão das impressoras 3D.

Palavras-chave: *modelos dentários, prótese dentária, impressão tridimensional.*

INTRODUCTION

The use of digital workflow in rehabilitation treatments is rapidly increasing. Given the practicality, biological safety, and comfort for the patient and health professional, this technology has gradually been replacing conventional workflow using stone models. Additive manufacturing (3D printing) enables the fabrication of provisional restorations and reliable preparations[1]. The accuracy and precision of these models are related to the final fit of prosthetic parts and, consequently, to the longevity of the restoration. Three-dimensional printers using the Direct Light Processing (DLP) technique have demonstrated good precision in obtaining dental models[2]. The impressions should be identical to the preparations (accuracy) and, if repeatedly printed, they should always have the same dimensions (precision). Recently, a wide variety of 3D printers has been introduced on the dental market, requiring technical assessments[3]. The present study was therefore designed to compare the accuracy and precision of four 3D printers used to obtain models of fixed dental prostheses.

MATERIAL AND METHODS

An acrylic resin model of a maxillary canine was prepared for a complete crown and then scanned (Trios, 3Shape S/A, Copenhagen, Denmark). Based on the generated dataset file (STL), models of the tooth were obtained from the impressions created using four 3D printers (Table 1). The specimens (n=8 for each printer) were scanned with a high-precision scanner (S600 ARTI, Zirkonzahn GmbH, Gais, Italy). The STLs that generated the impressions were superimposed on the STLs of the printed models using a specific software system (MeshLab 2016.12, Visual Computing Laboratory, Italy). Based on the overall superimposition prepared tooth, the discrepancies between the measurements were calculated by the Hausdorff method[4] and then qualitatively categorized according to their location. Incisal, mesial, distal, buccal and lingual areas were inspected for presence or absence of misfit (dichotomously, independently of the misfit area) and data were described in terms of misfit prevalence on each surface. The misfit was defined by the presence of red areas on superimposed images of original STL and STL of the printed models. Red areas mean that printed models are larger than original tooth. The mean overall discrepancy values of the 3D printed models were compared by Kruskal-Wallis test. The homogeneity of discrepancy variance was analyzed by the Levene test; differences in this analysis refer to the precision of the printer. The level of significance was set at 5% for all analyses.

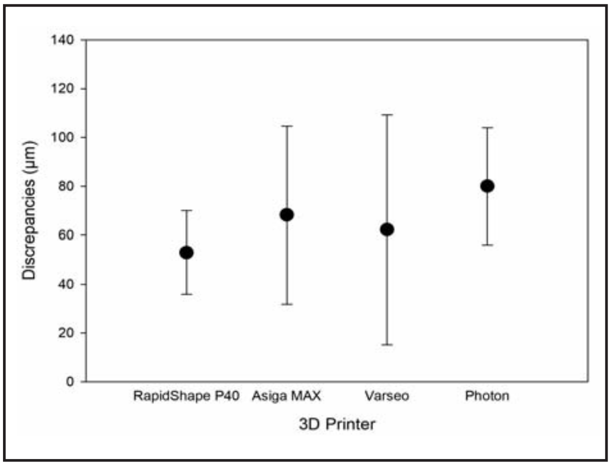

Fig. 1: Discrepancies (µm) ± standard deviation according to 3D printer. The circle indicates the mean value (accuracy) and the whiskers indicate the 95% confidence interval (precision). There was no difference in accuracy (p=0.587), but precision varied among printers (p=0.015).

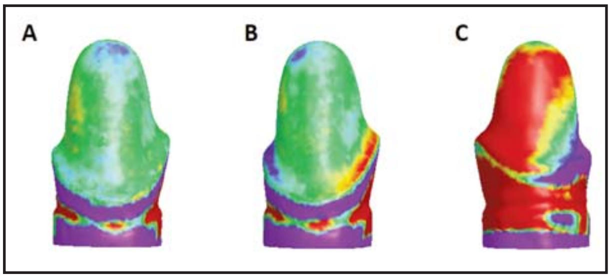

Fig. 2: Example of misfit (red areas) of models. A. no misfit; B. discrete misfit on the proximal surface; C. severe misfit on the buccal and proximal surfaces.

RESULTS

The mean discrepancy (µm) and standard deviations among the models were 52.97±20.48 (RapidShape P40), 68.27±43.53 (Asiga MAX), 62.22±56.21 (Varseo), and 80.03±28.67 (Photon), as shown in Fig. 1. Discrepancy values present a non-Gaussian distribution, and are shown in Table 1. There was no difference (p=0.314) in mean values, which

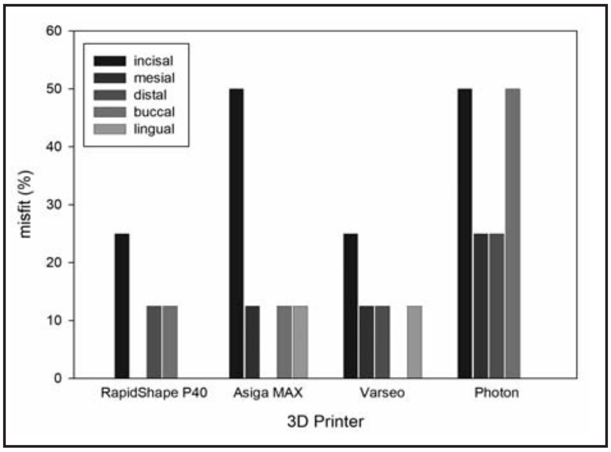

Fig. 3: Misfit (%) of models according to 3D printer and tooth surface.

Table 1: Detailed discrepancy values measured according printer evaluated.

Printer	Mean* ± sd#	Minimum discrepancy measured (µm)	Maximum discrepancy measured (µm)
RapidShape P40	52.97±20.48	31.86	84.65
Asiga MAX	68.27±43.53	29.59	126.57
Varseo	62.22±56.21	19.42	156.83
Photon	80.03±28.67	43.75	115.06

*arithmetic mean; #standard deviation of mean represent accuracy; however, the standard deviation distribution was distinct (0.015), which means that precision was different among the 3D printers. Fig. 2 shows images of superimposed original STL files on 3D printed models. Colors close to green indicate minimal discrepancy, while red areas indicate regions of greater superimposition. Fig. 3 shows the prevalence of discrepancies according to tooth surface.

DISCUSSION

Accuracy did not differ among the tested 3D printers, ranging from 52.97±20.48 to 80.03±28.67 µm. These values are consistent with the resolutions indicated by the manufacturers. Considering the distribution of these values, we can infer that is possible that all 3D printers can reproduce details in accordance with the ISO 6873 requirements for dental gypsum products, which establish a minimum detail reproduction of 75±8 µm for types 1 and 2 dental materials and of 50±8 µm for gypsum types 3, 4, and 5. The variability, given by the standard deviation, refers to the precision of the 3D printers, indicating that the most precise printer (the one with the lowest standard deviation for the mean value of discrepancy among models revealed by Levene test) was the one manufactured by Straumann.

The differences in precision could be related to the distinct resolutions, especially in the Z plane[4]. Larger deviations associated with the 3D printed model may be due to the thickness and shrinkage between the layers of the material that occur in the Z plane and to contraction of the material caused by post-curing[4]. The shrinkage in the Z plane may also be the cause of the higher prevalence of discrepancies on the incisal surface of the preparation. Positive discrepancies in this region, even without causing marginal misfit of prosthetic parts, may compromise the longevity of the restoration as a result of greater thickness of the cement line.

The large standard deviations of discrepancy values should be considered upon evaluation of the data presented. They are related to the precision of the printer, but random errors of methods used could also be present. Thus, the data presented in this study should be considered cautiously. Future clinical studies are welcome to evaluate the efficacy of oral rehabilitations where digital workflow is part of treatment.

Overall, all tested 3D printers appear to have sufficient accuracy and precision to be used in the digital workflow for patient rehabilitation. However, it should be noted that 3D printers with lower precision are more likely to lead to the misfit of prosthetic parts and consequently, to rework.

CONCLUSION

The printers had distinct precision but did not differ in accuracy.

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REFERENCES

1. Barazanchi A, Li KC, Al-Amleh, B, Lyons K, Waddell JN. Additive Technology: Update on Current Materials and Applications in Dentistry. J Prosthodont 2016; 26:156-163.
2. Kim SY, Shin YS, Jung HD, Hwang CJ, Baik HS, Cha JY. Precision and trueness of dental models manufactured with different 3-dimensional printing techniques. Am J Orthod Dentofacial Orthop 2018; 153:144-153.
3. Alharbi N, Wismeijer D, Osman RB. Additive Manufacturing Techniques in Prosthodontics: Where Do We Currently Stand? A Critical Review. Int J Prosthodont 2017; 30:474-484.
4. Sim JY, Jang Y, Kim WC, Kim HY, Lee DH, Kim JH. Comparing the accuracy (trueness and precision) of models of fixed dental prostheses fabricated by digital and conventional workflows. J Prosthodont Res 2019; 63:25-30.

Use of Antibiotics in early Childhood and Dental Enamel Defects in 6- to 12-year-old Children in Primary Health Care

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ABSTRACT

Dental enamel defects (DED) are lesions that occur due several factors. Proper care is needed to promote their treatment and prevention. The aim of this study was to evaluate the occurrence of DED in permanent teeth of children who used antimicrobial drugs in the first four years of life. This is a cross-sectional study carried out in a Primary Health Care (PHC) service, which included children from six to 12 years of age. DED were evaluated by oral examination, and data on the use of antimicrobials in early childhood were collected based on medical records. Data were analyzed with the chi-square test and Fisher's exact test. The sample included 144 children. In relation to DED, 50% (72) and 20.1% (29) presented opacity and hypoplasia, respectively. Amoxicillin was the most frequently prescribed drug, followed by sulfamethoxazole + trimethoprim. Among the children, 78.5% (113) were prescribed antimicrobial drugs at least once during the first 4 years of life, and 55% (79) of them presented some type of DED. There was no statistically significant association between the variables analyzed. In conclusion, there was high prevalence of children with DED, and amoxicillin was the most commonly prescribed antibiotic.

Received: December 2019; Accepted: January 2020

Keywords: *dental enamel hypoplasia, primary health care, anti-bacterial agents, amoxicillin, oral health.*

Uso de antibióticos na primeira infância e defeitos de esmalte dentário em crianças de 6 a 12 anos na Atenção Primária à Saúde

RESUMO

Os defeitos do esmalte dentário (DED) são lesões que ocorrem devido a vários fatores e é necessária atenção para promover seu tratamento e prevenção. O objetivo foi avaliar a ocorrência de DED em dentes permanentes de crianças que usaram antimicrobianos nos primeiros quatro anos de vida. Trata-se de um estudo transversal realizado em um serviço de Atenção Primária à Saúde (APS), que incluiu crianças de seis a 12 anos de idade. A DED foi avaliada por dados de exames bucais, e os dados sobre o uso de antimicrobiano na primeira infância foram coletados com base em prontuários médicos. A análise foi realizada com o teste do qui-quadrado e o teste exato de Fisher. A amostra foi composta por 144 crianças. Em relação ao DED, 50%(72) e 20,1%(29) apresentaram opacidade e hipoplasia, respectivamente. A amoxicilina foi o medicamento prescrito com mais freqüência, seguido pelo sulfametoxazol+ trimetoprim. Entre as crianças, 78,5%(113) receberam medicamentos antimicrobianos pelo menos uma vez nos primeiros 4 anos de vida e 55%(79) deles apresentaram algum tipo de DED. Não houve associação estatisticamente significante entre as variáveis analisadas. Em conclusão, houve uma alta prevalência de crianças com DED e a amoxicilina foi o antibiótico mais comumente prescrito.

Palavras-chave: *hipoplasia do esmalte dentário, atenção primária à saúde, antibacterianos, amoxicilina, saúde bucal.*

INTRODUCTION

Once dental enamel has formed, it lacks metabolic activity, which means that any disorders that occur during its development may be manifested as permanent defects in erupted teeth[1]. Such enamel defects are changes that may affect one tooth only or a group of similar teeth, in both dentitions[2]. Disorders in the early secretory phase of the amelogenesis matrix are likely to appear as quantitative or morphological defects (hypoplasia), whereas interruptions in the processes of calcification or maturation may produce morphologically normal enamel which is nevertheless structurally or qualitatively defective (hypomineralization/ hypomaturation)[1]. Any systemic, local or genetic factor that may affect the ameloblasts may cause defects on the surface of the dental enamel[3].

A major change is Molar-Incisor Hypomineralization (MIH), defined as a change in systemic etiology that affects one, two, three or all first permanent molars and permanent incisors[2]. Clinically, hypomineralization is seen as translucency and opacity of the enamel, well defined and not diffuse, which distinguishes it from fluorosis. Hypomineralized enamel has a porous, smooth, chalk-like consistency. Defect in coloring ranges from white to yellow-brown and may be easily differentiated from normal enamel[3]. The exact systemic nature of the lesion has not been fully explained, but disorders during pregnancy, some childhood illnesses and the frequent use of antimicrobials are conditions that are involved in this process. In addition, recent studies have concluded that genetic variations related to amelogenesis are associated with the possibility of developing MIH[4,5]. It should be noted that ameloblasts are very sensitive cells and the occurrence of any change during enamel maturation may lead to loss of tissue quality, causing defects such as hypomineralization[6].

The investigation of MIH etiology has focused on environmental accidents that occur during the 3 first years of life, which is the critical period for the formation of permanent molars and incisors[6]. Children with enamel defects have 15-fold higher chances of developing cavities than patients without this type of defect[7]. In addition, the first hypomineralized permanent molars are subject to enamel breakage after tooth eruption due to chewing forces[8]. Hypersensitivity is another common complication of MIH, making oral hygiene and eating more difficult, in addition to further compromising defective teeth, and possibly compromising the clinical management of MIH[9]. Solving this problem and its possible consequences can be a major challenge involving complex treatments.

Some studies show an association between the use of medicines, especially antimicrobials, and the development of MIH. One study investigated a disease related to MIH, to ascertain whether this association is due to the disease itself or to the drug used to treat it, finding an association between the use of amoxicillin in children and the development of MIH[10]. Another study also suggested this association, reporting that the use of amoxicillin from 6 weeks to 3 months and from 3 to 6 months significantly increases the risk of enamel defects in primary second molars, but that additional studies are needed to prove this association[11].

It is therefore important to investigate the use of medicines in early childhood in relation to dental alterations, as amoxicillin is one of the most commonly used antibiotics in pediatric patients, including the context of Primary Health Care (PHC). Thus, the aim of this study was to evaluate the association between the occurrence of dental enamel defects (DED) in permanent teeth of 6- to 12-year-old children who used antibiotics in the first 4 years of life at a Primary Health Care service.

MATERIALS AND METHODS

This is a cross-sectional study carried out in 2014 at two Basic Health Units of the Community Health Service of Conceição Hospital Group (SSC-GHC), located in the city of Porto Alegre, Rio Grande do Sul, Brazil. The present study used oral examination data for DED from another study carried out in 2012 with 228 children with the aim of evaluating the association between asthma and occurrence of caries, erosion, and enamel defects in children [12]. The research project was approved by the GHC Research Ethics Committee under the CAAE number 26083614.7.0000.5530, and the authors abide by the universal declarations and regulations of Brazil (CNS Resolution 466/12).

The study included children aged 6 to 12 years registered at the Health Units, and excluded any children who did not have regular follow-up in their respective units during the first four years of life, or any whose medical records were not found, either because they moved elsewhere or because care to the family was interrupted. All participants provided written informed consent.

In the original study in 2012[12], prevalence was estimated by considering the main oral changes, such as dental caries and enamel defects, found in previous studies[13,14]. Considering the statistical power of 80% and a p-value for rejection of the null hypothesis of $p < 0.05$, a minimum sample of 214 children was obtained. Out of 1,278 children, 362 children were selected at random and 228 were examined. In 2014, the medical records of 144 children were examined in order to gather information about the use of medicines and the occurrence of infections in early childhood.

The World Health Organization criteria for DED[15] were used. The modified DED index is a scale of 0 to 9 which considers enamel normality, presence of opacity (marked, diffuse, or both), presence/ absence of hypoplasia, presence of other defects, presence of all conditions simultaneously or possibility of non-existent records, according to the following codes: (0) Normal, (1) Marked opacity, (2) Diffuse opacity, (3) Hypoplasia, (4) Other defects, (5) Marked and diffuse opacities, (6) Marked opacity and hypoplasia, (7) Diffuse opacity and hypoplasia, (8) All three conditions and (9) No record.

Two dental surgeons were trained and then calibrated using photographs of the clinical conditions under study[16,17]. The Kappa correlation coefficient was used in the two calibrations to assess concordance between the images evaluated by the same examiner and between examiners. Intra-examiner 1, intra-examiner 2 and inter-examiner Kappa values were 1.00, 0.85 and 0.70, respectively.

The examinations were performed at the Health Units or at home visits with the aid of a mouth mirror under artificial lighting. To assess the frequency of infections and use of medicines, data were collected from the medical records of the children examined who had at least one visit at their health unit from the first months of life and over their first 4 years.

Data were collected by a pharmacist in 2014 from hardcopy medical records at the health units. A structured instrument was used to collect appointment data, patient age at the time of the appointment, drugs used according to the Anatomical Therapeutic Chemical international coding; defined daily dosage; and time of treatment (when reported in the medical record). Information on the reasons for

Table 1: Frequency of drug prescription in the first 4 years of life of 6- to 12-year old children in Primary Health Care, Porto Alegre - RS, 2014 (n = 144).

Drug	1st year N(%)	2nd year N(%)	3rd year N(%)	4th year N(%)
Amoxicillin	45 (31.2)	52 (36.1)	33 (22.9)	42 (29.2)
Amoxicillin + clavulanate	4 (2.8)	1 (0.7)	3 (2.1)	1 (0.7)
Ampicillin	4 (2.8)	1 (0.7)	4 (2.8)	1 (0.7)
Azithromycin	3 (2.1)	2 (1.4)	7 (4.9)	11 (7.6)
Benzylpenicillinbenzathine	3 (2.1)	6 (4.2)	8 (5.6)	7 (4.9)
Benzylpenicillin potassium	1 (0.7)	1 (0.7)	2 (1.4)	1 (0.7)
Benzylpenicillin procaine	3 (2.1)	2 (1.4)	2 (1.4)	2 (1.4)
Cefaclor	1 (0.7)	0 (0.0)	0 (0.0)	2 (1.4)
Cefadroxil	2 (1.4)	1 (0.7)	1 (0.7)	0 (0.0)
Cephalexin	6 (4.2)	2 (1.4)	3 (2.1)	2 (1.4)
Ceftriaxone	1 (0.7)	0 (0.0)	0 (0,0)	0 (0,0)
Cefuroxime	1 (0.7)	0 (0.0)	1 (0.7)	0 (0.0)
Ciprofloxacin	1 (0.7)	0 (0.0)	1 (0.7)	0 (0.0)
Erythromycin	4 (2.8)	6 (4.2)	11 (7.6)	5 (3.5)
Gentamicin	1 (0.7)	0 (0.0)	0 (0.0)	0 (0.0)
Metronidazole	0 (0.0)	4 (2.8)	4 (2.8)	4 (2.8)
Nystatin*	19 (13.2)	8 (5.6)	1 (0.7)	0 (0.0)
Nitrofurantoin	2 (1.4)	0 (0.0)	0 (0.0)	1 (0.7)
Oxacillin	2 (1.4)	0 (0.0)	0 (0.0)	0 (0.0)
Sulfamethoxazole + trimethoprim	12 (8.3)	17 (11.8)	16 (11.1)	10 (6.9)

*Topical use drug, but prescribed for oral mucosal infections, being included due to this reason.

appointments within the children's first 4 years of life was also included.

Concerning enamel defects, the children were classified as having normal teeth, presenting opacities (demarcated and/or diffuse) or presenting enamel hypoplasia. Conditions 4 (other defects) and 9 (No record) were not found among the children selected for the study.

To verify the association between exposure to medicines and the development of DED, chi-square and Fisher's exact tests were used. Associations with p values <0.05 were considered statistically significant. Data were organized and analyzed with the aid of the SPSS software version 16.0 (SPSS Inc, Chicago, IL).

RESULTS

The sample consisted of 144 children whose average age was 8.7 years. The most frequently prescribed antimicrobial drug during the first four years of life was Amoxicillin, with highest frequency of prescription in the second year, 36.1% (52), followed by sulfamethoxazole + trimethoprim (Table 1).

Table 2 shows that 70% (101) of the patients presented some type of DED, classified as opacity (demarcated and/or diffuse) or enamel hypoplasia. Even though there is no statistically significant difference, it can be seen that the patients who had had some episode of infectious disease were a slight majority among those who developed enamel

Table 2: Enamel defects and their relationship with infectious diseases and antimicrobial drug prescription per age group in the first 4 years of life of 6- to 12-year-old children in Primary Health Care, Porto Alegre - RS, 2014 (n = 144).

Infectious disease		Total N	Normal teeth N (%)	Opacities (demarcated and/or diffuse) N (%)	Hypoplasia N (%)	P
	144	43 (29.9)	72 (50.0)	29 (20.1)		
0 - 1 year	No	64	18 (28.1)	33 (51.6)	13 (20.3)	0.923
	Yes	80	25 (31.3)	39 (48.8)	16 (20)	
1 - 2 years	No	61	19 (31.1)	32 (52.5)	10 (16.4)	0.618
	Yes	83	24 (28.9)	40 (48.2)	19 (22.9)	
2 - 3 years	No	72	25 (34.7)	33 (45.8)	14 (19.4)	0.438
	Yes	72	18 (25.0)	39 (54.2)	15 (20.8)	
3 - 4 years	No	71	24 (33.8)	36 (50.7)	11 (15.5)	0.341
	Yes	73	19 (26.0)	36 (49.3)	18 (24.7)	
Antibiotic Prescription		**Total N**	**Normal teeth N (%)**	**Opacities (demarcated and/or diffuse) N (%)**	**Hypoplasia N (%)**	**P**
0 - 1 year	No	74	22 (29.7)	39 (52.7)	13 (17.6)	0.705
	Yes	70	21 (30.0)	33 (47.1)	16 (22.9)	
1 - 2 years	No	77	21 (27.3)	42 (54.5)	14 (18.2)	0.479
	Yes	67	22 (32.8)	30 (44.8)	15 (22.4)	
2 - 3 years	No	87	29 (33.3)	41 (47.1)	17 (19.5)	0.539
	Yes	57	14 (24.6)	31 (54.4)	12 (21.0)	
3 - 4 years	No	86	29 (33.7)	42 (48.8)	15 (17.4)	0.411
	Yes	58	14 (24.1)	30 (51.7)	14 (24.1)	
Cumulative of antibiotic prescriptions		**Total N**	**Normal teething N (%)**	**Opacities (demarcated and/or diffuse) N (%)**	**Hypoplasia N (%)**	**P**
Used at some point	No	31	9 (6.3)	18 (12.5)	4 (2.8)	0.461
	Yes	113	34 (23.6)	54 (37.5)	25 (17.4)	
Used all years	No	127	39 (27.1)	62 (43.1)	26 (18.0)	0.734
	Yes	17	4 (2.8)	10 (6.9)	3 (2.1)	

hypoplasia in each age group. In all age groups, the presence of opacity (demarcated and/or diffuse) was not related to the prescription of antimicrobial drugs (Table 2).

In the cumulative analysis of antibiotic prescriptions, 78.5% (113) of the children had used antibiotics at least once, and among these, 37.5% (54) had opacities (demarcated and/or diffuse) and 17.4% (25) presented hypoplasia (Table 2).

Table 3 shows that among patients with defects in enamel development, the most frequently prescribed antimicrobial drug was amoxicillin, with at least 6 patients having used it more than 6 times during their first 4 years of life. Sulfamethoxazole associated with trimethoprim was also prescribed more than 6 times in this age group in at least 1 patient. It is also worth mentioning that amoxicillin was the medicine most frequently used by patients without enamel defects.

DISCUSSION

This study was carried out in the context of Primary Health Care, which provides a children's health program with free access to medical and dental appointments. There are few evaluations of this type in the context of PHC with calibrated examiners for oral evaluation of the patients using a random sample. More than half the patients analyzed presented DED, with opacities and hypoplasia being the most prevalent. In addition to evaluating the prescription of antimicrobials, the study originally intended to evaluate medication timing and dosage. However, one of the difficulties of reviewing medical records is precisely the quality of the records, which can be considered a constraint of the study. Nevertheless, medical records provide more reliable data than the self-reported data provided by mothers regarding the medications used, which would be limited by memory bias.

The international literature reports widely varying prevalence of enamel defects around the world. One study mapped the occurrence of molar-incisor hypomineralization (MIH) in Europe through a questionnaire sent to members of the European

Table 3: Relationship between enamel defects and the frequency of the main antimicrobial drug prescription in the first 4 years of life of 6- to 12-year-old children in Primary Health Care, Porto Alegre - RS, 2014 (n = 144).

Infectious disease	Total	Normal teething	Opacities (demarcated and/or diffuse) N (%)	Hypoplasia	P*
Amoxicillin	144	43	72	29	0.442
Less than 4 times	114	35	55	24	
From 4 to 6 times	22	6	14	2	
More than 6 times	8	2	3	3	
Cephalosporin	144	43	72	29	-
Less than 4 times	144	43	72	29	
Penicillin	144	43	72	29	0.698
Less than 4 times	142	43	70	29	
From 4 to 6 times	2	0	2	0	
Sulfamethoxazole + trimethoprim	144	43	72	29	0.595
Less than 4 times	140	43	68	29	
From 4 to 6 times	3	0	3	0	
More than 6 times	1	0	1	0	
Azithromycin	144	43	72	29	0.45
Less than 4 times	142	43	71	28	
From 4 to 6 times	2	0	1	1	
Erythromycin	144	43	72	29	-
Less than 4 times	144	43	72	29	

Academy of Pediatric Dentistry[18]. Prevalence ranged from 3.6 to 25%, with the great majority of data coming from northern Europe[18]. Another study evaluated the prevalence of enamel defects in permanent teeth of portuguese children of 6 (n = 799) and 12 years of age (n = 800) in 1999, finding that 7.3% of 6-year-olds and 7.1% of 12-year-olds showed demarcated opacities. Numbers were lower for hypoplasia (0.3% and 0.6%, respectively)[19].

A Brazilian study showed that the prevalence of molar-incisor hypomineralization (MIH) among 5- to 12-year-olds was approximately 20% in both teething periods. In another study in Brazil, that prevalence was 24.4% in 3- to 5-year-olds[20]. These data show a trend to higher prevalence of DED in the Brazilian population compared to the European, which may be associated to different exposure to etiological factors. Conflicting results can be explained by certain factors such as ethnicity, disease history, socioeconomic level, diet, patient age and presence of pollutants in the region[20]. In addition, a recent study in Brazil with 8- to 12-year-olds found that enamel defects were common in this population, but found no association with pre-, peri- and postnatal factors[21]. In our country, developmental defects of dental enamel have not been sufficiently studied, even though they cause aesthetic problems, dental sensitivity, and are factors leading to predisposition to caries[22].

The occurrence of infectious disease episodes in the 6- to 12-year-old age group is quite common. In all age groups evaluated, most patients had had some infectious disease in this period resulting in the use of antimicrobial drugs for treatment. In the present study, the most frequently prescribed medicine was amoxicillin, which has been introduced as a broad-spectrum antimicrobial drug and has been available in the Brazilian public health system[23] for the past decades, which coincides with the age of the children participating in the study. Amoxicillin is one of the most commonly used antibiotics in pediatric patients for the treatment of upper respiratory tract infections and especially acute otitis, a common childhood disease that affects more than 80% of children at least once before they are 3 years old[24]. In addition, otitis was the most common reason for prescription of antibiotics. Amoxicillin was the most frequently prescribed antibiotic for children, followed by cephalosporins and sulfamethoxazole-trimethoprim[25]. The widespread use of amoxicillin during childhood may have a significant impact on oral health[26].

These results are in line with our study, in which cephalosporins and sulfamethoxazole-trimethoprim were among the most commonly prescribed drugs for children in the first 4 years of life. Sulfamethoxazole-trimethoprim was found to have been prescribed 4 to 6 times in at least 3 patients who developed opacities, and more than 6 times in one patient. However, it was not possible to establish a statistically significant association in any of the cases, which may be because the final sample was reduced by the exclusion criteria. A study in Pakistan evaluated the exposure of 367 children to penicillins and cephalosporins, which are widely used in children and considered low-risk for the development of amelogenesis[27]. The authors found out that 15.4% of those exposed to amoxicillin and 29.2% of those exposed to cephalosporins presented hypermineralization of permanent teeth and that the increase in the use of these medicines in the past had a statistically significant association ($p < 0.002$), especially among those who had used it more than 8 times.

Another study with 147 children with average age 10.7 years investigated whether the use of amoxicillin, penicillin V, cephalosporins, macrolides and sulfamethoxazole-trimethoprim could be associated with the development of molar-incisor hypomineralization (MIH). It found that 52.2% of the children with molar-incisor hypomineralization (MIH) had used antibiotics in the first year of life, and the condition was more common among children who had used amoxicillin or erythromycin than among those who had not used these drugs. In addition, the use of cephalosporins or sulfamethoxazole-trimethoprim was not correlated with molar-incisor hypomineralization (MIH)[10]. In parallel to the present work, we notice that among the 70 children who had used antibiotics in the first year of life, 49 (70%) presented some enamel defect and amoxicillin was the most frequently prescribed medicine, having been used in 31.2% of the cases.

Small sample size may be a limiting factor in the study, related to non-statistically significant associations between exposure and the outcomes studied, even though other papers in the literature also show this lack of association[6]. The current study included 144 children whose average age was 8.7 years, and found no significant difference between groups regarding use of antibiotics, age at which antibiotics were used for the first time, or average number of treatments. Moreover, there was no significant difference among those who used only erythromycin, penicillin, trimethoprim or some other unspecified antibiotic [6]. However, longitudinal studies with more robust samples may be necessary in order to ascertain such associations and to determine outcomes with the other antimicrobials mentioned in this study, since the literature does not present very consistent data, in general terms, that could support a definitive relationship.

It is possible to conclude that there was a high prevalence of children with DED, mainly opacities. It is therefore extremely important to reinforce the oral health care of this population with preventive and educational actions, since defects in the development of enamel can lead to the formation of cavities in the long term and thereby a significant loss of dental function, as well as causing aesthetic discomfort. Amoxicillin was the most frequently prescribed antibiotic for infectious diseases affecting children in early childhood, and its use was related, even though not statistically significantly, to the development of opacities and hypoplasia. Amoxicillin is known to be effective in the treatment of several infections, mainly those in the respiratory tract and the ear, and has been widely used in Primary Health Care during the past decades. When it is prescribed, therefore, attention should be given to any potential side effects that may arise, such as the development of enamel defects. Based on this information, physicians should take into account this possible association upon considering the risk-benefit ratio in each case.

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REFERENCES

1. Crombie F, Manton D, Kilpatrick N. Aetiology of molar-incisor hypomineralization: a critical review. Int J Paediatr Dent 2009; 19:73-83.
2. Silva MJ, Scurrah KJ, Craig JM, Manton DJ et al.. Etiology of molar incisor hypomineralization - A systematic review. Community Dent Oral Epidemiol 2016; 44:342-353.
3. Takahashi K, Correia A de SC, Cunha RF. Molar incisor hypomineralization. J Clin Pediatr Dent 2009; 33:193-197.
4. Jeremias F, Pierri RAG, Souza JF, Fragelli CMBet al. Family-Based Genetic Association for Molar-Incisor Hypomineralization. Caries Res 2016; 50:310-318.
5. Jeremias F, Koruyucu M, Kuchler EC, Bayram M, et al. Genes expressed in dental enamel development are associated with molar-incisor hypomineralization. Arch Oral Biol 2013; 58:1434-1442.
6. Whatling R, Fearne JM. Molar incisor hypomineralization: a study of aetiological factors in a group of UK children. Int J Paediatr Dent 2008; 18:155-162.
7. Oliveira AFB, Chaves AMB, Rosenblatt A. The influence of enamel defects on the development of early childhood caries in a population with low socioeconomic status: a longitudinal study. Caries Res 2006; 40:296-302.
8. William V, Messer LB, Burrow MF. Molar incisor hypomineralization: review and recommendations for clinical management. Pediatr Dent 2006; 28:224-232.
9. Daly D, Waldron JM. Molar incisor hypomineralisation: clinical management of the young patient. J Ir Dent Assoc 2009; 55:83-86.
10. Laisi S, Ess A, Sahlberg C, Arvio P, et al. Amoxicillin may cause molar incisor hypomineralization. J Dent Res 2009; 88:132-136.
11. Hong L, Levy SM, Warren JJ, Bergus GR, et al. Primary tooth fluorosis and amoxicillin use during infancy. J Public Health Dent 2004; 64:38-44.
12. Rezende G, dos Santos NML, Stein C, Hilgert JB et al. Asthma and oral changes in children: associated factors in a community of southern Brazil. Int J Paediatr Dent 2019; 29:456-463.
13. Allazzam SM, Alaki SM, El Meligy OAS. Molar incisor hypomineralization, prevalence, and etiology. Int J Dent 2014; 234-508.
14. Hoffmann RHS, de Sousa M da LR, Cypriano S. Prevalence of enamel defects and the relationship to dental caries in deciduous and permanent dentition in Indaiatuba, Sao Paulo, Brazil. Cad Saude Publica 2007; 23:435-444.

15. WHO. Oral health surveys: basic methods. World Health Organization. Geneva: 4th; 1997. https://apps.who.int/iris/handle/10665/41905
16. Ministry of Health. Examiner Calibration Manual. Brasília: Secretaria de Atenção à Saúde/Secretaria de Vigilância em Saúde. Departamento de Atenção Básica. Coordenação Geral de Saúde Bucal. SB Brasil 2010; 2010. http://bvsms.saude.gov.br/bvs/publicacoes/pesquisa_nacional_saude_bucal.pdf
17. Alves JC, da Silva RP, Cortellazzi KL, Vazquez F de L, et al. Oral cancer calibration and diagnosis among professionals from the public health in Sao Paulo, Brazil. Stomatologija 2013; 15:78-83.
18. Weerheijm KL, Mejare I. Molar incisor hypomineralization: a questionnaire inventory of its occurrence in member countries of the European Academy of Paediatric Dentistry (EAPD). Int J Paediatr Dent 2003; 13:411-416.
19. de Almeida CM, Petersen PE, Andre SJ, Toscano A. Changing oral health status of 6- and 12-year-old schoolchildren in Portugal. Community Dent Health 2003; 20:211-216.
20. Lunardelli SE, Peres MA. Prevalence and distribution of developmental enamel defects in the primary dentition of pre-school children. Braz Oral Res 2005; 19:144-149.
21. Vargas-Ferreira F, Peres MA, Dumith SC, Thomson WM et al. Association of Pre- Peri- and Postnatal Factors with Developmental Defects of Enamel in Schoolchildren. J Clin Pediatr Dent 2018; 42:125-134.
22. Cruvinel VRN, Gravina DBL, Azevedo TDPL, de Rezende CS, et al. Prevalence of enamel defects and associated risk factors in both dentitions in preterm and full term born children. J Appl Oral Sci 2012; 20:310-317.
23. Secretary of Health. Municipal list of essential medicines - REMUME. Porto Alegre: Coordenação de Assistência Farmacêutica; 2012. 4 p.
24. Klein JO. Is acute otitis media a treatable disease? Vol. 364, The New England Journal of Medicine. 2011. p. 168–169.
25. McCaig LF, Hughes JM. Trends in antimicrobial drug prescribing among office-based physicians in the United States. JAMA 1995; 273:214-219.
26. Ciarrocchi I, Masci C, Spadaro A, Caramia Get al. Dental enamel, fluorosis and amoxicillin. Pediatr Med Chir 2012; 34:148-154.
27. Tariq A, Alam Ansari M, Owais Ismail M, Memon Z. Association of the use of bacterial cell wall synthesis Inhibitor drugs in early childhood with the Developmental Defects of Enamel. Pakistan J Med Sci 2014; 30:393-397.

Radiographic Diagnosis of Simulated External Root Resorption in Multi-Rooted Teeth: The Influence of Spatial Resolution

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ABSTRACT

The aim of this study was to evaluate the influence of spatial resolution (line pairs per millimetre – lp/mm) on the diagnosis of simulated external root resorption (ERR) in multi-rooted teeth by using digital periapical radiography. Forty human mandibular molars (80 roots) were used. The roots were divided into the following groups (n = 10): control without root filling (WORF), control with root filling (WRF), small ERR-WORF, small ERR-WRF, moderate ERR-WORF, moderate ERR-WRF, extensive ERR-WORF and extensive ERR-WRF. Four digital radiographs (phosphor storage plates – PSP system) were taken of each tooth in three angulations. The PSPs were scanned with 10, 20, 25 and 40 lp/mm. All images were assessed by three endodontists who used a five-point scale for presence and absence of ERR and classified its location (cervical, middle or apical third). ROC curves and one-way ANOVA were performed (p < 0.01). Diagnosis of ERR in non-root-filled teeth showed higher values of sensitivity for 20 lp/mm and higher values of both specificity and accuracy for 40 lp/mm. In root-filled teeth, sensitivity and accuracy were higher for 25 lp/mm and spatial resolution had no influence on specificity. The best resolution for diagnosis of small and extensive ERR was 25 lp/mm, whereas for moderate ERR, it was 40 lp/mm. Cervical ERR was the most difficult to diagnose, regardless of the spatial resolution. Higher spatial resolutions have improved the radiographic diagnosis of simulated ERR in multi-rooted teeth and this should be considered when performing digital radiographs.

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Keywords: *digital dental radiography, root resorption, diagnostic imaging, endodontics.*

Diagnóstico radiográfico de reabsorção externa simulada em dentes multiradiculares: influência da resolução especial

RESUMO

O objetivo deste estudo foi avaliar a influência do número de pares de linhas em radiografia intra-oral digital, na precisão da detecção de reabsorção radicular externa. Quarenta molares inferiores (n=80 raízes) foram submetidos ao preparo químico mecânico e em então, metade da amostra foi obturada. Em seguida, as raízes dos dentes foram aleatoriamente divididas de acordo com o tamanho da reabsorção radicular a ser simulada e com a presença e ausência de tratamento endodôntico. As RRE foram realizadas com brocas esféricas diamantadas de tamanhos 1/2, 1, 2. Executou-se radiografias digitais por meio do sistema de aquisição semidireto com a utilização de placas de fósforo fotoestimuladas (PSP). Em cada dente, incidências orto, mésio e distorradial foram repetidas quatro vezes, para que pudessem ser digitalizadas com resoluções de 10, 20, 25, 40 pl/mm. Após análise, verificou-se que dentes obturados apresentaram menores valores de sensibilidade com 10, 20 e 25 pl/mm e maiores valores de especificidade e acurácia para as mesmas resoluções. Dentes sem obturação registraram maiores valores de sensibilidade para resolução 20 e menor para 40; no entanto, a especificidade e a acurácia, foram maiores com 40 e menores em 10. Em RRE pequena, as resoluções 10 e 25 pl/mm foram respectivamente menos e mais acuradas; RRE média, foi maior com 40 pl/mm e RRE grandes foram melhores identificadas com 25. Correlacionando acertos no diagnóstico com localização das RRE, verificou-se que o terço cervical apresentou-se menos detectável. Concluiu-se que resolução espacial influenciou a detecção de RRE simuladas em radiografias periapicais digitais.

Palavras-chave: *radiografia dentária digital, reabsorção da raiz, diagnóstico por imagem, endodontia.*

INTRODUCTION

External root resorption (ERR) is defined as the loss of dental mineral tissue, such as cement and dentine, as result of various factors (pathological or physiological) predisposing to alterations in osteoclast activity. Some of these factors are periapical lesions, orthodontic movements, tooth re-implantation, trauma, pressure from adjacent erupting teeth, and odontogenic and non-odontogenic cysts and tumours[1,2]. In general, ERR presents no clinical signal or symptom and is largely detected by means of routine radiographic examinations, being characterised by reduced root length or root surface defects. However, in most cases, these lesions are only diagnosed at an advanced stage, thus compromising the treatment itself[3, 4]. Early diagnosis is therefore key to preserving the teeth involved, which makes radiographic detection of ERR very important for the dental surgeon[5].

Digital radiographic systems have recently replaced radiographic films, as they emit lower radiation levels, use no chemical process, reduce work time, facilitate image acquisition/storage and enable image re-use, in addition to improving both practitioner-patient visual communication and the image itself[4,6,7].

Phosphor storage plates (PSP) are a type of digital image receptor commonly used intra-orally, which resemble conventional periapical films in size and thickness. The choice of the ideal system depends on factors related to the digital image which can directly influence image resolution and quality. Among these factors, we can highlight spatial resolution, which is directly related to the number of line pairs per millimetre (lp/mm) in the digital receptors. According to the manufacturers, the higher the number of lp/mm, the better the image resolution[8].

To date, studies assessing the influence of the number of lp/mm on the diagnostic accuracy of radiography have been limited to the diagnosis of root fractures[7,9] and caries lesions[10].

Considering that it is challenging to diagnose ERR correctly on periapical radiographs, especially in multi-rooted teeth, we can highlight the importance of performing studies to investigate digital system resources in order to improve the diagnosis of this endodontic complication. Thus, the aim of the present study was to assess the influence of spatial resolution on the diagnosis of simulated ERR in multi-rooted teeth by using digital periapical radiography. The null hypothesis to be tested was that spatial resolution does not influence the diagnosis of ERR.

MATERIALS AND METHODS

This research was approved by the Human Research Ethics Committee by protocol number 1.998.579/2017. It followed the recommendations of the National Health Council of the Ministry of Health for research in human subjects.

Sample Selection and Preparation

Forty newly-extracted lower molars (n=80 roots) were selected for reasons not inherent to the study and stored in formalin solution 10% at a temperature of 6º C. The teeth were clinically and radiographically inspected for further selection. Two-rooted lower molars with healthy roots were included, whereas those teeth with incomplete root formation, fused roots, supernumerary roots, obliterated root canals, root resorption (external or internal), fractured file inside the root canal, intra-radicular posts and pulp calcifications or which had been endodontically treated were excluded. Teeth with cracks and root fractures, which were confirmed by using trans-illumination technique with high-power LED at 1200 mW/cm^2 (Radii Cal, SDI, Victoria, Australia), were also excluded,

In order to eliminate the identification of each tooth by the examiners, all crowns were sectioned at the cementoenamel junction with a double-sided steel disc (#6702, Fava, São Paulo, Brazil) attached to a micro-motor (Beltec LB100, Araraquara, SP, Brazil).

The sample of roots was randomly divided into two groups, one (n = 40) without endodontic treatment (WORF) and other (n = 40) with endodontic treatment (WRF), which was performed in appropriate laboratory by a single endodontic specialist. Working length (WL) was determined 1 mm beyond the apical foramen by using a #'10 K-file (Dentsply/Maillefer, Ballaigues, Switzerland). Roots were instrumented with Protaper system by using files SX, S1, S2, F1 and F2 (Dentsply/Maillefer, Ballaigues, Switzerland), with the four latter being used until the WL was reached. A 2.5% sodium hypochlorite solution was used for irrigation of the root canals, which were then dried with absorbent paper points F2 (Protaper Point-

Maillefer, Dentsply) before being filled with the Tagger's hybrid technique using gutta-percha F2 (Dentsply/Maillefer, Ballaigues, Switzerland) and endodontic cement (Endofill, Dentsply/Maillefer, Petropolis, RJ, Brazil).

The roots were randomly divided into experimental groups for simulations of ERR, which were performed by using spherical diamond burs sizes 1/2, 1 and 2 (KG Sorensen, Cotia, São Paulo, Brazil) to create small (n = 20), moderate (n = 20) and extensive (n = 20) defects, respectively. The burs were mounted on a high-speed motor (Kavo, Joinville, Brazil), positioned, and placed perpendicularly on the root surface to simulate ERRs with symmetrical depths. Each molar root received only one defect on its surface, with location (i.e. cervical, middle or apical third and buccal, lingual or distal face) defined randomly by using Excel 16.0 software (Office 2016, Microsoft Corporation, USA). Each group had the same amount of mesial and distal roots and the same number of ERR on radicular thirds.

Experimental groups were defined as follows: control without root filling (WORF) (n = 10), control with root filling (WRF) (n = 10), small ERR-WORF (n = 10), small ERR-WRF (n = 10), moderate ERR-WORF (n = 10), moderate ERR-WRF (n = 10), extensive ERR-WORF (n = 10) and extensive ERR-WRF (n = 10).

In order to simulate the radiographic appearance of the periodontal ligament of the tooth within the alveolus, the entire root was covered with specific plastic wax (Plástica- Kotaimp Art's, São Paulo, SP, Brazil), resulting in a layer 0.3 mm thick. Thickness was confirmed using a digital calliper (Starrett® 799A, Starret, Itu, SP, Brazil). The teeth were placed individually and randomly into the residual alveoli of a macerated mandible.

Image Acquisition

Digital radiographs were taken by means of a semi-direct digital imaging system with the use of photostimulable phosphor plates (PSP) (Vista Scan® (Dürr Dental, Beitigheim-Bissingen, Germany). Kilo-voltage peak (kVp) and milliamperage (mA) were the acquisition parameters set according to the characteristics of the equipment (Gendex Dental Systems, Lake Zurich, IL, USA), namely, 70 kVp and 7 mA. Exposure time of 0.10 seconds (32.7 mGy.com^2) was determined for diagnosis after images with ideal contrast and density were obtained in pilot studies. This exposure time was used throughout the study. The distance between focus and PSP was standardised at 40 cm and followed the technique of parallelism by using an acrylic apparatus specially developed for periapical radiographs *in vitro* and soft tissue simulation. All images were obtained at ortho, mesial and distal angulations, ranging by 15° in relation to the horizontal angulation. The angulations were repeated four times in each tooth so that the PSPs could be digitalised with a scanner (VistaScan Mini View®, Dürr Dental, Bietigheim-Bissingen, Germany) at different resolutions of line pairs per millimetre: 10 lp/mm (500 dpi), 20 lp/mm (1000 dpi), 25 lp/mm (1270 dpi) and 40 lp/mm (2000 dpi).

Image Evaluation

After obtaining 480 radiographs, the images digitalised with different line pairs per millimetre were randomised in order to improve assessment reliability. The images were assessed by three endodontic specialists who were previously instructed regarding the assessments and blinded to the study's methodology. They examined the images individually for presence or absence of ERR in each root according to a 5-point scale, namely: 1 – totally absent; 2 – probably absent; 3 – uncertain; 4 – probably present; and 5 – totally present. For cases in which the examiners considered presence of ERR (scores 4 and 5), it was further rated according to its location in the root thirds, i.e., cervical, middle or apical. Any discordant cases were jointly re-assessed by all three examiners so that agreement was achieved between at least two of them, thereby enabling the calculation of the response mode.

Periapical radiographs were assessed on Windows® photo viewer before being exported to .TIFF file format (uncompressed) by using the system's image acquisition software (DBS Win, Dürr Dental, Bietigheim-Bissingen, Germany). All assessments were performed on a 24-inch LCD monitor (MDRC 2124 (Barco Inc. Duluth, GA, USA) under ideal light conditions. Zoom, brightness and contrast tools could be used to improve the images.

After 30 days, a period considered to be long enough for the examiners to forget the images, 20% of the sample was re-assessed in order to calculate intra- and inter-rater reliability[11].

Statistical Analysis

Statistical analysis was carried out using the SPSS statistics software version 21.0 (IBM Corp, Armonk, New York, USA) at a significance level of 1%. Weighted kappa test was used for intra- and inter-rater reproducibility. For comparison of the results of the images obtained at different resolutions (lp/mm) with the gold standard, ROC (receiver operating characteristics) curves were built to determine the values of sensitivity, specificity and areas under ROC curve (accuracy). One-way analysis of variance (ANOVA) and *post-hoc* Tukey's test were used to compare the area values.

RESULTS

Kappa test showed that values for intra- and inter-rater reliability ranged from significant to almost perfect agreement[12] (0.66 to 0.81 and 0.77 to 0.86), respectively.

ROC curves were calculated for each spatial resolution in the WORF and WRF groups. The values of sensitivity, specificity and areas under the ROC curves are shown in Table 1.

Analysis of diagnostic tests showed that spatial resolution and presence of root filling material significantly influenced the detection of simulated external root resorptions on digital periapical radiographs. Fig. 1 shows periapical radiographs of mandibular molars with and without endodontic treatment and presence of simulated RRE in different thirds of root canal.

By comparing the values of sensitivity, specificity and accuracy between the WORF and WRF groups regarding the different spatial resolutions, it was observed that root-filled teeth had lower values of sensitivity at resolutions of 10, 20 and 25 lp/mm ($P < 0.001$) and higher values of specificity and accuracy at the same resolutions ($P < 0.001$). On the other hand, by comparing the values of sensitivity, specificity and accuracy obtained at each spatial resolution, it was found that the WORF group presented higher values of sensitivity at a resolution of 20 lp/mm (0.917) and lower values at 40 lp/mm (0.611). Moreover, non-root-filled teeth had higher values of specificity and accuracy at a resolution of 40 lp/mm, respectively 1.00 and 0.873, and lower values at 10 lp/mm, respectively,

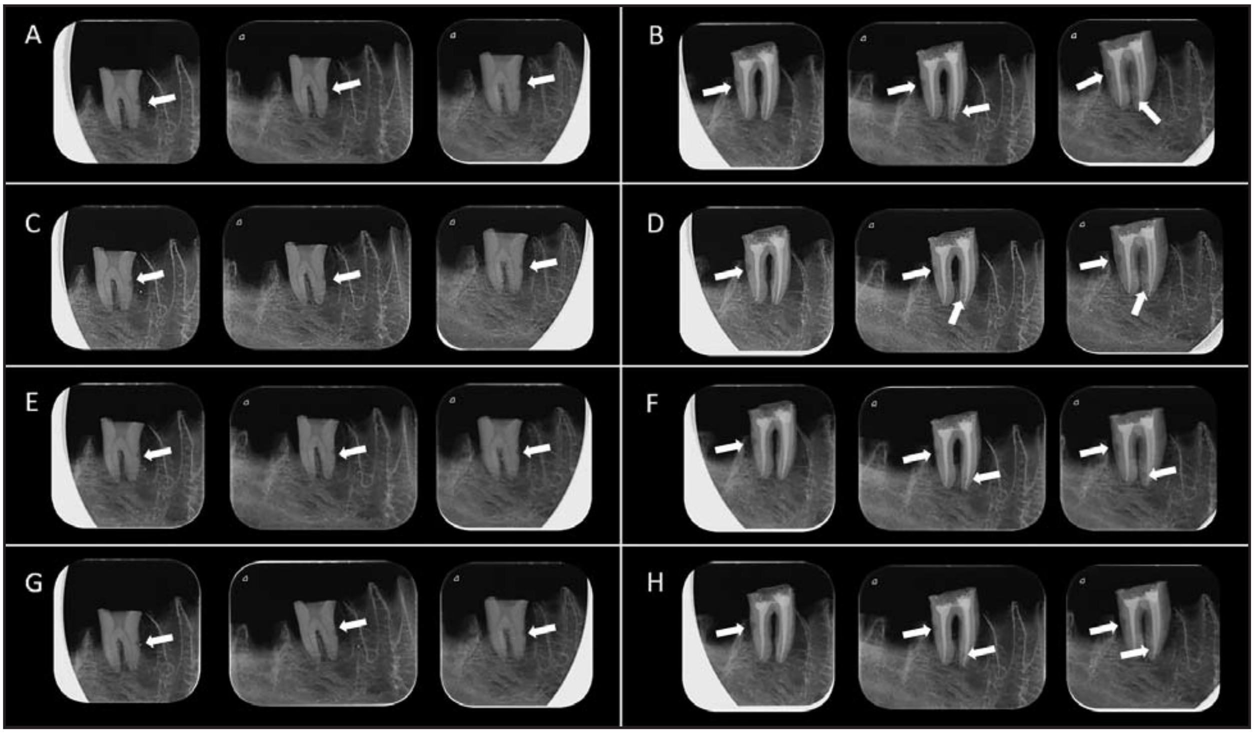

Fig. 1: Periapical radiographs of mandibular molars. (A) Absence of endodontic treatment and presence of simulated RRE in the middle third of the mesial root, 10 lp/mm. (B) Presence of endodontic treatment and simulated RRE in the middle third of the distal root and in apical third of the mesial root, 10 lp/mm. (C) and (D) - Spatial resolution 20 lp/mm. (E) and (F) - Spatial resolution 25 lp/mm. (G) and (H) - Spatial resolution 40 lp/m (white arrows).

0.714 and 0.823. For root-filled teeth, the values of sensitivity and accuracy at a resolution of 25 lp/mm were higher, 0.769 and 0.887 respectively, whereas no difference was found in specificity between different spatial resolutions (Table 1).

With regard to the extent of ERR, it was found that different types of spatial resolution produced different results ($P < 0.001$). For small ERRs, spatial resolution of 10 lp/mm was less accurate (0.755) than that of 25 lp/mm (0.848). For moderate ERRs, on the other hand, accuracy was higher at a resolution of 40 lp/mm (0.885) than at other resolutions. Extensive ERRs were significantly better identified at a resolution of 25 lp/mm (0.891), whereas a resolution of 40 lp/mm enabled less accurate identification (0.876) (Table 2).

By correlating the percentage of diagnostic hits to localization of ERR at different spatial resolutions, it was possible to verify that the percentage of hits was lower in the cervical third than that in the other thirds for all resolutions tested, whereas ERRs located in the middle third were more easily detected (Table 3).

Table 1: Values of diagnostic tests for the different spatial resolutions, in the presence and absence of root filling.

		Spatial Resolution				
		10 lp/mm	**20 lp/mm**	**25 lp/mm**	**40 lp/mm**	**P Value**
Sensitivity	WORF	$0.889^{A,a}$	$0.917^{B,a}$	$0.806^{C,a}$	$0.611^{D,a}$	P < 0.001
	WRF	$0.718^{A,b}$	$0.744^{B,b}$	$0.769^{C,b}$	$0.744^{B,b}$	P < 0.001
Specificity	WORF	$0.714^{A,a}$	$0.857^{B,a}$	$0.786^{C,a}$	$1.000^{D,a}$	P < 0.001
	WRF	$0.923^{A,b}$	$0.923^{A,b}$	$0.923^{A,b}$	$0.923^{A,b}$	P = 1.000
AZ	WORF	$0.823^{A,a}$	$0.836^{B,a}$	$0.832^{C,a}$	$0.873^{D,a}$	P < 0.001
	WRF	$0.841^{A,b}$	$0.848^{B,b}$	$0.887^{C,b}$	$0.864^{D,b}$	P < 0.001

AZ, Area under the ROC curves. Lp/mm, line/pair per millimetre. WORF, Without root filling. WRF, With root filling.
For each diagnostic parameter value, different capital letters indicate significant difference (P < .01) between the different spatial resolutions, in the WORF and WRF conditions.
For each diagnostic parameter value, different lowercase letters indicate significant difference (P < .01) between the WORF and WRF conditions.

Table 2: AZ values for the different sizes of simulated external root resorption in the different spatial resolutions.

	Spatial Resolution				
Size of ERR	**10 lp/mm**	**20 lp/mm**	**25 lp/mm**	**40 lp/mm**	**P Value**
Small ERR	0.755^{A}	0.827^{B}	0.848^{C}	0.831^{B}	P < 0,001
Medium ERR	0.833^{A}	0.829^{A}	0.826^{A}	0.885^{B}	P < 0,001
Extensive ERR	0.876^{A}	0.849^{B}	0.891^{C}	0.847^{B}	P < 0,001

ERR, external root resorption. Lp/mm, line/pair per millimetre.
P Value, One-way ANOVA, Tukey Post-Hoc (P < 0.01).
Different capital letters indicate significant difference (P < 0.01) between the different spatial resolutions for the three sizes of simulated ERR.

Table 3: Relative frequency of correct diagnosis of the location of the simulated external root resorptions in the different spatial resolutions.

	Spatial Resolution			
Location of ERR	**10 lp/mm**	**20 lp/mm**	**25 lp/mm**	**40 lp/mm**
Absence of ERR	88.90%	92.60%	85.20%	88.90%
Cervical third ERR	45.80%	41.70%	45.80%	41.70%
Middle third ERR	77.80%	88.90%	96.30%	85.20%
Apical third ERR	45.80%	50.00%	50.00%	58.00%

DISCUSSION

The aim of the present study was to ascertain whether spatial resolution (line pairs per millimetre) has any influence on the diagnosis of simulated ERRs in multi-rooted teeth by using digital periapical radiography.

The results show that both spatial resolution and different root canal conditions (i.e. root-filled or non-root-filled) had a significant influence on the detection of simulated ERRs on digital periapical radiographs.

Based on the diagnostic efficacy of spatial resolution for detection of simulated EERs on digital radiographs, high spatial resolutions were expected to have higher values of specificity, sensitivity and accuracy. For non-root-filled teeth, however, these expected results were found for specificity and accuracy as they were higher at a resolution of 40 lp/mm and lower at 10 lp/mm.

These findings corroborate the literature, showing that images acquired at high spatial resolutions enable better detection of radiographic details[9,10,13,14]. Nevertheless, contrary to our expectations, better results for accuracy were found for root-filled teeth at a resolution of 25 lp/mm. This finding is important because it shows that it is possible to make a precise diagnosis without spending a long time on scanning, as occurs during the use of line pairs in high spatial resolution imaging. Another inherent advantage is small image file size, which also enables easy data export.

No statistical difference was observed for specificity in root-filled teeth. Although it is not feasible to perform a direct comparison due to methodological differences, a previous study corroborated our finding by demonstrating that different systems with different spatial resolutions showed no statistical difference in the detection of vertical root fracture[15]. On the other hand, analysis of sensitivity showed better results in non-root filled teeth at a resolution of 20 lp/mm, whereas higher sensitivity was found in root-filled teeth at a resolution of 25 lp/mm. For this reason, considering the diagnostic difficulties minimised due to the lack of filling material, the use of a lower resolution was enough for a precise diagnosis, whereas a higher resolution enabled safer diagnosis in teeth whose images were affected by the filling material.

These data are similar to those reported by a recent study assessing different intra-radicular conditions during the diagnosis process. Teeth filled with gutta-percha or restored with intra-radicular posts were more poorly diagnosed than those without root filling[15,16].

With regard to the extent of ERRs, the present study has demonstrated that higher spatial resolutions improve accuracy in detecting small to moderate-sized ERRs, although it has been suggested that the higher the spatial resolution, the better the identification of radiographic details[17]. Studies using different spatial resolutions were also performed to detect morphological alterations such as vertical root fractures [9] and deep caries lesions[18], reporting better results for higher resolutions, which corroborates our findings.

For extensive ERRs, accuracy was better at a resolution of 25 lp/mm and worse at 40 lp/mm. This can be explained by the fact that an increase in spatial resolution without altering the exposure time, i.e., a proportionally longer exposure is needed for a higher spatial resolution in order to keep the same quality of the image acquired with lower spatial resolution[17]. Because extensive ERRs show a greater number of radiographic details and the study was performed with the same exposure time despite the different spatial resolutions, such details were more easily identified at lower resolutions.

In addition, greater wear of the root structure leads to a larger radiolucent area. Therefore, the accurate diagnosis of root resorption was higher for larger cavities[4]. The same finding was reported by a study on ERR with different sizes, showing that extensive resorptions were not considered a diagnostic challenge as they could be easily identified in all modalities tested[19].

With regard to the location of ERRs, middle third was the region enabling the most precise diagnosis, whereas the lowest percentage of diagnostic hits was observed in the cervical third for all spatial resolutions. Statistical differences between different root thirds have also been reported in the literature[5,20], with the middle third enabling the best results[5], which corroborates our study.

These findings can be attributed to a higher density and greater thickness of the structures superimposed in the cervical region, such as surrounding bone, root dentine and, in cases of root-filled teeth, greater amount of filling material.

On the other hand, a study using other digital methods with PSPs did not show any statistically

significant superiority in detecting ERRs with different sizes and in different sites[3].
The failure in timely diagnosis of ERR may lead to loss of dental structure and decrease the likelihood of preserving the tooth, since resorptive lesions are the first ones to be diagnosed on intra-oral radiographs. Safe radiographic techniques for diagnosis of such lesions is therefore necessary[6].
Spatial resolution, usually expressed as line pairs per millimetre (lp/mm), is a characteristic of digital receptors that enables differentiation of image details. To date, however, there is no study describing the effect of different spatial resolutions using a semi-direct digital imaging system (phosphor storage plates) on the detection of ERR.
The present study used two-rooted lower molars with healthy roots. Multi-rooted teeth were selected because their morphology is largely affected by necessary endodontic procedures, thus posing a great challenge during the diagnosis of ERR due to root superimposition, which may result in poor therapeutic outcome. Different intra-radicular conditions (i.e. root-filled and non-root-filled teeth) were prepared in order to create diagnostic difficulties resulting from the filling material.
In the present study, teeth with simulated resorption cavities were considered to be the gold standard, which enabled the calculation of the exact percentage of positive and negative readings. It should be emphasised that simulated ERRs have relatively distinct cavity ridges, making them fairly easy to detect compared to naturally formed ERRs, which have more diffuse ridges[6].
We also decided to simulate ERRs on the whole surface of the root and in three different regions to reproduce the likelihood of finding them in dental practice. Moreover, the teeth were placed within the alveoli of dried mandibles in order to better simulate the condition of their roots, i.e., surrounded by bone trabeculae and peripheral bone contour[2]. In addition, tissue attenuation and x-ray dispersion were precisely simulated in the acrylic apparatus[21].
To follow the ALARA principle (as low as reasonably achievable), exposure time was determined by means of a pilot study in which the image quality was obtained for all radiographs and maintained constant for all exposures, regardless of the spatial resolution. The aims of this procedure were to enable clinical reproducibility in terms of radiation exposure level and to ensure that any statistical differences were exclusively related to spatial changes.
Similarly, based on specific guidelines for radiologic diagnosis of tooth fractures[22], in which at least two radiographs should be taken for diagnosis, the present work has extrapolated this recommendation by performing a radiographic triad for each tooth at different spatial resolutions in order to increase diagnostic sensitivity.
ERRs not viewed on orthoradial images were thus more likely to be detected after altering the horizontal angles[23].
With regard to image evaluation criteria, it has been reported that subjective analysis of the image quality of a digital radiographic system is rather complex because it is directly related to sensitivity and dynamic scale of each examiner and related to the importance of the object of analysis[8]. In the present study, nevertheless, the examiners were previously trained and their analyses tested by means of a ROC curve. This approach enabled outlier data to be eliminated in order to increase the reliability of the study.
However, the present study has limitations inherent to the laboratory method and the results should be extrapolated to clinical practice with caution. In addition to the fact that simulated ERRs were relatively sharper than naturally formed ERRs, which tend to be rather diffuse and calcified, data on painful symptoms, crown colour and patient's dental history can help contextualise the observations made during interpretation of the images.
Therefore, further research should be carried out *ex vivo* by using a methodology similar to ours in order to precisely compare specificity, sensitivity and accuracy of digital imaging systems in the detection of ERR, as well as studies using irregular resorption cavities to better simulate clinical features.
In conclusion, it has been found that spatial resolution influenced the detection of simulated ERRs on digital periapical radiographs, as well as their location, size, root canal configuration and presence of filling material.

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REFERENCES

1. Vaz ASL, Vasconcelos TV, Neves FS, de Freitas DQ, Haiter-Neto F. Influence of cone-beam computed tomography enhancement filters on diagnosis of simulated external root resorption. J Endod 2012; 38:305-308.
2. Creanga A, Geha H, Sankar V, Teixeira F. McMahan C, Noujeim M. Accuracy of digital periapical radiography and cone-beam computed tomography in detecting root resorption. Imaging Sci Dent 2015; 45:153-158.
3. Shokri A, Mortazavi H, Salemi F, Javadian A, Bakhtiari H, Matlabi H. Diagnosis of simulated external root resorption using conventional intraoral film radiography, CCD, PSP, and CBCT: a comparison study. Biomed J 2013; 36:18-22.
4. Mesgrani A, Haghanifar S, Ehsani M, Yaghub SD, Bijani A.Accuracy of conventional and digital radiography in detecting external root resorption. Iran Endod J 2014; 9:241-245.
5. Kamburoğlu K, Tsesis I, Kfir A, Kaffe I. Diagnosis of artificially induced external root resorption using conventional intraoral film radiography, CCD, and PSP: an ex vivo study. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2008; 106:885-891.
6. Sakhdari S,Khalilak Z,Najafi E,Cheraghi R. Diagnostic accuracy of charge-coupled device sensor and photostimulable phosphor plate receptor in the detection of external root resorption in vitro J Dent Res Dent Clin Dent Prospects 2015; 9:18-22.
7. Nejaim Y, Gomes AF, Silva EJ, Groppo FC, Neto H. The influence of number of line pairs in digital intra-oral radiography on the detection accuracy of horizontal root fractures. Dental Traumatol 2016; 32:180-184.
8. Bóscolo FN, Oliveira AEF, Almeida SM, Haiter CFS. Haiter N. A comparative clinical study of the image quality of the three digital radiographic systems, E-speed film and digital film. Pesqui Odontol Bras 2001; 15:327-333.
9. Wenzel A, Kirkevang LL. High resolution charge coupled device sensor vs. medium resolution photostimulable phosphor plate digital receptors for detection of root fractures in vitro. Dental Traumatol 2005; 21:32-36.
10. Li G, Berkhout WER, Sanderink GCH, Martins M. Stelt PF. Detection of in vitro proximal caries in storage phosphor plate radiographs scanned with different resolutions. Dentomaxillofac Radiol 2008; 37:325-329.
11. Ferreira LM, Visconti MAPG, Nascimento HA, Dallemolle RR, Ambrosano GM, Freitas DQ. Influence of CBCT enhancement filters on diagnosis of vertical root fractures: a simulation study in endodontically treated teeth with and without intracanal posts. Dentomaxillofac Radiol 2015; 44:1-6.
12. Landis JR, Koch GG. The measurement of observer agrement for categorical data. Biometrics 1977; 33:159-174.
13. Møystad A, Svanaes DB, Risnes S, Larheim TA. Gr€ondahl HG. Detection of approximal caries with a storage phosphor system. A comparison of enhanced digital images with dental X-ray film. Dentomaxillofac Radiol 1996; 25:202-206.
14. Kositbowornchai S, Basiw M, Promwang Y, Moragorn H. Sooksuntisakoonchai N. Accuracy of diagnosing occlusal caries using enhanced digital images. Dentomaxillofac Radiol 2004; 33:236-240.
15. Nascimento HA, Neves FS, de-Azevedo-Vaz SL, Duque TM. Ambrosano GM, Freitas DQ. Impact of root filling and posts on the diagnostic ability of three intraoral digital radiographic systems in detecting vertical root fractures. Int Endod J 2014; 48:864-871.
16. Lima TF, Gamba TO, Zaia AA, Soares AJ. Evaluation of cone beam computed tomography and periapical radiography in the diagnosis of root resorption. Australian Dent J 2016; 61:425-431.
17. Lin LM, Rosenberg PA, Lin J. Do procedural errors cause endodontic treatment failure? J Am Dent Assoc 2005; 136: 187-193.
18. Janhom A, van Ginkel FC, van Amerongen JP, van der Stelt PF. Scanning resolution and the detection of approximal caries. Dentomaxillofac Radiol 2001; 30:166-171.
19. Patel S, Dawood A, Wilson R, Horner K.Mannocci F. The detection and management of root resorption lesions using intraoral radiography and cone beam computed tomography- an in vivo investigation. Int Endod J 2009; 42:831-838.
20. Andreasen FM, Sewerin I, Mandel U, Andreasen JO. Radiographic assessment of simulated root resorption cavities. Endod Dent Traumatol 1987; 3:21-27.
21. Durack C, Patel S, Davies J, Wilson R. Mannocci F. Diagnostic accuracy of small volume cone beam computed tomography and intraoral periapical radiography for the detection of simulated external inflammatory root resorption. Int Endod J 2011; 44:136-147.
22. Sonnabend E. The role of x-ray films in diagnosis in operative dentistry. Dtsch Zahnarztl Z 1990; 45:691-695.
23. Westphalen VP, Gomes de Moraes I, Westphalen FH, Martins WD. Souza PH.Conventional and digital radiographic methods in the detection of simulated external root resorptions: a comparative study. Dentomaxillofac Radiol 2004; 33:233-235.

Vestibular Alveolar bone height measurement: Accuracy and Correlation between direct and indirect techniques

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ABSTRACT

Cone Beam Computed Tomography (CBCT) has modified the perspective of dentistry images, providing manipulable three-dimensional images with a 1:1 patient:image ratio. Treatments and diagnosis are modified or corroborated by CBCT; however, its accuracy in thin structures such as cortical bone has been subjected to critical review. The aim of this study is to correlate the measurement of vestibular alveolar bone height using direct measurements and measurements performed with cone-beam tomographic images with standard (SD) voxel resolution. Thirty incisor and premolar teeth of patients undergoing open curettage were measured with a high-precision caliper and with Cone Beam Computed Tomography (CBCT) at an SD resolution of 0.16 mm voxels in a 3D Orthophos XG Sirona scanner. Intra-observer evaluation was performed using the intraclass correlation coefficient (ICC). Direct measurements and CBCT measurements were correlated using Pearson correlation (PCC). The mean difference between indirect and direct measurements was 3.15 mm. Paired t test and Pearson Correlation coefficient determined that all measurements differed statistically from each other with p< 0.05. With the CT scanner and protocol used in this study, CBCT images do not enable accurate evaluation of vestibular alveolar bone height.

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Keywords: computed tomography, cone-beam, diagnostic imaging, alveolar bone loss, tooth.

Medición de la altura del hueso alveolar vestibular: precisión y correlación entre técnicas directa e indirecta

RESUMEN

La tomografía de haz cónico (CBCT) ha modificado la perspectiva de la imagenología en odontología que brinda una imagen tridimensional manipulable con una relación 1:1, paciente: imagen. Los tratamientos y diagnósticos se ven modificados o corroborados por el CBCT; sin embargo, la exactitud que presenta en estructuras delgadas como las corticales óseas ha sido sometida a críticas. El objetivo fue correlacionar la medición de la altura del hueso alveolar vestibular mediante mediciones directas y las realizadas con imágenes tomográficas de haz cónico con resolución de vóxel estándar (SD). Treinta dientes incisivos y premolares de pacientes sometidos a un curetaje abierto se midieron con un calibrador de alta precisión y una tomografía computarizada de haz cónico (CBCT) a una resolución SD de 0,16 mm de vóxeles en un escáner 3D Orthophos XG Sirona. La evaluación intraobservador se realizó utilizando el coeficiente de correlación intraclase (ICC), y las mediciones directas y las mediciones CBCT se correlacionaron utilizando la correlación de Pearson (PCC). La diferencia media entre las mediciones indirectas y directas fue de 3,15 mm. La prueba t pareada y el Coeficiente de Correlación de Pearson determinaron que todas las mediciones fueron estadísticamente diferentes entre sí con una p < 0.05. Con el escáner de TC y el protocolo utilizado en este estudio, las imágenes CBCT no permiten una evaluación precisa de la altura del hueso alveolar vestibular.

Palabras clave: tomografía computarizada, haz cónico, diagnóstico por imagen, pérdida de hueso alveolar, diente.

INTRODUCTION

Orthodontic treatments use 2D X-rays as a fundamental part of diagnosis to determine dental or bone injury, pathologies, root parallelism, tooth inclinations, and growth during treatment[1]. In dentistry, 3D radiology complements this tool. The company Quantitative Radiology (Verona, Italy) introduced the first Cone Beam Computed Tomography (CBCT) for dental use in the year 2000 in Loma Linda University, USA[2]. This new tool has significant advantages such as low radiation dose, a 1:1 ratio of bone structures and shorter shooting times than 2D X-rays and computed axial tomography (CAT). The companies involved in 3D tomography have provided different options to the orthodontist, making it important to analyze each tomogram and corroborate the accurate measurement of anatomical structures. The 1:1 ratio has been demonstrated in many cases; however, when highly accurate measurement of thin structures is required for cortical bone, the reliability of 3D tomography decreases[3, 4].

The alveolar bone is formed by the projection of the external and internal cortical bone and provides support to the teeth and soft tissues. The determination of alveolar bone height helps to establish the limitations in orthodontic treatments, e.g., in biomechanical expansion or closure of spaces, movements could cause or aggravate fenestrations or dehiscences in the alveolar crest, especially if the diagnosis was incorrect. Biomechanical and biological factors are closely related and determine the collateral effects of potential treatment such as root resorption, gingival recession, dehiscence and fenestrations[5, 6].

Three-dimensional imaging through a CBCT scan is important because it can reveal fenestrations and dehiscences. These types of alveolar defects are common, especially in hyper-divergent patients and increased lower facial height[7,8]. A previous study of 1872 teeth reported high prevalence of these defects in hyper-divergent (8.35%) and normo-divergent (8.18%)[6] subjects. In another study, i-CAT™ was performed on 123 scans, showing that the presence of fenestrations and dehiscences did not vary significantly between skeletal I, II or III malocclusion[9].

In terms of measurements on CBCT, it has been found that linear measurements are reliable, but the presence of soft tissue and voxel size influenced the results. Even when using a 0.125 mm voxel, the thin vestibular cortex of some external layers is not represented, hence, there is a considerable risk of overestimating the dehiscences or fenestrations viewed in a three-dimensional image[10].

The correlation between linear measurements performed on a skull and the same measurements taken from tomographic images analyzed using Dolphin® software has been assessed in the literature. It was demonstrated that many of the linear measurements differed significantly[11]. This highlighted the limitations of using this technology for studies in skulls. Several factors can influence the accuracy of the results, such as the attenuation of soft tissues, metal-based restorations and patient movement.

CBTC scans (0.4 mm voxel) were performed in pig jaws to measure the height of the vestibular alveolar bone in the molar region. The results showed that tomographic images can overestimate alveolar bone loss associated with rapid palatal expansion by 1.5 to 2 mm[12].

The aim of this study was to investigate whether there is a significant correlation in the accuracy of the vestibular alveolar bone height measurement between direct and indirect techniques (0.16 mm voxel resolution CBCT), with the goal of validating this methodology and establishing its limitations.

The importance of the present study is the observation of variables in living patients, and analysis of the representation of clinical reality in CBCT scans.

MATERIALS AND METHODS

This study was reviewed and approved by the Faculty of Dentistry Research Committee at the Benemérita Universidad Autónoma de Puebla (Mexico). The procedures adhered to ethical standards and there was no risk in the investigation according to the Regulation of the General Health Law on Research and Helsinki Declaration guidelines for research involving human subjects.

The intraclass correlation coefficient for the vestibular alveolar bone height measurements of both researchers was 0.980. The inter-researcher's Pearson's correlation coefficient for the direct technique (Vernier caliper measurement) was 0.990, and for the indirect technique (CBCT at 0.16 mm) it was 0.986. Thus, direct and indirect measurements were consistent and reproducible for each researcher.

Eight patients were invited to participate in the study, under informed consent, to receive open curettage treatments. The protocol included a sample of 30 teeth that were exposed to a full-thickness flap during debridement. The study included incisors and premolars of patients of different ages and genders, and excluded

teeth with extensive restorations that could affect the tomographic image.
The procedure included patients who required flap elevation for their periodontal procedure which enabled us to perform the measurements. A copy of the CBCT scan was delivered to the specialist who was treating the patients for diagnostic purposes without additional cost.
Patients were exposed to one CBCT scan after clinical procedures. Precautions were taken to minimize the radiation dose, in view of the "As Low as Reasonably Achievable" (ALARA) principles. Exposure time was kept as short as possible, good hygiene practices were followed and proper use was made of personal protective equipment such as lab coats, disposable gloves and safety glasses. The design and execution of this study were subjected to Institutional Review Board oversight (IRB). Patients who were scheduled to receive radiation from cone-beam tomographic images were prospectively enrolled in an IRB-approved study.

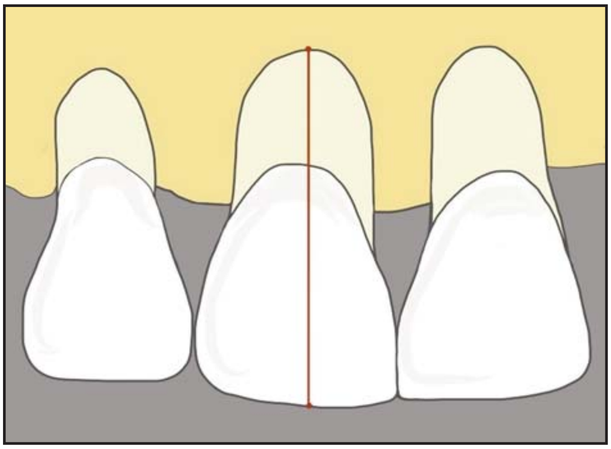

Fig. 1: Direct measurement technique.

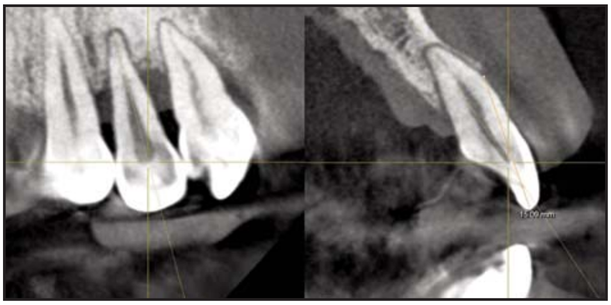

Fig. 2: Indirect measurement technique.

Direct measurement technique

Once the flap was performed and after elimination of dental calculus, the height of the vestibular alveolar bone was measured from the incisal, occlusal edge or the vestibular cusp, along the longitudinal axis of the tooth to the end of the observable vestibular alveolar bone using a digital Vernier caliper Stainless Steel Professional, with an error range of 0.01 mm. Three independent measurements were carried out by two researchers before the periodontist placed the suture (Fig. 1).

Indirect measurement technique

Subsequently, the patient was taken to the radiology department to perform the image study using an Orthophos XG 3D Sirona® scanner. This study was conducted according to the specifications suggested by the manufacturer: 12 mA, 90 kVp and 14.9-second exposure[13]. The field of view of the CBCT scans was 8x8x8 cm, with a resolution of 0.16 mm. The tooth was placed in axial, frontal and transverse views, taking care that the resulting tomographic sections always followed the longitudinal axis of the tooth. Using the measurement tool in the software, the digital ruler was placed from the incisal or occlusal edge to the end of the observable vestibular alveolar bone, as shown in Fig. 2. This measurement was repeated three times. An indirect measurement of the vestibular alveolar bone height of each tooth was previously measured directly.
The Kolmogorov-Smirnov test was employed to assess the data distribution. Means and standard deviations were calculated, and bivariate analysis, Pearson's correlation coefficients and paired t test were used to establish the significance of differences between the measurements. All the data were analyzed using IBM SPSS Statistics version 22 software. Tests were applied with $p \leq 0.05$ level of significance.

RESULTS

The vestibular alveolar bone height based on 30 measurements for the direct technique (Vernier caliper measurement) was 11.92±2 mm, while for the CBCT indirect technique at 0.16 mm, it was 15.07±4 mm (Table 1). Mean dehiscences were overestimated by 3.15 mm (26%) on CBCT.

Paired t test demonstrated significant differences between direct measurement and SD CBCT (p= 0.001), as shown in Table 2.

Pearson's correlation coefficient (PCC) between direct and indirect measurements was 0.68, which is statistically significant (P<0.001) and indicates that linear correlation between parameters was moderate.

DISCUSSION

Cone beam computed tomographic (CBCT) imaging has emerged as an important supplemental radiographic technique in different areas. In this study, low-resolution CBCT values differed significantly from the values measured using Vernier caliper. Even though the voxel size of the CBCT Orthophos XG 3D is one of the smallest and most accurate available, the results of this study are consistent with those of Sun et al.[12], who reported discrepancies of up to 2 mm between direct and indirect measurements. The current study showed discrepancies of up to 3 mm between techniques, probably due to the thinness of the cortical layer.

Leung et al.[4] compared direct and indirect measurements, finding a threefold higher incidence of fenestrations when measuring using CBCT compared to direct measurement, and more false positives. There were also false negatives in which CBCT showed no real defects, with more than half of the dehiscences not detected by CBCT.

However, Patcas et al.[10] and Timock et al.[14] found no significant difference between direct and indirect measurements. The importance of the current study is that it considers the variables present in a living patient and shows the clinical reality of the tomograms. These variables may generate discrepancies between studies, as may other factors related to the *in vitro* technique, such as alcohol, glutaraldehyde or formaldehyde fixation, the presence or absence of restorations and soft tissues, or the nonexistent movement of the corpse or skull, all of which can generate changes in the density and architecture of the periodontium at the time of the scan[1, 3, 4, 10, 14]. However, the position of the skull does not affect the accuracy of the linear measurements[2].

This study used the standard specifications provided by the manufacturer: 12 mA, 90 kVp and 14.9-second exposure, according to the scanner user manual[13]. Misch *et al.* suggested that *in vivo* studies should be undertaken as in previous studies that employed 47.7 mA, 120 kVp and a 20-second exposure[15]; or 98 mA, 120 kVp and a 20-second exposure[16]. Both studies showed that all alveolar defects (artificially created *in vitro*) were detected with high precision, although the radiation level was significantly increased. However, other studies used different values, such as: 1–3 mA, 7 mA, 2 mA and for example, 80 kVp and a 17-second exposure, or 0.5 mA, 110 kVp and a 5–7-second exposure, and obtained results with low accuracy and sensitivity[12, 17, 18].

In addition, the use of CBCT as a reference standard is not appropriate, since all scanners showed errors in the measurements. The study explains that the lack of soft tissue affects the final image and the presence of tissue generates attenuation coefficients that affect image sharpness.

This study was conducted in periodontally compromised patients. Healthy patients might have longer

Table 1: Descriptive statistics of direct and indirect measurements of vestibular alveolar bone height.

	Direct measurement X=30 mm	Indirect measurement X=30 mm
Mean± standard deviation	(11.92 ± 2)	(15.07 ± 4)
Minimum	(8.58 ± 0.01)	(9.04 ± 0.01)
Maximum	(15.92 ± 0.01)	(20.18 ± 0.01)
Range	(7.34 ± 0.01)	(11.14 ± 0.01)
Mean (average) difference	Reference standard	+ (3.15)

Table 2: Paired t test between measurement groups and limits of the confidence interval (95%) for the mean difference.

Technique comparison	Mean value	Standard deviation	Standard error	Lower limit	Upper limit	t	Bilateral significance
Vernier – CBCT	-3.1	2.7	0.50	-4.2	-2.1	-6.3	0.001

and thinner cortical bones, which would be even more difficult to detect by CBCT.

The use of CBCT images to measure the vestibular alveolar bone of incisors and premolars provides inaccurate results when using SD resolutions in the Orthophos XG 3D (0.16 mm voxel), since it over estimates the size of dehiscences by an average 3.15 mm. SD CBCT measurements differed significantly from values measured with Vernier caliper.

Likewise, the Pearson correlation between the two techniques was 0.68, indicating a moderate correlation. This result confirms the overestimation of the descriptive statistics.

CBCT has proven its accuracy for the measurement of bone structures, but its ability to detect the thin cortical bones and determine alveolar bone height is poor. It is therefore recommended that this tool, which still has limitations, should not be used as a determining factor in treatment, but as an aid to diagnosis.

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REFERENCES

1. Fleiner J, Hannig C, Schulze D, Stricker A, Jacobs R. Digital method for quantification of circumferential periodontal bone level using cone beam CT. Clin Oral Investig 2013; 17:389-396.
2. Berco M, Rigali Jr PH, Miner RM, DeLuca S, Anderson NK, Will LA. Accuracy and reliability of linear cephalometric measurements from cone-beam computed tomography scans of a dry human skull. Am J Orthod Dentofacial Orthop 2009; 136:17-18.
3. Mischkowski R A, Pulsfort R, Ritter L, Neugebauer J, Brochhagen H G, Keeve E, Zöller J. E. Geometric accuracy of a newly developed cone-beam device for maxillofacial imaging. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2007; 104:551-559.
4. Leung CC, Palomo L, Griffith R, Hans MG. Accuracy and reliability of cone-beam computed tomography for measuring alveolar bone height and detecting bony dehiscences and fenestrations. Am J Orthod Dentofacial Orthop 2010; 137: 109-119.
5. Karring T, Nyman S, Thilander B, Magnusson I. Bone regeneration in orthodontically produced alveolar bone dehiscences. J Periodontal Res 1982; 17:309-315.
6. Enhos S, Uysal T, Yagci A, Veli İ, Ucar FI, Ozer T. Dehiscence and fenestration in patients with different vertical growth patterns assessed with cone-beam computed tomography. Angle Orthod 2012; 82:868-874.
7. Beckmann S, Kuitert RB, Prahl-Andersen B, Segner D, The R, Tuinzing D. Alveolar and skeletal dimensions associated with lower face height. Am J Orthod Dentofacial Orthop 1998; 113:498-506.
8. Gracco A, Lombardo L, Mancuso G, Gravina V, Siciliani G. Upper incisor position and bony support in untreated patients as seen on CBCT. Angle Orthod 2009; 79:692-702.
9. Yagci A, Veli İ, Uysal T, Ucar FI, Ozer T, Enhos S. Dehiscence and fenestration in skeletal Class I, II, and III malocclusions assessed with cone-beam computed tomography. Angle Orthod 2011; 82:67-74.
10. Patcas R, Müller L, Ullrich O, Peltomäki T. Accuracy of cone-beam computed tomography at different resolutions assessed on the bony covering of the mandibular anterior teeth. Am J Orthod Dentofacial Orthop 2012; 141:41-50.
11. Periago DR, Scarfe WC, Moshiri M, Scheetz JP, Silveira AM, Farman AG. Linear accuracy and reliability of cone beam CT derived 3-dimensional images constructed using an orthodontic volumetric rendering program. Angle Orthod 2008; 78:387-395.
12. Sun Z, Smith T, Kortam S, Kim D-G, Tee BC, Fields H. Effect of bone thickness on alveolar bone-height measurements from cone-beam computed tomography images. Am J Orthod Dentofacial Orthop 2011; 139:117-127 .
13. Company STD. Manual del operador ORTHOPHOS XG 3D / Ceph, ORTHOPHOS XG 3 Dready / Ceph. Germany: Dental Systems GmbH. 2013.
14. Timock A M, Cook V, McDonald T, Leo M C, Crowe J, Benninger B L, Covell Jr D A. Accuracy and reliability of buccal bone height and thickness measurements from cone-beam computed tomography imaging. Am J Orthod Dentofacial Orthop 2011; 140:734-744.
15. Misch KA, Erica SY, Sarment DP. Accuracy of cone beam computed tomography for periodontal defect measurements. J Periodontol 2006; 77:1261-1266.
16. Pinsky H, Dyda S, Pinsky R, Misch K, Sarment D. Accuracy of three-dimensional measurements using cone-beam CT. Dentomaxillofac Radiol 2006; 35:410-416.
17. Hilgers ML, Scarfe WC, Scheetz JP, Farman AG. Accuracy of linear temporomandibular joint measurements with cone beam computed tomography and digital cephalometric radiography. Am J Orthod Dentofacial Orthop 2005; 128: 803-811.
18. Hintze H, Wiese M, Wenzel A. Cone beam CT and conventional tomography for the detection of morphological temporomandibular joint changes. Dentomaxillofac Radiol 2007; 36:192-197.

Is it necessary to pre-treat Dentine before GIC Restorations? Evidence from an in Vitro Study

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ABSTRACT

The aim of this study was to assess the influence of different pre-treatment approaches on glass ionomer cement (GIC) bond strength (BS) to dentine. Sixty bovine incisors were allocated into six groups according to substrate – sound or caries-affected dentine; and substrate pre-treatment approach – no conditioning (control), polyacrylic acid for 10 s and phosphoric acid for 7 s. Teeth in the caries-affected dentine group were previously submitted to cariogenic pH-cycling challenge. After dentine pre-treatment, according to experimental groups, polyethylene tubes were placed on flat dentine surfaces and filled with GIC. Teeth were stored in distilled water for 24 h at 37 °C and then submitted to microshear test (0.5 mm/min). Failure pattern analysis was performed under stereomicroscope (400x). Data were analysed using two-way ANOVA and Tukey's test (α=5%). Statistically significant differences were found for the pre-treatment approach, regardless of substrate (p<0.001). The polyacrylic acid group and control group had similar BS values, and were both better than the phosphoric acid group. In general, GIC had better bonding performance in sound dentine than in caries-affected dentine. In conclusion, dentine pre-treatment with polyacrylic acid did not improve the performance of GIC restoration on clinically relevant substrates.

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Keywords: *shear strength, glass ionomer cements, dental caries, dental atraumatic restorative treatment.*

É necessário pré tratar a dentina antes das restaurações de CIV? Evidência de um estudo in vitro

RESUMO

O objetivo deste estudo foi avaliar a influência de diferentes pré-tratamentos na resistência de união (RU) de cimentos de ionômero de vidro (CIV) a dentina. Sessenta incisivos bovinos foram alocados em 6 grupos de acordo com o substrato – hígido ou cariado; e com a abordagem de pré-tratamento – sem condicionamento (controle), ácido poliacrílico por 10 s, e ácido fosfórico por 7 s. Os dentes pertencentes aos grupos de dentina cariada foram previamente submetidos ao desafio cariogênico por meio da ciclagem de pH. Após o pré-tratamento da dentina, de acordo com os grupos experimentais, tubos de polietileno foram colocados sobre superfícies planas de dentina e preenchidos com CIV. Os dentes foram armazenados em água destilada por 24 h a 37°C e então submetidos ao teste de microcisalhamento (0,5 mm/min). A análise do padrão de fratura foi realizada em estereomicroscópio (400x). Os dados obtidos foram analisados usando ANOVA de dois fatores e teste de Tukey (α=5%). Diferença estatisticamente significante foi encontrada para as diferentes abordagens de pré-tratamento, independente do substrato (p<0,001). Aplicação de ácido poliacrílico resultou em valores de RU similares aos do grupo controle. Entretanto, ambos os grupos mostraram um melhor desempenho quando comparado a aplicação de ácido fosfórico. De forma geral, CIV apresentou melho5 desempenho adesivo em dentina sadia quando comparada a dentina cariada. Em conclusão, o pré-tratamento em dentina com ácido poliacrílico não melhora o desempenho das restaurações de CIV em substratos clinicamente relevantes.

Palavras-chave: *resistência ao cisalhamento, cimentos de ionômeros de vidro, cárie dentária, tratamento dentário restaurador sem trauma.*

INTRODUCTION

Caries prevalence today is still high in many populations[1]. Due to the negative impact on patients' lives associated to more severe stages of the disease,[2] efforts have focused on developing more effective treatments associated to preventive measures[3]. As a result, the World Health Organization (WHO) has recommended Atraumatic

Restorative Treatment (ART) as part of the Basic Package of Oral Care (BPOC)[3].

Glass Ionomer Cements (GIC) have been proposed as the material of choice for ART. Although GIC longevity is similar to that of other restorative materials in primary teeth[4], it still poses a challenge for the survival of occlusal-proximal restorations. In this regard, the use of protocols to improve substrate-material adhesion may enhance the longevity of restorations. However, there is still no consensus regarding which substrate pre-treatment protocol is best when using GIC.

The GIC bonding mechanism can be explained as an ionic interaction with bipolar electrostatic forces between the cement and the dental structure, with an important role of the initial wetting, promoted by the carboxylic free radicals, for effective adhesion[5]. Knowing that this adhesion is more critical in dentine than in enamel, several authors have assessed methods to improve it[6-11], understanding that the frequently used technique of leaving a "smear layer" on the cavity walls in the carious tissue removal process may help. Two of the main discussion points are whether or not the smear layer should be removed and which is the best pre-treatment alternative[6-11].

Previous studies have used different products for surface pre-treatment, ranging from different polyacrylic acid concentrations to phosphoric acid, which has a conditioning/cleaning effect. However, it has been suggested that in addition to removing the smear layer, strong acids used as pre-treatment agents could also cause enamel and dentine decalcification, considerably reducing the amount of calcium available for adequate adhesion, leading to a decrease in bond strength[9]. Regarding the use of polyacrylic acid, the literature has shown some advantages in relation to GIC properties[10,11]. Other studies have shown that some products do not interfere in adhesion quality or even that mechanical cleaning procedures only may be enough[6].

Thus, there is a lack of evidence regarding the influence of pre-treatment on the materials' bond strength to dental substrates, especially those submitted to cariogenic challenges, which are clinically relevant because they are commonly found during restorative procedures in the current scenario of minimal intervention.

Thus, the aim of this *in vitro* study was to assess the influence of different pre-treatment approaches on GIC bond strength to both sound and carious dentine. The hypothesis is that there is no difference in bond strength values according to the pre-treatment of dentine.

MATERIALS AND METHODS

Study design and ethics

This *in vitro* study received ethical and legal approval from the Santa Cecilia University Ethics Committee (Protocol #04/2016). Experiments were conducted following the Ethical Principles on Animal Experimentation, adopted by the Brazilian Laboratory Animal Science Association (COBEA) and certified by the Use of Animals Ethics Committee of the Cruzeiro do Sul University according to Law 11.794/2008.

Teeth were randomly assigned to six experimental groups according to substrate – sound or caries-affected dentine; and substrate pre-treatment approach – no conditioning (control), polyacrylic acid for 10 s or phosphoric acid for 7 s.

Sample selection

The sample consisted of 60 bovine teeth (n=10), according to a previous study of GIC bond strength to bovine dentine[12]. Inclusion criteria were the absence of structural defects and/or cracks and fractures. Teeth were cleaned with pumice slurry and stored in chloramine solution T 0.5% at 4 ºC for 30 days, with the solution being changed weekly.

Tooth preparation

Roots were removed with diamond discs in a cutting machine (Labcut 1010, Extec Co, Enfield, USA) and crowns were embedded in PVC tubes with chemically activated acrylic resin, (JET Clássico®, São Paulo, SP, Brazil).

The dentine surface was abraded with silicon carbide water sandpaper #180 for 60 sec to obtain a flat surface, and then with #600 for 60 sec to standardise the smear layer.

Cariogenic Challenge

Caries-affected dentine specimens were submitted to a pH-cycling cariogenic challenge as follows: 14 immersion cycles (8 hours) in a demineralising solution (2.2 mM $CaCl_2$, 2.2 mM NaH_2PO_4, 0.05M acetic acid, pH adjusted at 4.5 with 1M KOH); and then, immersion (16 hours) in a remineralising solution (1.5 mM $CaCl_2$, 0.9 mM NaH_2PO_4, 0.15 mM KCL, pH =7.0), at room temperature without shaking[13].

Restorative procedures

Pre-treatment substrate approaches were performed following manufacturers' recommendations according to allocation group. After surface treatments, three polyethylene tubes 1.0 mm tall and 0.76 mm in diameter (*micro-bore® Tygon S-54-HL Medical Tubing*, Saint-Gobain Performance Plastics, Akron, OH) were placed on the exposed surfaces. Tubes were filled with an encapsulated GIC (Riva Self Cure, SDI, Victoria, Australia) inserted with an applicator. A thin petroleum jelly layer was applied over the material to prevent both water absorption and loss.

Specimens were stored in distilled water at 37°C for 24 h. After this time, tubes were removed using a #15 blade. All samples were analysed under 10x microscope, excluding those presenting bubbles, interface failures and other defects. All specimens were prepared by a single trained operator at room temperature (24 °C).

Microshear test

After 24h storage, an external examiner, blinded to the experimental groups, fixed the specimens to a device, previously adapted to a testing machine (Kratos, Kratos Dinamômetros, Brazil). Thin wires (0.20 mm) were used to make a loop around the load cell projection and the GIC cylinder, maintaining the contact with the dentine surface as close as possible to the bonding interface. A shear force (0.5 mm/min crosshead speed) was applied until failure occurred.

Maximum load values supported by the dentine/material bond were expressed in Newtons (N) and later converted to megapascals (MPa), considering the inner diameter area of the polyethylene tube used as matrix.

Failure Mode

After testing, the specimens were assessed under stereomicroscope at 400x magnification (HMV II, Shimadzu, Kyoto, Japan) to establish the failure mode. Failures were classified as adhesive (failure in the substrate/material interface), cohesive in the dentine or the GIC (failure in the substrate or the material), and mixed (combination between adhesive and cohesive, with any type of cohesive failure of up to 25% on the interface).

Statistical Analysis

The experimental unit was the tooth. Bond Strength values (BS) in MPa were initially assessed for normality distribution and variances homogeneity using Kolmogorov-Smirnov and Levene's tests, respectively. To analyse whether the substrate pre-treatment approaches influence the BS, two-way analysis of variance – pre-treatment and substrate condition – was conducted. Chi-square was used to compare premature failures among groups. Descriptive analyses of the failure modes in relation to experimental groups were also performed. A significance level of 5% was adopted for all analyses. SPSS V16 for Windows (SPSS Inc., Chicago, IL, USA) was used.

RESULTS

Microshear bond strength

Table 1 shows the results, including BS means and standard deviations, for the experimental groups in sound and caries-affected dentine.

ANOVA showed statistically significant differences between the main factors *pre-treatment* ($p<0.001$) and *substrate condition* ($p=0.025$). However, interaction among factors did not show any differences ($p=0.058$). GIC had better bond performance in sound dentine than caries-affected dentine. In addition, pre-treatment with phosphoric acid resulted in lower BS values when compared to both polyacrylic acid and control groups. Polyacrylic acid application led to similar BS values to those in the control group.

Table 1: Microshear bond strength means and standard deviations (MPa) for all experimental groups according to substrate.

	Substrate pre-treatment		
Substrate condition	**No conditioning**	**Polyacrylic acid**	**Phosphoric acid**
Sound dentine	$12.60 \pm 2.68^{a,A}$	$16.01 \pm 4.38^{a,A}$	$9.29 \pm 1.47^{b,A}$
Caries-affected dentine	$12.20 \pm 5.55^{a,B}$	$10.43 \pm 4.36^{a,B}$	$8.68 \pm 2.25^{b,B}$

*Different superscript lowercase letters indicate significant differences among the types of substrate pre-treatment.
**Different superscript uppercase letters indicate significant differences between substrate condition.

Failure patterns analysis

Fig. 1 presents the failure pattern distribution in the experimental groups. In general, there was predominance of adhesive and mixed failures. Cohesive failures in the substrate were only seen, though less frequently, in caries-affected dentine.

Table 2 shows the distribution of premature failures in the experimental groups. Regardless of substrate, there was no statically significant difference among groups.

DISCUSSION

The field of dental materials still lacks evidence of the influence of pre-treatment approaches on GIC bonding to dental substrates. Clinically, substrates subjected to cariogenic challenges are the most relevant because they are commonly found during restorative procedures in the current scenario of minimal intervention.

In this study, the application of polyacrylic acid led to BS values similar to those in the control group, regardless of the substrate, corroborating previous studies that showed no benefit of surface pre-treatment with 10-25 % polyacrylic acid in terms of GIC bond strength to the dentine[14-16]. However, other reports refute such findings by showing several advantages of polyacrylic acid pre-treatment for enhancing GIC properties[10,11].

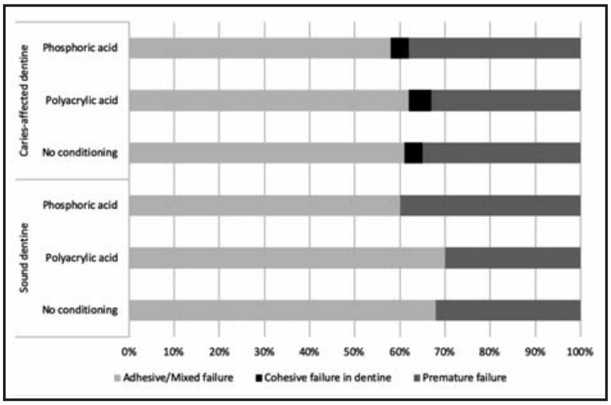

Fig. 1: Failure pattern distribution in the experimental groups.

GIC chemical bonding to dental substrates has been recognised as one of the main characteristics of these materials[5-7]. However, the effective validity of these bonds is still poorly understood, with only some explanatory theories. Bond strength is basically influenced by two factors: substrate composition and surface pre-treatment (conditioning). The presence of contaminating agents may alter the surface energy and therefore the material wettability to the dental surface[17]. Thus, the *smear layer* interferes with the GIC bonding to the dental structures,[18] which is why pre-treatment is advocated[7,19,20]. Still, this theory was not confirmed by the present study.

Pre-treatment with phosphoric acid resulted in lower BS values than those in the polyacrylic acid group and even those in the control group. This could be explained by the excessive demineralisation and consequent lack of minerals, which are essential for the GIC chemical bonding. Results from this study complement those found by Kokmaz et al.[20], who did not observe better GIC bonding to either enamel or dentine after the application of phosphoric acid.

These findings may be explained by the GIC bond mechanism itself, which is mainly produced by ionic exchange with calcium free radicals. Thus, strong acids such as citric or phosphoric acid, in addition to removing the *smear layer*, (rich in calcium radicals and capable of forming a bonding bridge between the cement and the dentinal walls), also act as enamel and dentine decalcifying agents, greatly reducing the amount of available calcium required for adequate bonding[7,17].

As has also been observed in previous studies, the GIC that we used had better adhesive performance on sound dentine[21-23]. It has been hypothesised that differences in chemical compositions and morphology may be responsible for the poorer restorative materials performance, especially GIC, which, as mentioned above, needs calcium bonds to the substrate to support the chemical reaction.

Table 2: Distribution of premature failures according to experimental groups.

		Sound dentine			Caries-affected dentine	
Experimental groups	No conditioning	Polyacrylic acid	Phosphoric acid	No conditioning	Polyacrylic acid	Phosphoric acid
Premature failures n (%)	14 (46.7)	13 (43.3)	20 (66.7)	16 (53.3)	15 (50)	18 (60)

It should be highlighted that the caries-affected dentine in this study was artificially developed. This method for developing carious lesions produces dentinal lesions resembling natural ones[13]. It has proven to be effective, especially for BS tests, and has been suggested for use because it allows a greater area of exposed dentine than other methods of artificial carious lesion development[13]. Additionally, other factors, such as operator variability, may affect mechanical tests results. Indeed, Adebayo et al.[24] observed that as the operator gained experience, there was a gradual increase in mean values and decrease in standard deviations and variation coefficient from tests results. This reflects the importance of training in the methodology used. The present study found a high number of premature failures, possibly as a result of the stress applied to the specimens, which may have caused a fracture when the polyethylene tubes were removed before microshear testing, as previously suggested by Tedesco et al.,[25] who verified a clear trend to premature failures as a result of removing polyethylene and/or starch tubes.

To conclude, dentine pre-treatment showed no benefit for the GIC bond to either sound or affected dentine. However, this study was conducted using a single GIC brand, and the results cannot be extrapolated to other GIC brands. Studies assessing the bond stability of GIC when submitted or not to pre-treatment approaches are needed to contribute to the evidence to support the best protocol for restorations with Glass Ionomer Cements, which are effective, cheaper, less time-consuming materials.

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REFERENCES

1. Gimenez T, Bispo BA, Souza DP, Viganó ME, et al. Does the Decline in Caries Prevalence of Latin American and Caribbean Children Continue in the New Century? Evidence from Systematic Review with Meta-Analysis. PLoS One 2016;11:e0164903. doi:10.1371/journal.pone.0164903. eCollection 2016.
2. Guedes RS, Ardenghi TM, Piovesan C, Emmanuelli B, Mendes FM. Influence of initial caries lesions on quality of life in preschool children: a 2-year cohort study. Community Dent Oral Epidemiol 2016;44:292-300. doi:10.1111/cdoe.12217
3. Frencken JE, Holmgren CJ, van Palenstein Helderman WH. Basic package of oral care. WHO Collaboration Centre for Oral Health Care Planning and Future Scenarios. Them Netherlands: Nijmegen; 2003. http://www.chdentalinstitute.org/images/BPOC.pdf
4. Tedesco TK, Calvo AF, Lenzi TL, Hesse D, et al. ART is an alternative for restoring occlusoproximal cavities in primary teeth - evidence from an updated systematic review and meta-analysis. Int J Paediatr Dent 2017; 27:201-209. doi:10.1111/ipd.12252
5. Mount GJ. Adhesion of glass-ionomer cement in the clinical environment. Oper Dent 1991; 16:141-148.
6. Hoshika S, De Munck J, Sano H, Sidhu SK, van Meerbeek B. Effect of Conditioning and Aging on the Bond Strength and Interfacial Morphology of Glass-ionomer Cement Bonded to Dentin. J Adhes Dent 2015;17:141-146. doi:10.3290/j.jad.a33994
7. Powis DR, Folleras T, Merson SA, Wilson AD. Improved adhesion of a glass ionomer cement to dentin and enamel. J Dent Res 1982; 61:1416-1422. doi:10.1177/00220345820610120801
8. Shalabi HS, Asmussen E, Jorgensen KD. Increased bonding of a glass of a glass ionomer cement to dentin by means of FeC13. Scand J Dent Res 1981; 89:348-353. doi:10.1111/j.1600-0722.1981.tb01693.x
9. El Wakeel AM, Elkassas DW, Yousry MM. Bonding of contemporary glass ionomer cements to different tooth substrates; microshear bond strength and scanning electron microscope study. Eur J Dent 2015; 9:176-182. doi:10.4103/1305-7456.156799
10. Aboush YEY, Jenkins CBG. The effect of poly acrylic acid cleanser on adhesion of a glass polyalkenoate cement to enamel and dentine. J Dent 1987; 15:147-152. doi:10.1016/0300-5712(87)90138-2
11. Hewlett ER, Caputo AA, Wrobel DC. Glass ionomer bond strenght and treatment of dentin with polyacrylic acid. J Prosthet Dent 1991; 66:767-772. doi:10.1016/0022-3913(91)90412-p
12. Olegário IC, Malagrana APVFP, Sun Ha Kim S, Hesse D, et al. Mechanical Properties of High-Viscosity Glass Ionomer Cement and Nanoparticle Glass Carbomer. J Nanomater 2015; 2015:472401. doi.org/10.1155/2015/472401
13. Lenzi TL, Tedesco TK, Calvo AFB, Ricci HA, Raggio DP. Does the method of caries induction influence the bond

strength to dentin of primary teeth?. J Adhes Dent 2014; 16:333-338. doi:10.3290/j.jad.a31799

14. Glasspoole EA, Erickson RI, Davidson CL. Effect of surfece treatment on the bond strength of glass ionomer to enamel. Dent Mater 2002; 18:454-462. doi:10.1016/s0109-5641(01)00068-9
15. Banamyong D, Palamara JEA, Burrow MF, Messer HH. Effect of dentin conditioning on dentin permeability and micro-shear strength. Eur J Oral Sci 2007; 115:502-509. doi:10.1111/j.1600-0722.2007.00483.x
16. Ekworapoj P, Sidhu SK, McCabe JF. Effect of surface conditioning on adhesion of glass ionomer cement to Er,Cr:YSGG laser-irradiated human dentin. Photomed Laser Surg 2007; 25:118-123. doi:10.1089/pho.2006.2004
17. Van Meerbeek B, Yoshida Y, Inoue S, De Muck J, Van Landuyt K, Lambrechts P. Glass-ionomer adhesion: The mechanism at the interface. J Dent 2006; 34:615-616.
18. Nakanuma K, Hayakama T, Tomita T, Yamazaki M. Effect of the application of dentin primers and a dentin bonding agent on the adhesion between the resin-modified glass-ionomer cement and dentin. Dent Mater 1998; 14:281-286. doi:10.1016/s0109-5641(98)00040-2
19. Pereira PN, Yamada T, Tei R, Tagami J. Bond strength and interface micromorphology of an improved resin-modified glass ionomer cement. Am J Dent 1997; 10:18-32.
20. Kokmaz Y, Ozel E, Attar N, Bicer CO. Influence of different conditioning methods on the shear bond strength of novel light-curing nano-ionomer restorative to enamel and dentin. Lasers Med Sci 2010; 25:861-866. doi:10.1007/s10103-009-0718-8
21. Ceballos L, Camejo DG, Fuentes MV, Osório R, et al. Microtensile bond strenght of total-etch and self-etching adhesives to caries affected dentine. J Dent 2003; 31:469-477. doi:10.1016/s0300-5712(03)00088-5
22. Hosoya Y, Kamada E, Ushigome T, Oda Y, Garcia-Godoy F. Micro-tensile bond strenght of sound and caries-affected primary tooth dentin measured with original designed jig. J Biomed Mater Res B Apll Biomater 2006; 77:241-248. doi:10.1002/jbm.b.30433
23. Tedesco TK, Bonifacio CC, Hesse D, Klerverlaan CJ, Lenzi TL, Raggio DP. Bonding longevity of flowable GIC layer in artificially carious dentin. Int J Adhes Adhes 2014; 51:62-66. doi.org/10.1016/j.ijadhadh.2014.02.011
24. Adebayo OA, Burrow MF, Tyas MJ. Bond strength test: role of operator skill. Aust Dent J 2008; 53:145-150. doi:10.1111/j.1834-7819.2008.00024.x
25. Tedesco TK, Montagner AF, Skupien JA, Soares FZM, Susin AH, Rocha RO. Starch Tubing: An alternative method to buid up microshear bondst specimens. J Adhes Dent 2013; 15:311-315. doi:10.3290/j.jad.a28602

Internal Lower Incisor Morphology revealed by Computerized Microtomography

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ABSTRACT

This study evaluated the internal morphology of lower incisors using computerized microtomography (micro-CT) images. Eighty-nine lower incisors were scanned by micro-CT and reconstructed with NRecon software. 2D parameters (perimeter, root length, circularity and canal diameter) and 3D parameters (volume, surface area and structure model index) were evaluated with CTAn and CTVol software. The results are presented descriptively. It was found that 89.9% of the canals had a single main root canal (type I), followed by type II (6.7%) and III (3.4%), while 5.6% of the specimens presented lateral canals and 1.1% had an apical delta. Mean volume and surface area were 31.80mm³ and 90.58mm², respectively. The most prevalent shape of the root canal at CEJ level was circular (41.6%) and 1mm from the apex, 73% of the samples were classified as oval. Lower incisors with internal anatomical variations may offer a high degree of technical complexity and may result in treatment failure.

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Keywords: *computerized microtomography, X-ray microtomography, endodontics, incisors.*

Morfologia interna de incisivos inferiores reveladas por microtomografía computadorizada

RESUMO

Este estudo avaliou a morfologia interna dos dentes incisivos inferiores utilizando imagens de microtomografia computadorizada (micro-TC). 89 incisivos inferiores foram escaneados por micro-CT e reconstruídos com o programa NRecon. Parâmetros 2D - perímetro, comprimento da raiz, circularidade e diâmetro do canal - e parâmetros 3D - volume, área superficial e índice de modelo de estrutura - foram avaliados com os softwares CTAn e CTVol. Os resultados foram apresentados de forma descritiva. 89,9% dos canais apresentaram um único canal radicular principal (tipo I), seguido pelo tipo II (6,7%) e III (3,4%). 5,6% dos espécimes apresentavam canais laterais e 1,1% delta apical. O volume médio e a área de superfície foram 31,80mm³ e 90,58mm², respectivamente. A forma mais prevalente do canal radicular no nível da CEJ foi circular (41,6%) e a 1 mm do ápice, 73% das amostras foram classificadas como ovais. Incisivos inferiores com variações anatômicas internas podem oferecer um alto grau de complexidade técnica e resultar em falha do tratamento.

Palavras-chave: *microtomografia computadorizada, microtomografia por raios-X, endodontia, incisivos.*

INTRODUCTION

The root canal system (RCS) has complex internal anatomy, which needs to be clearly understood in order to ensure successful endodontic treatment. Since anatomical variations offer a high degree of technical complexity and may even result in treatment failure, the practitioner should be fully aware of both internal tooth anatomy and anatomical variations observed in daily practice[1,2]. Incisors have rectilinear roots, so their anatomy is not considered to be complex, nevertheless, mandibular incisors may present variations such as oval and flattened root canals, as well as a second root canal in 11% to 45% of teeth[2].

Several imaging methods have been used to evaluate root canal anatomy, but many of these techniques are time consuming and present certain difficulties such as artifacts and distortion[3]. Thus, in order to ensure successful root canal treatment, more accurate imaging methods should be used to analyze internal canal morphology [2].

Although computerized microtomography (micro-CT) is not yet available for daily clinical use, it is rapidly gaining popularity for scientific research in the field of dentistry. The micro-CT imaging method has several advantages compared to other methods, mainly because it is fast, reproducible and non-invasive, provides high-quality results which can be viewed in 2D and 3D, and enables simultaneous observations of both internal and external tooth anatomy[3].

In this context, the present study aims to evaluate the internal morphology of incisors using computed microtomography (micro-CT) images.

MATERIALS AND METHODS

This study was approved by the Research Ethics Committee of Federal University of Juiz de Fora (approval number 2.754.921). Forty lower central incisors and 49 lower lateral incisors were selected from the Biobank, totaling 89 teeth presenting healthy crowns and closed apices.

After cleaning the external surfaces using ultrasound (ProfiClass, Dabi Atlante Ltda. - Ribeirão Preto, SP, Brazil), the teeth were stored in universal collectors (Farmax - Distribuidora Amaral, Divinópolis, MG, Brazil) and identified. Images were acquired using a SkyScan 1173 microtomograph (Bruker Micro-CT Kontich, Belgium) applying the following parameters: 50Kv, 160 µA, 1mm thickness aluminum filter with 360° rotation, 0.8º rotation step, pixel size 12.11µm, in 2240 x 2240 (small pixel size) camera mode, totaling a scanning time of approximately 28 minutes per specimen.

Subsequently, three-dimensional (3D) specimen images were reconstructed using NRecon v1.6.6.0 software (Brucker MicroCT, Kontich, Belgium) and reduction of possible artifacts. This process resulted in an average 794 slices per specimen (range 636 to 918 slices), from the apex to the level of the cemento enamel junction, enabling internal microstructures to be viewed. The reconstructed images were then saved in bitmap format (bmp).

The following quantitative 3D parameters were evaluated: volume, root canal surface area, structure model index (SMI). When the SMI was close to 0, the root canal was considered flat, a value close to 3 was considered cylindrical and a value close to 4 spherical[4,5]. The following two-dimensional (2D) parameters were assessed: perimeter, root length from cementoenamel junction (CEJ) to the apex and shape of the canal mouth, cervical, middle and apical thirds, circularity, smallest and largest anatomical canal diameter at 1 mm from the apex. The cross sections of the root canals were classified as round, oval or long oval according to the smaller and main canal diameter at 1 mm from the apex[6]. The 3D analysis plug-in and morphometric analysis tools from CTAn software were used to obtain these measurements. The presence and location of accessory canals and the amount of foramina at 4 mm from the apex were also evaluated.

The following anatomical parameters were evaluated using the CTAn V.1.13 and CTVol v.2.2.1 software (Bruker microCT) in the qualitative analysis: number of root canals, main root canal classification, occurrence and location of lateral root canals[7], curvature degree of the main canal[8], position of the anatomical foramen in relation to the apex, and presence or absence of apical constriction and its classification, when present[9].

The qualitative and quantitative results of the anatomical parameters were presented descriptively, without statistical analyses.

RESULTS

The analysis of 89 human incisors indicated that 89.9% of the canals had a single main root canal and were therefore classified mostly as Vertucci type I canals. Type II (6.7%) and type III (3.4%) conformations were found only in lower central incisors (Fig. 1). Only 5.6% of the specimens presented lateral root canals, 4.5% and 1.1% in the apical and middle third, respectively. However, no lateral root canal was observed in the cervical third. Only 1.1% of the sample presented an apical delta (Fig. 2).

Regarding the quantitative three-dimensional parameters, the central incisor canals had mean volume and surface area of 27.31 ± 7.68 mm³ and 82.81 ± 15.04 mm², respectively. For lateral incisors, average volume was 35.88 ± 11.11 mm³ and average surface area was 97.64 ± 18.83 mm². The SMI indicated that the root canal geometry of the central and lateral incisors resembled a cylinder, with SMI = 2.12 ± 0.39 and $1.94 + 0.55$, respectively.

The two-dimensional analysis results (perimeter, circularity, and largest and smallest diameter) of the

central and lateral incisors are shown in Table 1. Root length measured from the pulp chamber to the apical foramen ranged from 11.76 to 20.84 mm (15; 89 ± 1.96mm) and the average number of apical foramina 4mm below the apex was 1.10 ± 0.45.
Regarding the shape of the root canal mouths at CEJ level, central incisors had prevalence of circular (58%), followed by oval (20%), "bowling pin" (10%), kidney-shaped (5 %), triangular, fish and hourglass (3% each). In lateral incisors, prevalence was oval (35%), followed by circular (27%), fish and bowling pin (12% each), triangular (6%), kidney-shaped (4%) and hourglass-shaped (2%). (Fig. 3).

DISCUSSION

Knowledge of internal tooth anatomy is paramount for successful endodontic treatment[10]. In this context, the aim of this study was to analyze the internal morphology of mandibular and maxillary incisors by means of computed microtomography.
Several studies have reported the prevalence of a single main root canal (Vertucci Type I) in incisors, ranging from 64% to 100%[11-13]. In the present study, 89.9% of the samples were classified as Vertucci type I, similar to the results reported by Lin et al.[12] The divergences regarding the prevalence of a single root

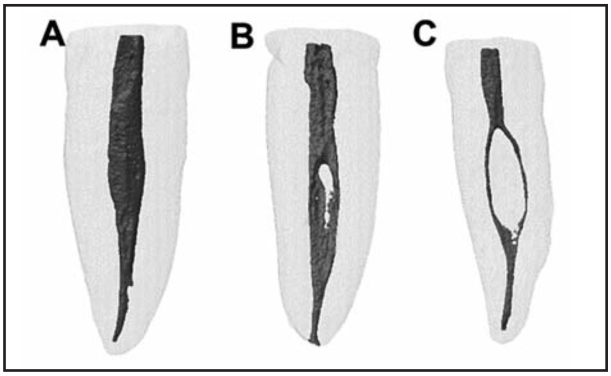

Fig. 1: Representative image of the most common main root canal classifications: A- type I, B- type II and C- type III.

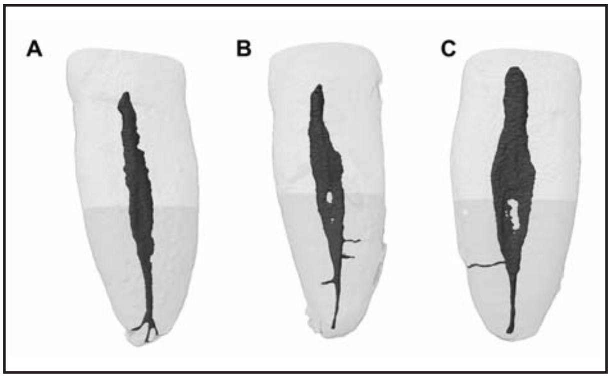

Fig. 2: Representative 3D images of A- apical delta, B and C - lateral canals.

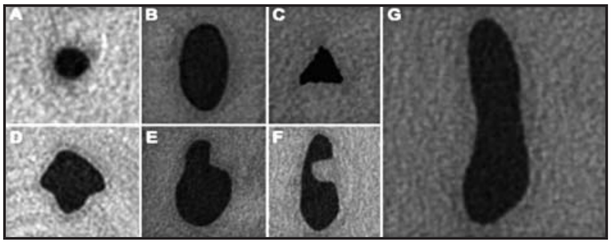

Fig. 3: Representative image of the observed cross-sections: A- circular, B- oval, C- triangular, D- fish, E- bowling pin, F- kidney-shaped and G- hourglass.

Table 1: 2D analysis of root canal morphology in 89 central and lateral mandibular incisors.

	Cervical	Middle	Apical	1mm from the apex
Central incisors				
Perimeter (mm)	2.70 (1.10)	2.06 (0.77)	1.05 (0.35)	0.91 (0.20)
Circularity	0.63 (0.13)	0.31 (0.18)	0.45 (0.18)	0.48 (0.13)
Largest diameter (mm)	0.95 (0.35)	0.94 (0.37)	0.38 (0.19)	0.36 (0.11)
Smallest diameter (mm)	0.49 (0.22)	0.24 (0.14)	0.21 (0.04)	0.20 (0.04)
Lateral incisors				
Perimeter (mm)	3.64 (1.06)	2.80 (0.98)	1.31 (0.52)	0.94 (0.22)
Circularity	0.49 (0.12)	0.26 (0.17)	0.43 (0.18)	0.62 (0.11)
Largest diameter (mm)	1.26 (0.40)	1.30 (0.43)	0.52 (0.18)	0.35 (0.09)
Smallest diameter (mm)	0.65 (0.26)	0.27 (0.15)	0.22 (0.08)	0.20 (0.05)

canal in incisors can be explained due to different populations, sample size and the proposed evaluation method, such as the use of diaphanization[11], cone beam computed tomography[12,13] and computed microtomography[1]. In addition, unlike in the present study, other assessment articles do not separate central incisors from lateral incisors, which increases the range of anatomical variations compared to the current analysis, which distinguishes between different teeth.

The presence of two separate root canals that join in the apical portion (type II) and a single root canal that divides in the middle third and then joins to form a single outlet root canal (type III) accounted for 6.7% and 3.4%, respectively, in lower central incisors only. These findings may be explained by the fact that a dentin bridge is formed inside the lower incisor pulp, creating two root canals within a single root, which can either merge and terminate in a single apical foramen or remain separate from each other throughout their length[10].

Types IV, V, VI, VII and VIII were not observed in the current study, in agreement with previous studies[11-13]. However, the aforementioned studies indicate the presence of type IV cases – two root canals separated from the crown to the apex – and/or type V cases – a single root canal that divides to form two root canals in the apical region. The analysis of different populations and the evaluation methodology applied may explain these differences.

Root length measured from the pulp chamber to the apical foramen ranged from 11.76 to 20.84 mm, in agreement with the result of a previous study[1]. The largest and smallest root canal diameter 1mm below the apex contrasts between studies, ranging from 0.13 to 1.49 (largest diameter) and 0.02-0.43 (smallest diameter)[1,14,15]. In the present study, the largest and smallest diameters in the apical third were, respectively, 0.38 ± 0.19 and 0.21 ± 0.04 for central incisors and 0.52 ± 0.18 and $0.22. \pm 0.08$ for lateral incisors. These differences between studies may be explained by the fact that anatomical groups were separated in the present study, thereby reducing variations. By grouping upper (larger) incisors with lower (smaller) incisors, average diameter variation tends to increase.

The presence of an accessory canal, a branch that exits the main canal and communicates with the external surface of the root, an apical delta and several apical foramens enables the passage of pulp irrigants to the periodontium[16] and, if not properly cleaned, may lead to endodontic treatment failure[17]. In the present study, only 5.6% of the specimens presented lateral canals, 4.5% and 1.1% in the apical and middle third, respectively, in agreement with findings reported in previous studies[1,9,18]. No lateral root canal was found in the cervical third of the teeth.

According to a previous study, only 1.1% of the sample had an apical delta, accessory canals were observed only in the apical third, and most of the central (60%) and lateral (74%) incisors had no accessory canals[1]. The average number of apical foramina 4 mm below the apex was $1.10 \text{ mm} \pm 0.45 \text{ mm}$.

Canals may have different cross-sectional shapes at different levels of the root in the same tooth[15]. In the present study, 73% of the incisors were classified as oval in the cross section 1mm below the apex. In the root canal mouth at CEJ level, the most prevalent central incisor type was circular (58%), followed by oval (20%), while for the lateral incisors there was prevalence of oval (35%), followed by circular (27%).

According to the literature, mandibular incisors usually have flat and oval canals, and oval canals in this group lead to difficulties and reduce the quality of endodontic treatment[2]. However, although the cross-sections in the apical third and canal mouth are oval and/or circular, the SMI along the entire canal in the assessed incisors indicates that root canal geometry varies from oval to cylindrical (SMI = 2.02 ± 0.49), facilitating extensive endodontic canal treatment in some in canals with this characteristic.

The root canal morphology of anterior teeth is variable and may present additional canals and a variety of canal configurations, as observed herein. The dentist should be aware of possible variations, as they may lead to errors in the attempt to locate root canals and, consequently, endodontic treatment failure.

Although incisors have a single root with relatively simple anatomy, internal anatomical variations may offer a high degree of technical complexity, leading to treatment failure if not well understood by dental professionals.

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REFERENCES

1. Leoni GB, Versiani MA, Pécora JD, Damião de Sousa-Neto M. Micro–computed tomographic analysis of the root canal morphology of mandibular incisors. J Endod 2014; 40: 710-716.
2. Paes da Silva Ramos Fernandes LM , Rice D, Ordinola-Zapata R, Alvares Capelozza AL, Bramante CM, Jaramillo D, Christensen H. Detection of various anatomic patterns of root canals in mandibular incisors using digital periapical radiography, 3 Cone-beam Computed Tomographic Scanners, and Micro-Computed Tomographic Imaging. J Endod 2014; 40:42-45.
3. Grande NM, Plotino G, Gambarini G, Testarelli l, D'Ambrosio F, Pecci R, Bedini R. Present and future in the use of micro-CT scanner 3D analysis for the study of dental and root canal morphology. Ann Ist Super Sanita 2012; 48: 6-34.
4. Peters OA, Laib A, Rüegsegger P, Barbakow F. Three-dimensional analysis of root canal geometry by high-resolution computed tomography. J Dent Res 2000; 79: 1405-1409.
5. Versiani MA, Pécora JD, Sousa-Neto MD. Microcomputed tomography analysis of the root canal morphology of single-rooted mandibular canines. Int Endod J 2013; 46:800-807.
6. Jou YT, Karabucak B, Levin J, Liu D. Endodontic working width: current concepts and techniques. Dent Clin North Am 2004; 48:323-335.
7. Vertucci FJ. Root canal anatomy of the mandibular anterior teeth. J Am Dent Assoc 1974; 89:369-371.
8. Schneider S.W. A comparison of canal preparations in straight and curved root canals. Oral Surg Oral Med Oral Patol 1971; 2:271-275.
9. Dummer PM, McGinn JH, Rees DG. The position and topography of the apical canal constriction and apical foramen. Int Endod J 1984; 17:192-198.
10. Assadian H, Dabbaghi A, Gooran M, Eftekhar B, Sharifi S, Shams N, Dehghani Najvani A, Tabesh H.. Accuracy of CBCT, Digital Radiography and Cross-Sectioning for the Evaluation of Mandibular Incisor Root Canals. Iran Endod J 2016; 11:106-110.
11. Rahimi S, Milani AS, Shahi S, Sergiz Y, Nezafati S, Lotfi M. Prevalence of two root canals in human mandibular anterior teeth in an Iranian population. Indian J Dent Res 2013; 24:234-236.
12. Lin Z, Hu Q, Wang T, Ge J, Liu S, Zhu M, Wen S. Use of CBCT to investigate the root canal morphology of mandibular incisors. Surg Radiol Anat 2014; 36:877-882.
13. Kamtane S, Ghodke M. Morphology of mandibular incisors: a study on CBCT. Pol J Radiol 2016; 81:15-16.
14. Mauger MJ, Schindler WG, Walker WA 3rd. An evaluation of canal morphology at different levels of root resection in mandibular incisors. J Endod 1998; 24:607-609.
15. Wu MK, R'oris A, Barkis D, Wesselink PR. Prevalence and extent of long oval canals in the apical third. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2000; 89:739-743.
16. Vertucci FJ. Root canal morphology and its relationship to endodontic procedures. Endod Top 2005; 10:3-29.
17. Siqueira JF Jr. Reaction of periradicular tissues to root canal treatment: benefits and drawbacks. Endod Top 2005; 10: 123-147.
18. Gomes BP, Rodrigues HH, Tancredo N. The use of a modelling technique to investigate the root canal morphology of mandibular incisors. Int Endod J 1996; 29: 29-36.

Odontogenic Infection and dental Pain negatively impact Schoolchildren's Quality of Life

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ABSTRACT

The aim of this study is to assess the prevalence of odontogenic infection in low-income Brazilian schoolchildren and evaluate its association with the subjective variables of oral health-related quality of life and dental pain. In this cross-sectional study, 230 schoolchildren aged eight to ten years old underwent a clinical oral survey in which the DMFT/dmft and PUFA/pufa indexes were measured. Afterward, children responded individually to the Child Perceptions Questionnaire (CPQ_{8-10}) and self-reports of dental pain were collected. Data were statistically analyzed using Mann-Whitney or Kruskal-Wallis test with a post-test by Dunn's and Pearson correlation. Of the children evaluated, 42.6% had odontogenic infection and 80% reported experiencing dental pain. Children's age ($p = 0.034$) and past experience of dental pain ($p < 0.002$) were associated with odontogenic sepsis, in addition to impairment of their emotional well-being ($p = 0.008$), social welfare ($p = 0.009$) and overall impact on quality of life ($p = 0.019$). Toothache intensity ($p < 0.001$), frequency ($p < 0.001$) and duration ($p < 0.001$) were correlated to the overall impact on children's quality of life. The prevalence of odontogenic infection remains high among low-income Brazilian schoolchildren. Pediatric infection and its related pain induce not only various biological disorders but also impair children's self-perception of quality of life.

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Keywords: *child, odontogenic infection.*

Infecção odontogênica e odontalgia impactam negativamente a qualidade de vida de crianças escolares

RESUMO

O objetivo deste estudo foi avaliar a prevalência de infecção odontogênica em crianças escolares brasileiras de baixa renda e analisar a sua associação com as variáveis subjetivas da auto-percepção de qualidade de vida relacionada à saúde bucal e dor dentária. Neste estudo transversal, 230 crianças escolares com 8 a 10 anos de idade realizaram exame clínico bucal no qual os índices CPO-D/ceo-d e PUFA/pufa foram mensurados. Com isso, as crianças responderam individualmente ao Child Perceptions Questionnaire (CPQ_{8-10}) e relatos de dor dentária foram coletados. Os dados foram estatisticamente analisados utilizando-se o teste Mann-Whitney ou Kruskal-Wallis, com posterior teste da correlação de Dunn e Pearson. Dentre as crianças examinadas, 42,6% apresentavam infecção odontogênica e 80% reportou experiência de dor dentária. A idade (p=0,034) e experiência de dor dentária (p<0,002) das crianças foram associadas à infecção odontogênica, bem como o seu bem-estar emocional (p=0,008) e social (p=0,009) e impacto geral na qualidade de vida (p=0,019). Além disso, a intensidade (p<0,001), frequência (p<0,001) e duração (p<0,001) da odontalgia foram correlacionadas com o impacto geral na qualidade de vida da criança. A prevalência de infecção odontogênica permanece elevada em crianças escolares brasileiras. Infecção pediátrica e sua dor relacionada induzem não apenas diversas desordens biológicas, mas também afetam negativamente a auto-percepção de qualidade de vida das crianças.

Palavras-chave: *criança, infecção odontogênica.*

INTRODUCTION

Although there has been a worldwide reduction in the prevalence, incidence and severity of dental caries among children aged five to twelve years old over the past 30 to 40 years, the disease remains widespread. Untreated cavitated dentine carious lesions are still the most common disease that affects humans worldwide[1,2] and, especially in poor communities, children's oral health status remains a challenge yet to be resolved[1].

Untreated carious lesions and their clinical consequences are the main cause of pediatric dental pain[3,4]. Toothache is a complex phenomenon and its occurrence is relatively frequent in dental diseases, resulting in school absenteeism and impairing the child's quality of life[5,6]. The progression of untreated dental caries to odontogenic infection is the result of lack of public policies and children's lack of access to health services[3].

Dental pain measurements enable better assessment of the need for oral health care and foster the establishment of treatment priorities[7]. The implementation of public health strategies for vulnerable populations is essential, especially in low- and middle-income countries, where caries disease and its related painful episodes continue to be polarized and strongly influenced by the socioeconomic status of the population[3].

Even though it is well known that dental sepsis causes biological disorders in children, there are few studies analyzing it effect on children's self-perceived quality of life. Therefore, the present study was designed to assess the prevalence of untreated carious lesions which developed odontogenic infection and to evaluate its association with the subjective variables of quality of life and dental pain among low-income schoolchildren aged eight to ten years.

MATERIALS AND METHODS

Ethical approval

This study was approved by the Ethics Committee of the Federal University of Alagoas (Protocol No. 2.970.527 /18) and conducted in accordance with the World Medical Association Declaration of Helsinki. Consent for undertaking the research was obtained from the school director, all children, and their parents or caregivers. Only students whose parents or caregivers signed a written informed consent were included in the study.

Participants

A cross-sectional study was conducted with children enrolled in a public school in the city of Maceió, Alagoas, located in the Northeast Region of Brazil. Sample size was calculated using an 18.3% prevalence rate from a previous study[8], with 95% confidence level and 5% standard error. Hence, the minimum sample size was defined as 227 schoolchildren.

The public school was chosen randomly, and the students were randomly selected from the school attendance lists. Inclusion criterion was children aged eight to ten years old at the time of the clinical examination. Exclusion criteria were children with physical or mental disability or developmental dental anomalies, in order to avoid incorporation bias.

Oral examinations

Data were collected in a classroom from January to May 2019. The oral survey was performed with children sitting on regular school chairs, under natural light, by a single dentist calibrated for visual examination, following the criteria established by the World Health Organization for dental caries[9]. The examinations were carried out with the aid of a dental mirror (Golgran®, São Paulo, Brazil) and wooden spatula. Tooth surfaces were dried with sterile gauze.

The Kappa coefficient for intra-rater agreement was $K = 0.85$. Prevalence of dental caries was determined using the DMFT/dmft index. The decayed component (D/d) was used as an indicator of untreated carious teeth. The existence of infection as a consequence of untreated dental caries were evaluated using the PUFA/pufa index[10], $K = 0.84$. The PUFA/pufa score per person was calculated in the same cumulative way as the DMFT/dmft. The PUFA for permanent teeth and pufa for primary dentition were reported separately. A combination of the decayed component and PUFA/pufa score was applied to measure the severity of untreated dental caries. Thus, the untreated caries-pufa ratio was calculated using the following equation, providing the percentage of teeth with untreated caries that developed odontogenic infection[10].

$$\frac{(PUFA+pufa)}{(D+d)} \times 100$$

Quality of life questionnaire (CPQ_{8-10})

After the clinical survey, children were invited to respond individually to an interview performed by a trained examiner. The independent variable of this study was children's oral health-related quality of life (OHRQoL), assessed using the Child

Perceptions Questionnaire (CPQ_{8-10})[11], validated to Brazilian Portuguese by Martins et al.[12]. The interview script involved information on the child's name, age and sex, followed by the OHRQoL instrument.

The questionnaire contains 25 items arranged into four subscales: oral symptoms (n = 5), functional limitations (n = 5), emotional well-being (n = 5) and social welfare (n = 10). The responses are graded on a five-point Likert scale as: "never" (score = zero); "once or twice" (score = one); "sometimes" (score = two); "often" (score = three); and "every day or almost every day" (score = four). The scores for all questions were added to provide the overall CPQ_{8-10}, with possible values ranging from zero to 100. The scores for each domain were also calculated. Higher scores evidenced a greater negative impact on OHRQoL[8].

Dental pain measurement

The experience of dental pain was evaluated in four aspects: presence, frequency, duration and intensity, the latter by means of a verbal scale and a visual analogue scale (VAS).

The child was asked whether he/she had ever had toothache, and the answer categorized as present/absent. Frequency was recorded as the recurrence of painful events during the week, and duration was assessed as the period presenting painful episodes. To analyze intensity, the child was asked to describe the dental pain using the verbal scale responses: "mild" (score = zero), "uncomfortable" (score = one), "stressful" (score = two), "horrible" (score = three) and "intolerable" (score = four). At this point, the child was shown a numerical/VAS tool to register the severity of the disease-related symptom on a scale from zero to ten.

Data analysis

After data collection and variables categorization, a database was created for statistical analysis using SPSS (Statistical Package for Social Sciences), version 23. Normal data distribution was verified by applying the Kolmogorov-Smirnov test. To test the association between variables, Mann-Whitney or Kruskal-Wallis test was used with post-test by Dunn's nonparametric comparison. The correlation between the impact on quality of life and aspects of dental pain was determined by Pearson's correlation test. The error margin used in the decisions of the statistical tests was 5%, with intervals at a 95% confidence level.

RESULTS

Two hundred and thirty children were surveyed. Mean age was 9±0.85 and 53.5% were male. Average dental decay was 2.59±2.59, and 74.3% (n = 171) of the children had cavitated carious lesions. The filling (F/f) component of DMFT/dmft was 0.20±0.806, showing that 91.7% of the evaluated children had not had any teeth restored.

Of the surveyed children, 42.6% (n = 98) had odontogenic infection and the large majority (80%; n = 184) reported having had dental pain sometimes. Table 1 shows the association between

Table 1: Variables associated with odontogenic infection.

Variables	Odontogenic infection			
	N	Mean – SD	P value	P value
Sex				
Male	123	22.23 ± 32.47	0.558*	
Female	107	25.66 ± 35.63		
Age				
8 (a)	78	15.09 ± 26.29	0.034**	axb 0.044*
9 (b)	74	27.17 ± 36.26		axc < 0.013*
10 (c)	78	29.38 ± 33.94		bxc 0.694*
Past experience of dental pain				
Absent (a)	46	12.00 ± 27.20	< 0.002**	
Present (b)	184	26.84 ± 34.89		

SD, standard deviation. Significant association at 5.0%. *Mann-Whitney test. **Kruskal-Wallis test. The letters a, b and c represent different groups – post-test by Dunn's nonparametric comparison.

Table 2: Odontogenic infection associated with impact on overall and domain-specific CPQ scores.

	Odontogenic infection		
	Absence (n)	Presence (n)	
	134	96	
Variables	**Mean – SD**	**Mean – SD**	**P value***
Subscale 1 (Oral symptoms)	5.43 ± 3.61	6.25 ± 3.70	0.079
Subscale 2 (Functional limitations)	3.73 ± 3.62	4.30 ± 3.87	0.303
Subscale 3 (Emotional well-being)	3.72 ± 3.90	5.31 ± 4.54	0.008
Subscale 4 (Social welfare)	4.93 ± 5.97	7.22 ± 7.52	0.009
Overall impact (CPQ8-10)	17.81 ± 14.64	23.08 ± 17.03	0.019

CPQ, Child Perceptions Questionnaire; SD, standard deviation. Significant association at 5.0%. *Mann-Whitney test.

odontogenic infection and sex ($p = 0.558$), age ($p = 0.034$) and past experience of toothache ($p < 0.002$). Among the self-reported quality of life variables analyzed, the presence of odontogenic infection was significantly associated with the child's emotional well-being ($p = 0.008$), social welfare ($p = 0.009$) and overall impact on quality of life ($p = 0.019$). (Table 2).

Using the verbal scale of pain responses, children reported the intensity of painful episodes as "horrible" (24.3%; n = 56), "mild" (18.3%; n = 42), "uncomfortable" (18.7%; n = 43), "stressful" (14.8%; n = 34) and "intolerable" (5.7%; n = 13). The mean score on the numerical pain scale was 5.17±3.63. Table 3 shows the correlation between the overall impact on children's quality of life and toothache intensity assessed by the verbal pain scale ($r = 0.184$; $p = 0.005$) and numerical scale of pain ($r = 0.504$; $p < 0.001$), toothache duration ($p < 0.001$) and frequency ($p < 0.001$).

Table 3: Correlation between the overall impact on children's quality of life and intensity, duration and frequency of dental pain

Variables	**Overall impact (CPQ_{8-10})**	
	Pearson correlation	P value*
Intensity		
Numerical Pain Scale	0.504	< 0.001
Verbal Pain Scale	0.184	0.005
Duration	0.510	< 0.001
Frequency	0.388	< 0.001

CPQ, Child Perceptions Questionnaire. Significant association at 5.0%. *Pearson correlation test.

DISCUSSION

Most orofacial infections are believed to have odontogenic origin, in which untreated dental caries plays a major role. The occurrence of dental decay has a contextual nature, in addition to its infectious progression[13,14]. The present school-based cross-sectional survey evaluated the prevalence of odontogenic infection and its relation with children's OHRQoL and dental pain. It was observed that children's age and experience of dental pain were associated with the occurrence of dental sepsis, impacting negatively on their quality of life.

Of the evaluated children, 42.6% had odontogenic infection. The prevalence of infectious process in schoolchildren reported in the literature ranges from 7.1% to 85%[15-21]. These widely differing results are believed to result from differences in the diagnostic methods used by the examiners, study design and population surveyed. It is still important to emphasize that the prevalence of dental infection may be underestimated since the manifestation of dental abscesses might be episodic. Our study found no significant association between odontogenic infection and sex. In contrast, previous studies demonstrated an association between these two variables, arguing that odontogenic infection and tooth decay are significantly higher in boys[17,21].

The child's age was found to be associated with the occurrence of odontogenic infection, with prevalence and severity of dental infectious process increasing with age. This might be explained by the early complete formation of the primary dental arches and consequently, their longer exposure time to cariogenic diet in the dynamic oral environment. The long time elapsed between the tooth eruption and examination may enable the development of extensive carious lesions that may lead to odontogenic sepsis[21]. Family beliefs and attitudes are determinant factors in children's oral health status, possibly placing them at risk for disease. Parent's past adverse experiences during dental treatment and the fact that they do not value deciduous teeth potentially lead to the lack of care for caries prevention in their children's primary dentition, enabling pathology to advance as teeth mature[22,23]. Nevertheless, from a biomedical perspective, nobody should have odontogenic sepsis[20].

The dental infectious process revealed an association with the past experience of dental pain among the surveyed children, of whom 80% reported having felt dental pain at some time in their lives. The prevalence of toothache reported in previous papers varies widely, ranging from 16.5% to 60%[3-6,15,18,24]. However, it should be noted that there were differences among these studies in the manner of assessing and measuring painful episodes, such as sample size, age surveyed and time elapsed since toothache.

In our study, the child's emotional well-being and social welfare were associated with the occurrence of odontogenic infection. Schoolchildren's emotions and social coexistence are still maturing and clearly affect their self-concept and self-esteem. These psychosocial variables have been shown to interfere in oral health behavior, determining clinical oral conditions[25,26]. Similarly, the incidence of dental decay and toothache impair children's individual and social confidence, disturbing their educational performance and social interactions, thereby leading to a chain of circumstances[6,15,27,28]. Poor access to oral health services in socioeconomically disadvantaged groups can lead to the progression of the untreated disease, trapping children in a vicious circle with evident disturbances in their emotional and biological well-being, such as deficient development and growth, odontogenic sepsis and pain[3,5,13,16].

The domains of functional limitations and oral symptoms were not associated with odontogenic infection. This suggests that the infectious process analyzed may not have occurred at a time close to the time of examination (memory bias), and may also be related to the following: (1) the sensation of pain is inherently peculiar and private for children, manifesting as an essentially subjective multifactorial event; (2) as the pediatric infection can rapidly become chronic, it may produce unperceived painful sensation and this could also be related to the child's cognition level; and (3) the special anatomical and physiological characteristics of children's bones allow the suppurative complications of pulp infection diffuse more rapidly than in adults[7,14,29]. However, the current study found that the overall self-reported impairment of children's quality of life was associated with odontogenic infection.

It is interesting to note that painful episodes in pediatric patients include the interaction of psychological, physiological and developmental circumstances. In this context, we verified a strong correlation between the numerical/visual analogue scale (VAS) format and self-reported impact on children's quality of life, whilst the verbal pain scale demonstrated low association. The greater effectiveness of the numerical/VAS tool as a measurement of toothache intensity may be due to the age of the children in the group surveyed. The psychometric scale may have achieved more accurate responses than the verbal model, because the latter requires certain cognitive and linguistic development[7]. Toothache frequency and duration were also associated with the overall impact on children's quality of life, with relation to disturbances in psychological well-being, mood, self-perception and school environment caused by intense dental pain[30], possibly inducing school absenteeism[6].

Studies on the effect of odontogenic infection and its related pain on psychosocial elements of children are scarce, even though they would provide significant understanding of the population evaluated and its interaction with social aspects. Although the present cross-sectional survey suggests that dental sepsis and painful symptoms have a direct influence on determinants of quality of life in children, further studies are still needed to identify cause-effect determinants. This survey also shows that previous approaches to public health services have not been successful or did not even reach the examined children, thus reinforcing the assumption that disparities in socioeconomic and environmental levels still affect the incidence of odontogenic infection and toothache among schoolchildren. Restructuring public health policies and designing community-based dental services should be desirable targets in order to reduce the persistent inequalities in children's access to health and pain relief.

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REFERENCES

1. Frencken JE, Sharma P, Stenhouse L, Green D, Laverty D, Dietrich T. Global epidemiology of dental caries and severe periodontitis – a comprehensive review. J Clin Periodontol 2017; 44S94-S105.
2. Ministério da Saúde (BR). Secretaria de Vigilância em Saúde. SB Brasil 2010: Pesquisa Nacional de Saúde Bucal: resultados principais. Brasília, DF: SVS; 2012.
3. Schuch HS, Correa MB, Torriani DD, Demarco FF, Goettems ML. Perceived dental pain: determinants and impact on Brazilian schoolchildren. J Oral Facial Pain Headache 2015; 29:168-176.
4. Boeira GF, Correa MB, Peres KG, Peres MA, Santos IS, Matijasevich A, Barros AJ, Demarco FF. Caries is the main cause for dental pain in childhood: Findings from a birth cohort. Caries Res 2012; 46:488-495.
5. de Lacerda JT, de Bem Pereira M, Traebert J. Dental pain in Brazilian schoolchildren: a cross-sectional study. Int J Paediatr Dent 2013; 23:131-137.
6. Guskuma RC, Lages VA, Hafner MB, Rando-Meirelles MPM, Cypriano S, Sousa MLR, Batista MJ. Factors associated with the prevalence and intensity of dental pain in children in the municipalities of the Campinas region, São Paulo. Rev Paul Pediatr 2017; 35:322-330.
7. Jain A, Yeluri R, Munshi AK. Measurement and Assessment of Pain In Children - A Review. J Clin Pediatr Dent 2012; 37:125-136.
8. Corrêa-Faria P, Daher A, Freire MDCM, de Abreu MHNG, Bönecker M, Costa LR. Impact of untreated dental caries severity on the quality of life of preschool children and their families: a cross-sectional study. Qual Life Res 2018; 27: 3191-3198.
9. World Health Organization Basic Epidemiological survey of oral health. Manual. 4th Ed. Geneva: World Health Organization; 1997.
10. Monse B, Heinrich-Weltzien R, Benzian H, Holmgren C, van Palenstein Helderman W. PUFA—An index of clinical consequences of untreated dental caries. Community Dent Oral Epidemiol 2010; 38:77-82.
11. Jokovic A, Locker D, Tompson B, Guyatt G. Questionnaire for measuring oral health-related quality of life in eight- to ten-year-old children. Pediatr Dent 2004; 26:512-518.
12. Martins MT, Ferreira FM, Oliveira AC, Paiva SM, Vale MP, Allison PJ, Pordeus IA. Preliminary validation of the Brazilian version of the child perceptions questionnaire 8-10. Eur J Paediatr Dent 2009; 10:135-140.
13. Ardenghi TM, Piovesan C, Antunes JL. Inequalities in untreated dental caries prevalence in preschool children in Brazil. Rev Saude Publ 2013; 47:129-137.
14. Al-Malik M, Al-Sarheed M. Pattern of management of oro-facial infection in children: A retrospective. Saudi J Biol Sci 2017; 24:1375-1379.
15. Santos PS, Martins-Júnior PA, Paiva SM, Klein D, Torres FM, Giacomin A, Gonçalves BM, Konrath AC, et al. Prevalence of self-reported dental pain and associated factors among eight- to ten-year-old Brazilian schoolchildren. PLoS One 2019 Apr 8;14(4):e0214990. doi: 10.1371/journal.pone.0214990. eCollection 2019.
16. Benzian H, Monse B, Heinrich-Weltzien R, Hobdell M, Mulder J, van Palenstein Helderman W. Untreated severe dental decay: a neglected determinant of low Body Mass Index in 12-year-old Filipino children. BMC Public Health 2011 13;11:558. doi: 10.1186/1471-2458-11-558.
17. Dziwak M, Heinrich-Weltzien R, Limberger K, Ifland S, Gottstein I, Lehmann T, Schuler IM. Dental health and odontogenic infections among 6- to 16-year-old German students with special health care needs (SHCN). Clin Oral Invest 2017; 21:1997-2006.
18. Rogers HJ, Tariq U, Olsson L, Riaz SA, Miah MR. Caries prevalence, clinical consequences and self-reported pain experienced by children living in the West Bank. Eur Arch Paediatr Dent 2019; 20:333-338.
19. Kamran R, Farooq W, Faisal MR, Jahangir F. Clinical consequences of untreated dental caries assessed using PUFA index and its covariates in children residing in orphanages of Pakistan. BMC Oral Health 2017;17:108. doi: 10.1186/s12903-017-0399-9.
20. Monse B, Benzian H, Araojo J, Holmgren C, van Palenstein Helderman W, Naliponguit EC, Heinrich-Weltzien R. A Silent Public Health Crisis: Untreated Caries and Dental Infections Among 6- and 12-Year-Old Children in the Philippine National Oral Health Survey 2006. Asia Pac J Public Health 2015; 27:2316-2325.
21. Grund K, Goddon I, Schuler IM, Lehmann T, Heinrich-Weltzien R). Clinical consequences of untreated dental caries in German 5- and 8-year-olds. BMC Oral 2015 4;15(1):140. doi: 10.1186/s12903-015-0121-8.
22. Bagińska J, Rodakowska E, Wilczyńska-Borawska M, Jamiołkowski J. Index of clinical consequences of untreated dental caries (pufa) in primary dentition of children from north-east Poland. Adv Med Sci 2013; 58:442-447.
23. Riedy CA, Weinstein P, Milgrom P, Bruss M. An ethnographic study for understanding children's oral health in a multicultural community. Int Dent J 2001; 51:305-312.
24. Schuch HS, Costa FS, Torriani DD, Demarco FF, Goettems ML. Oral health-related quality of life of schoolchildren:

impact of clinical and psychosocial variables. Int J Paediatr Dent 2015; 25:358-365.
25. Macgregor ID, Regis D, Balding J. Self-concept and dental health behaviours in adolescents. J Clin Periodontol 1997; 24:335-339.
26. Chin LSH, Chan JCY. Self-esteem, oral health behaviours, and clinical oral health status in Chinese adults: An exploratory study. Health Educ J 2013; 72:684-694.
27. Gomes MC, Perazzo MF, Neves ET, Martins CC, Paiva SM, Granville-Garcia AF. Oral Problems and Self-Confidence in Preschool Children. Braz Dent J 2017; 28:523-530.
28. Maroneze MC, Ardenghi DM, Brondani M, Unfer B, Ardenghi TM. Dental treatment improves the-related quality of life of adolescents: A mixed-methods approach. Int J Paediatr Dent 2019; 29:765-774.
29. Caviglia I, Techera A, García G Antimicrobial therapies for odontogenic infections in children and adolescents. Literature review and clinical recommendations. J Oral Res 2014; 3:50-56.
30. Haraldstad K, Christophersen KA, Helseth S. Health-related quality of life and pain in children and adolescents: a school survey. BMC Pediatr 2017;17:174. doi: 10.1186/s12887-017-0927-4.

Decontamination of Gutta-percha Cones employed in Endodontics

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ABSTRACT

The gutta-percha cones used in endodontic treatment are produced in aseptic conditions and their composition includes zinc oxide, which is responsible for antibacterial activity. However, there is the possibility of microbial contamination by manipulation, aerosol or during storage. Although several chemical agents have been tested for their decontamination, there is no consensus on the best disinfection protocol to be used. The aim of this study was to evaluate the decontamination of gutta-percha cones contaminated with the bacteria Enterococcus faecalis, by using chlorhexidine digluconate (CHX) and sodium hypochlorite (NaClO) at different concentrations for short exposure times. For this purpose, gutta-percha cones (size 40) were selected at random from a sealed box and immersed for 1 min in a microbial suspension. Then they were immersed in specific Petri dishes for different groups containing: CHX 2%, NaClO 1% or NaClO 2.5% for 30 s or 1 min, and subsequently placed in tubes containing BHI broth. After incubating the tubes for 48 h, it was observed that 1% and 2.5% NaClO and 2% CHX were effective for decontaminating the cones at those exposure time intervals. Microbial growth was detected in one of the replicates of the group with CHX applied for 30 s. To prevent the possibility of failures at this stage, the exposure time of gutta-percha cones to the decontaminating agent should not be reduced.

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Keywords: *gutta-percha, decontamination, enterococcus faecalis.*

Descontaminação de cones de guta-percha empregados em endodontia

RESUMO

Os cones de guta-percha utilizados no tratamento endodôntico são produzidos em condições assépticas e possuem óxido de zinco em sua composição, responsável pela atividade antibacteriana. No entanto, existe a possibilidade de contaminação microbiana por manipulação, aerossol ou seu armazenamento. Embora vários agentes químicos já tenham sido testados para sua descontaminação, não há consenso sobre o melhor protocolo de desinfecção a ser usado. Nosso objetivo foi avaliar a descontaminação de cones de guta-percha contaminados com a bactéria Enterococcus faecalis, utilizando digluconato de clorexidina (CHX) e hipoclorito de sódio (NaClO) em diferentes concentrações e tempos de exposição curtos. Para esse fim, 40 cones de guta-percha foram selecionados aleatoriamente, de uma caixa selada e imersos por 1 min em uma suspensão microbiana. Em seguida, foram imersos em placas de Petri específicas para diferentes grupos contendo: CHX 2%, NaClO 1% ou 2,5%, nos tempos de exposição de 30s e 1min e subseqüentemente imersos em tubos contendo caldo BHI. Após incubação dos tubos por 48 h, observou-se que NaClO 1% e 2,5% e CHX 2% foram eficazes para a descontaminação dos cones nesses intervalos de tempo de exposição. Em uma das réplicas do grupo com CHX aplicado por 30s foi detectado crescimento microbiano. O tempo de exposição dos cones de guta-percha ao agente de descontaminação não deve ser reduzido para evitar a possibilidade de falhas nesse estágio.

Palavras-chave: *guta-percha, descontaminação, enterococcus faecalis.*

INTRODUCTION

The success of endodontic therapy is strictly related to the careful aseptic performance of all its phases, including opening, cleaning, disinfecting, modeling and subsequent sealing of the entire root canal system[1-3]. However, during this process there may be failures in instrumentation, infiltration of bacteria present in saliva and/or use of contaminated instruments and/or materials[4].

Microorganisms may still persist after biomechanical preparation due to variations in root canal internal anatomy or as a result of the defense mechanisms of the microorganisms themselves, so the need for good filling and sealing of the root canal system is imperative[5,6].

For an adequate sealing procedure, the obduration material should ensure three-dimensional filling of the root canal system, thereby preventing bacterial percolation and the appearance or maintenance of periapical lesions[7].

Gutta-percha cones have several properties that make them an excellent material for canal obturation, including biocompatibility, size stability, radiopacity, thermoplasticity, easy removal from the root canal[8,9] and antibacterial activity[10].

These cones are based on a polymer obtained from the coagulation of latex produced by trees in the *Sapotaceae* family, mainly *Palaquiumgutta*. Commercial gutta-percha cones consist of an inorganic part containing barium sulphate (BaSO4) and zinc oxide (ZnO) and an organic part composed of waxes/resins and gutta-percha. Their percentage content varies according to the manufacturer, and will directly influence rigidity, tensile strength, brittleness and radiopacity[10].

Although they are produced under aseptic conditions and contain about 69.8 - 81.9% of zinc oxide, which is responsible for the antibacterial activity[10], there is still a chance of contamination by manipulation, aerosol or storage[8,11].

Gutta-percha cones cannot withstand sterilization processes, so they must undergo a decontamination step to ensure the maintenance of the aseptic chain. Although several chemical agents have been tested, there is no consensus in the literature on which is the best protocol to disinfect these materials[2,3,12,13].

Under these circumstances, the use of NaOCl for rapid decontamination of gutta-percha cones was proposed by Senia et al.[14], who demonstrated that when contaminated with the bacteria *Staphylococcusepidermidis*, *Corynebacteriumxerosis*, *Escherichia coli* and *Enterococcus faecalis,* they were decontaminated after immersion in Clorox® (5.25% sodium hypochlorite) for 30, 45 and 60 seconds.

Another chemical agent that has been widely applied in endodontics is chlorhexidine digluconate, which was first used for root canal irrigation in 1964, and is currently therapeutically categorized as having long-acting antimicrobial action, especially against *E. faecalis*, being a topical antiseptic anddisinfecting agent[15,16].

The aim of this paper is to verify the efficacy of decontamination protocols of gutta-percha cones employed in root canal obturation, using NaOCl and chlorhexidine digluconate as disinfecting agents, with variations in their concentrations and short times of action.

MATERIALS AND METHODS

This was an exploratory, observational laboratory study with a qualitative approach. *E. faecalis* (ATCC 29212) was inoculated on Brain Heart Infusion (BHI) agarin Petri dishes and incubated for 24 hours at 37°C under aerobic conditions. After that, bacterial colonies were collected with the aid of a platinum loop and inoculated in sterile saline solution (0.9% w/v NaCl) until a turbidity of 0.5 on the McFarland scale ($1\text{-}2\times10^{8}$ CFU/ml) was obtained. This suspension was used to contaminate gutta-percha cones.

Thirty-two size 40 gutta-percha cones (Dentsply)™ were used. The sealed package of cones was opened inside a laminar flow hood previously sterilized by UV light and the cones were removed in random order with the aid of sterile tweezers. Each cone was immersed completely in 6 mL of microbial suspensionin Petri dishes for 1 minute to ensure contamination. For the subsequent disinfection process, the cones were removed from the contaminated solution with the aid of sterile tweezersand transferred to Petri dishes (one for each group) containing the disinfecting solutions to be evaluated. Six groups were formed, each containing 4 cones (experiment performed in quadruplicate) individually immersed in solutions of 2% chlorhexidine digluconate (CHX), 1% sodium hypochlorite (NaOCl or NaClO) or 2.5% sodium hypochlorite, at exposure times of 30 s and 1 min each. After this time, the cones were removed from the disinfecting solution and immersed for 10 seconds in sterile distilled water to remove excess disinfectant (Table 1). Finally, the cones were placed inside threaded tubes containing 3 mL BHI broth supplemented with 1% yeast extract and incubated for 48h in an oven at 37ºC under aerobic conditions. After this time, the results were recorded based on turbidity (bacterial growth) or absence of turbidity in the culture medium. A positive control group, to attest to *E. faecalis* viability, was prepared by immersing contaminated cones only in saline solution. A negative control group, to certify that the cones in the sealed box

were sterile, was prepared by removing cones from the carton using sterile tweezers and placing them immediately in the culture medium.
Nonparametric Kruskall-Wallis and Mann-Whitney tests were used for statistical evaluation of results.

RESULTS

After a 48h incubation period at 37°C under aerobic conditions, bacterial growth was observed in all tubes of the positive control group, indicating that the bacteria were viable and in sufficient quantity for growth in the tubes, as well as the effectiveness of the culture medium in providing nutrients for growth and multiplication. In the negative control group, no turbidity occurred, evidencing that the cones coming from the sealed box were without microbial contamination.
There was no bacterial growth in three of the four incubated samples in group G1 (CHX 2% -30s), but turbidity of the medium was observed in one of the replicates. In group G2 (CHX 2% -1min, with the same chemical solution but longer exposure time than G1), effective decontamination was observed in all samples (Table 2). In groups G3, G4, G5 and G6, which corresponded to the application of sodium hypochlorite, no bacterial growth was observed in any of the four samples in each group (Table 3).

In order to identify whether there are statistically significant differences regarding the presence of microbiological contamination in the groups, nonparametric tests were performed, considering the non-normal distribution of the data, ascertained by the significant value of the Kolmogorov-Smirnov test ($p < 0.001$). Thus, at first, the Kruskal Wallis test was performed to compare the eight

Table 1: Experimental groups and the treatments.

Group	Treatment
G1	Immersion in chlorhexidine digluconate 2% - 30 seconds
G2	Immersion in chlorhexidine digluconate 2% - 1 minute
G3	Immersion in sodium hypochlorite 1% - 30 seconds
G4	Immersion in sodium hypochlorite 1% - 1 minute
G5	Immersion in sodium hypochlorite 2.5% - 30 seconds
G6	Immersion in sodium hypochlorite 2.5% - 1 minute
Positive control	Immersion in saline solution
Negative control	Direct immersion in the culture medium

Table 2: Decontamination capacity of immersion of gutta-percha cones in 2% chlorhexidine digluconate for 30 seconds and 1 minute.

Sample	Group 1 CHX 2% (30s)	Group 2 CHX 2% (1min)	Positive Control	Negative Control
1	0	0	1	0
2	0	0	1	0
3	0	0	1	0
4	1	0	1	0

0- absence of turbidity in the culture medium; 1- presence of turbidity in the culture medium.

Table 3: Decontamination capacity of immersion of gutta-percha cones in 1% sodium hypochlorite and 2.5% sodium hypochlorite for 30 seconds and 1 minute.

Sample	Group 3 NaClO 1% (30 s)	Group 4 NaClO 1% (1 min)	Group 5 NaClO 2,5% (30 s)	Group 6 NaClO 2,5% (1 min)	Positive Control	Negative Control
1	0	0	0	0	1	0
2	0	0	0	0	1	0
3	0	0	0	0	1	0
4	0	0	0	0	1	0

0-absence of turbidity in the culture medium; 1-presence of turbidity in the culture medium.

groups together, finding a statistically significant difference among them (p = 0.01), with the highest average for gutta-percha cones in the positive control group, followed by G1 and the other groups. Mann-Whitney tests were performed to compare the groups in pairs considering the different substances and times. No statistically significant difference was found upon comparing groups G1 and G2 (U = 6.01; p = 0.69); G3 and G4 G2 (U = 8.00; p = 1.00) and G5 and G6 (U = 8.00; p = 1.00). Statistical difference was found upon comparing the control groups (U = 10.00; p = 0.03), with the positive control group having the highest average.

DISCUSSION

Amaralet al.[17] report that NaOCl has been widely applied in endodontics in various concentrations in the chemical preparation of root canals and as an effective method to disinfect gutta-percha cones. The high active chlorite content is a predominant factor to maintain product quality, in which decontamination is inversely proportional to concentration. Chlorhexidine has also been used as an antimicrobial solution in endodontic therapy and is indicated for patients who are allergic to NaOCl or for refractory treatments, in which the use of intracanal medication has not been successful.

According to Gomes et al.[18], several antiseptic chemicals have been described in the literature but chlorhexidine and NaOCl have been highlighted because they are considered potent antimicrobial agents.

Rocha et al.[19] evaluated the decontamination of cones previously contaminated with strains of *E. faecalis* (alcohol 70%, chlorhexidine 0.12% and 2%, NaClO 1%, 2% and 2.5% salinefor 1, 2, 3, 4 and 5 minutes), and found that all agents were effective in decontamination for one minute or longer. In the positive control group, turbidity was observed, indicating that the bacteria remained viable until the end of the experiment. The negative control tube did not present growth of microorganisms, ensuring the sterility of the cones in that experiment, corroborating our results. A divergent result was observed by Sayãoet al.[5], who observed that 6.67% of the cones from sealed or manipulated boxes were contaminated.

Amaralet al.[17] evaluated the effectiveness of gutta-percha and Resilon cone decontamination with the following groups: NaOCl 5.25%, NaOCl 2.5%, chlorhexidine 2% for 1 minute; phenolic glycerin for 24 hours. The agents were effective as decontaminants, although 2% chlorhexidine was ineffective in 20% of both gutta-percha and Resilon cones, differing from our study on the 1-minute test time using chlorhexidine 2%, which showed efficacy in 100% of the samples. However, with reservations for the 30-second time, in which there was contamination in one replicate, the action of NaClO corroborates our study in the one-minute test.

Cardoso et al.[20] tested 1% NaOCl and showed that it was efficient for the decontamination of cones contaminated with *E. faecalis* in a contact time of 5 minutes. In the current study, we demonstrated that 1% NaOCl is capable of disinfecting in a time frame of 30 seconds. Schmidt et al.[3] tested the effect of NaOCl at 2.5% and chlorhexidine gel at 2%, observing that they could both promote complete elimination of *E. faecalis*, *Candida albicans*, and *Staphylococcus aureus* after 30 seconds of exposure time.

Redmerski et al.[2] demonstrated that 2% chlorhexidine digluconatewas effective in the decontamination in 5 min of cones contaminated with *S. aureus*, *E. faecalis*, *E. coli*, *C. albicans*, and spores of *B. subtilis*. In our research, the same solution was effective against *E. faecalis* as from 1 minute exposure time.

There is interest in reducing decontamination time because the surface of gutta-percha cones may change after decontamination with sodium hypochlorite. It is known that 5.25% NaOCl causes changes in the topography of gutta-percha cones in 20-minute exposure times and changes in elasticity in only one minute, while 2% chlorhexidine does not cause structural change in gutta-percha cones [21].

CONCLUSION

The results indicated that 1% and 2.5% NaOCl and 2% chlorhexidine digluconate were effective for the decontamination of gutta-percha cones contaminated with *E. faecalis,* in short contact time intervals of 30 s and 1 min. It should be recognized, however, that microbial growth was observed in one of the replicates of the CHX experiment applied for 30s, which provides a warning that the disinfection protocols must still be followed, with special care to not unduly reduce the exposure time to the decontaminating agent.

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REFERENCES

1. Ferraz CC, Gomes BP, Zaia AA, Teixeira FB, Souza-Filho FJ. Comparative study of the antimicrobial efficacy of chlorhexidine gel, chlorhexidine solution and NaOClas endodontic irrigants. Braz Dent J 2007; 18:294-298.
2. Redmerski R, Bulla JR, MorenoT, Garcia LB, Cardoso CL. Disinfection of gutta-percha cones with chlorhexidine. Braz J Microbiol 2007; 38:649-655.
3. Schmidt MHM, Sallenave RF, Demarchi A, Farina AP, Cecchin D. Effectiveness of different auxiliary chemical substances in the rapid disinfection of gutta-percha points - an in vitro study. RFO 2015; 20:64-68.
   URL:http://revodonto.bvsalud.org/pdf/rfo/v20n1/a12v20n1.pdf
4. Pereira ER, Nabeshima CK, Machado MEL. Analysis of contamination of endodontic absorbent paper points. Ver Odonto Ciênc 2011; 26:56-60.
   URL: https://doi.org/10.1590/S1980-65232011000100013
5. Sayão DM, Barros RR, Camões ICG, Freitas LF, Gomes CC, Pinto SS. Análise Microbiológica de Cones de Guta-Percha Disponíveis no Mercado Brasileiro. Pesqui. Bras Odontopediatria Clín Integr 2010; 10:265-269.
   DOI: 10.4034/1519.0501.2010.0102.0020
6. Decúrcio DA, Grosara MB, Silva JA, Amorim LFG, Estrela CRA. Avaliação antimicrobiana de cones de guta-percha associados ao hidróxido de cálcio e ou clorexidina. ROBRAC 2010; 18:30-33.
   URL: https://www.robrac.org.br/seer/index.php/ROBRAC/article/view/411
7. Ricucci D, Loghin S, Siqueira JJF. Exuberant Biofilm infection in a lateral canal as the cause of short-term endodontic treatment failure: report of a case. J Endod 2013; 39: 712-718.
8. Salvia ACRD, Teodoro RG, Balducci I, Koga-Ito CY, Oliveira SHG. Effectiveness of 2% peracetic acid for the disinfection of gutta-percha cones. Braz Oral Res 2011; 25: 23-27.
9. Silva EM, Júnior ECS, Marques AAF. Microbiological assessment of contamination of gutta-percha cones used by pos- graduation students. J HealthSci Inst 2010; 28: 235-236.
   URL: https://www.researchgate.net/publication/255730608_
10. Maniglia-Ferreira C, Silva JB Jr, Paula RC, Feitosa JP, Cortez DG, Zaia AA, Souza-Filho FJ.Brazilian gutta-percha points. Part I: chemical composition and X-ray diffraction analysis. Braz Oral Res 2005; 19:193-197.
11. Prado M, Gusman H, Gomes BPFA, Simão RA. The importance of final rinse after disinfection of gutta-percha and Resilon cones. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2011; 111:21-24.
12. Siqueira JJF, Pereira CHS, Cerqueira MDO, Lopes HP, Uzeda M. Effectiveness of four chemical solutions in eliminating Bacillus subtilis spores on gutta- percha cones. Endod Dent Traumatol. 1998;19: 124-129.
13. Souza ER, Souza EA, Neto MDS, Pietro RCLR. In vitro evaluation of different chemical agents for the de contamination of gutta-percha cones. Pesqui Odontol Bras 2003; 17:75-77.
    URL: https://www.scielo.br/scielo.php?script=sci_arttext&pid=S1517-74912003000100014
14. Senia ES, Marraro RV, Mitchell JL, Lewis AG, Thomas L. Rapid sterilization of gutta- percha cones with 5.25% sodium hypochlorite. J Endod 1975; 1:136-140.
15. Zamany ASK, Spangberg LSW. The effect of chlorhexidine as an endodontic disinfectant. Oral Surg Oral Med Oral Pathol Oral Radiol Endod2003;5:578-581.
16. Basrani B, Santos JM, Tjardehane L, Grad H, Gordusys Oet al. Substantive antimicrobial activity in chlorhexidine - treated human root dentin. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2002; 94:240-245.
17. Amaral G, Carraz R, Freitas LF, Fidel SR, Castro AJR. Efetividade de três soluções na descontaminação de cones de guta-percha e de resilon. Rev Bras Odontol 2013; 70:54-58.
    URL: http://revodonto.bvsalud.org/scielo.php?script=sci_arttext&pid=S0034-72722013000100013
18. Gomes CC, Camoes ICG, Freitas LF, Pinto SS, Saraiva SM, Sambati S. Avaliação do hipoclorito de sódio e da clorexidina na desinfecção de cones de guta-percha. Rev Odontol Univ São Paulo 2010; 22:94-103.
    URL: http://publicacoes.unicid.edu.br/index.php/revistadaodontologia/article/view/403
19. Rocha EALDSS, Limeira FIRL, Carvalho AVOR, Santos KSA, Medeiros ACD. Avaliação da eficácia de diversas substâncias químicas na descontaminação de cones de guta-percha. Odontol Clín-Cient 2013; 12:35-38.
    URL: http://revodonto.bvsalud.org/scielo.php?script=sci_arttext&pid=S1677-38882013000100008
20. Cardoso CL, Redmerski R, Bittencourt NRL, Kotaka CR. Effectiveness of different chemical agents in rapid decontamination of gutta-percha cones. Braz J Microbiol 2010; 31:67-71.
    URL: https://doi.org/10.1590/S1517-83822000000100016
21. Valois CRA, Silva LP, Azevedo RB. Structural effects of NaOCl solutions on gutta-percha cones: atomic force microscopy study. J Endod 2005;749-751.

Effect of Obesity and/or Ligature-induced Periodontitis on Aortic Wall Thickness in Wistar Rats

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ABSTRACT

The purpose of this study was to evaluate aortic wall thickness after periodontal disease and/or obesity induction in a Wistar rat model. Sixty male Wistar rats were randomly divided into four groups: control (CT), periodontal disease (PD), obesity (OB), and obesity plus periodontal disease (OB+PD). Groups OB and OB+PD received cafeteria diet for 17 weeks. After they had acquired obesity (week 12), periodontal disease was induced by placing a silk ligature on the maxillary right second molar of groups PD and OB+PD. During the experimental period, body weight and Lee index were assessed. Mean alveolar bone loss (ABL) was evaluated, and aortas were prepared for histometric analysis of the aortic wall by ImageJ software. Body weight and Lee index increased in rats exposed to cafeteria diet. Mean ABL was higher in Groups PD and OB+PD than in control and OB ($p<0.05$). ABL was 18% higher in Group OB+PD than in Group PD, with statistically significant difference ($p<0.001$). Aortas were thicker in Groups OB and OB+PD than in control and PD groups, respectively (2.31mm ± 0.28 and 2.33 ± 0.29 vs. 2.18 ± 0.26 and 2.14 ± 0.27). Group OB differed significantly from the control group ($p=0.036$), and OB+PD and OB differed significantly from PD ($p=0.004$ and $p=0.001$, respectively). Obesity alters aortic wall thickness in Wistar rats. However, the presence of periodontal disease did not affect the aortic wall thickness under the conditions of the present study.

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Keywords: *obesity, alveolar bone loss, atherosclerosis, rats.*

Efeito da obesidade e/ou periodontite induzida por ligadura na espessura da parede da aorta em ratos wistar

RESUMO

O objetivo deste estudo foi avaliar a espessura da parede da aorta após modelos de indução de doença periodontal e/ou obesidade em ratos Wistar. Sessenta ratos Wistar machos foram aleatoriamente divididos em quatro grupos: controle (CT), doença periodontal (DP), obesidade (OB), obesidade mais doença periodontal (OB+DP). Os grupos OB e OB+DP receberam dieta de cafeteria por 17 semanas. Após de adquirirem obesidade, (semana 12), doença periodontal foi induzido pela colocação de ligaduras de seda no segundo molar superior direito dos grupos DP e OB+DP. Durante o período experimental, o peso corporal e índice de Lee foram obtidos. Média de perda óssea alveolar (POA) foi avaliada e as aortas preparadas para análise histométrica da parede aórtica (em mm) pelo software ImageJ. Ratos expostos a dieta de cafeteria demonstraram um aumento do peso corporal e do índice de Lee. Uma POA media maior foi observada nos grupos DP e OB+DP comparado aos grupos controle e OB ($p<0.05$). O grupo OB+DP, quando comparado ao grupo DP, apresentou POA 18% maior e essa diferença foi estatisticamente significativa ($p<0.001$). Os grupos OB e OB+DP exibiram uma espessura de aorta maior comparado aos grupos DP e controle, respectivamente (2.31 ± 0.28 e 2.33 ± 0.29 vs. 2.18 ± 0.26 e 2.14 ± 0.27). Diferenças significativas foram observadas nas comparações dos grupos OB e controle ($p=0,036$), e OB+DP e OB comparado ao grupo DP ($p=0.004$ e $p=0.001$, respectivamente). A obesidade parece afetar a espessura da parede da aorta em ratos Wistar. Entretanto, a presença de doença periodontal não afetou a espessura da parede da aorta sob as condições do presente estudo.

Palavras-chave: *obesidade, perda óssea alveolar, aterosclerose, ratos.*

INTRODUCTION

Obesity is defined as a chronic multifactorial disease characterized by abnormal or excessive fat accumulation that represents a risk to health[1,2]. Genetic and behavioral aspects are the main etiological factors.

Obesity is known to be a risk factor for cardiovascular diseases (CVD)[3,4], the main cause of death

worldwide, caused mainly by atherosclerosis. Atherosclerosis involves the deposition of cholesterol and other lipids on arterial walls, followed by inflammation and fibrosis, which increase arterial wall thickness and protrude into the vessel lumen, thus affecting normal irrigation. Studies suggest that periodontitis may influence the development of atherosclerosis[5,6]. In addition, studies report that obese individuals with periodontal disease may have an increased risk of cardiovascular events[7]. The biological plausibility is that both conditions lead to prothrombotic and proinflammatory conditions[8]. Some animal studies have evaluated the correlation between periodontal disease and obesity regarding atherosclerotic events, reporting diverse results[9,10]
This study aims to evaluate aortic wall thickness, taken as an indicator of atherosclerosis, in rats exposed to induced periodontal disease (PD) and/or obesity (OB). We intend to contribute to assessing periodontal disease as an additional risk factor for the development of atherosclerosis.

MATERIALS AND METHODS

Study design and ethical considerations

This is a secondary analysis of a blinded, randomized, controlled animal model study[11]. The Animal Research Reporting In Vivo Experiments (ARRIVE) guidelines were followed[12]. The research protocol was approved by the Animal Research Ethics Committee of the Hospital de Clínicas de Porto Alegre, Brazil (protocol number 110051 at 26/04/2011). Fig. 1 shows the study flowchart.

Animals

We used sixty male 60-day-old Wistar rats, weighing approximately 350g each. Animals were housed in groups of 4-5 under a 12-hour light/dark cycle at room temperature (22°C ± 2°C) with free access to water and the assigned diet. Two animals were lost during the experimental period, (one from Group CT and one from Group OB), for reasons unrelated to the protocol, as confirmed in the necropsies.

Randomization and allocation to groups

All animals were weighed and arranged in ascending order according to weight. Based on the distribution of this weight, rats were stratified into tertiles and randomized into 4 experimental groups, as follows:
Control (CT): received standardized rat chow diet and water.
Periodontal Disease (PD): exposed to ligature-induced periodontal disease in the upper right 2nd molar, with the same diet as CT.
Obesity (OB): obesity induction using a high-fat hypercaloric cafeteria diet (CAF).
Obesity and Periodontal Disease (OB+PD): exposed to obesity and periodontal disease induction, as described in Groups OB and PD.

Cafeteria diet-induced obesity

The CAF diet, used to induce obesity in rats, was composed of 55% carbohydrates, 20% lipids, 20% protein and 5% other constituents (sodium, calcium, vitamins, preservatives and minerals, among others). This high-calory diet included condensed milk, soda, sandwich cookies, wafer, sausage, cheese and ham snacks, provided at libitum following the criteria of Sampey et al.[13] The control group animals were fed with standard chow. Water was available *ad libitum* for all groups. The CAF diet model is able to raise body weight and glucose levels, and to cause hyperlipidemia[13]. All foods were replaced daily to ensure freshness.

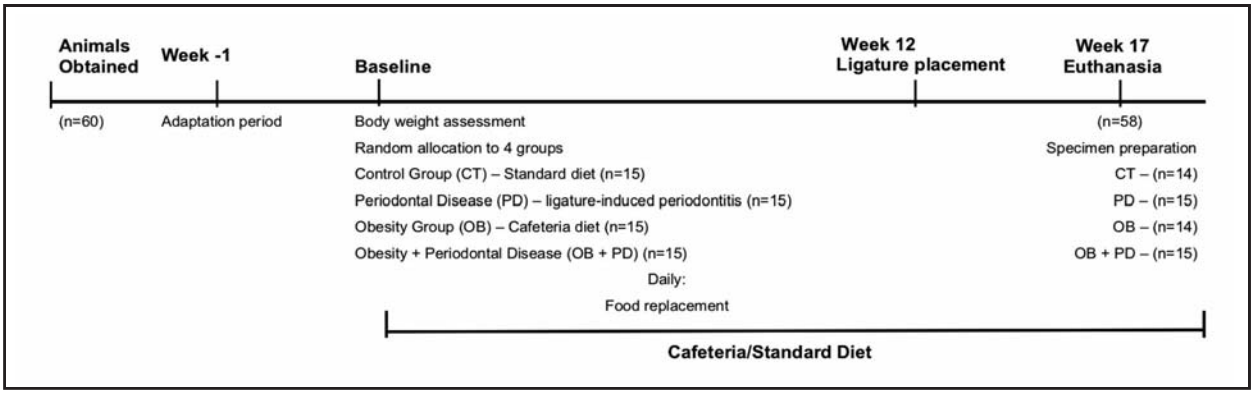

Fig. 1: Study Flowchart.

Body weight and Lee index assessments
Animals were weighed on an electronic scale. The Lee index method[14] was applied for rapid, accurate measurement of obesity in the groups fed on the cafeteria diet. The Lee index was calculated by dividing the cubic root of the body weight in grams by the naso-anal length in centimeters and multiplying by ten.

Periodontal disease model
At week 12, after groups under CAF diet had acquired obesity, periodontal disease was induced by placing a silk ligature on the maxillary right second molar, under general anesthesia with inhaled 5V% isoflurane (Isoforine™ Cristália, SP, Brazil) vaporized in 100% oxygen by facemask. All anesthetic procedures were performed by a veterinarian. The contralateral molar was used as intra-group control.

Specimen preparation
Animals were euthanized by decapitation 30 days after placing the ligature. Maxillaries were removed, sectioned, and de-fleshed in 9% sodium hypochlorite for 2 hours. The aortic arteries were carefully removed, washed abundantly with saline solution and fixed with 10% buffered formalin for histological processing.

Alveolar bone loss morphometric analysis
Morphometric analysis was performed using standard digital photographs. Pictures were taken using a 6.1-megapixel digital camera (Nikon™ Coolpix, Ayutthaya, Thailand) attached to a tripod and equipped with 100 mm macro-lenses with minimal focal distance. Specimens were fixed to an endodontic ruler, parallel to the ground. Photographs were taken of the buccal and palatal aspects of right and left hemimaxillae.
Linear distances were measured from the cemento enamel junction to the bone crest, using Adobe Photoshop™ CS4 software (Adobe Systems Inc., San Jose, CA, USA). An external researcher kept track of the groups to ensure that the examiner was blinded to them. Five measurements were taken on each surface of the second molar, both buccally and palatally (two on the distal root, two on the mesial root and one on the furcation). The measurements in pixels were converted into millimeters using the markings of the endodontic ruler to which the hemimaxillae were attached as reference.

Histometric analysis of aortas
The aortas were sectioned into four parts and embedded in paraffin, and 3 mm sections were cut for hematoxylin and eosin staining.
Microphotographs were taken at 100x magnification. The aortic wall (including intima and media) was measured (mm) by means of ImageJ software. The thickest point of each section was selected for measurement. The examiner was blinded to group allocation during histometric analysis.
The examiner was calibrated before starting the histometric analysis. Twenty percent of the aorta slides were selected randomly and the examiner measured them twice, with a one-week interval between measurements. Their means were compared using Pearson's correlation coefficient. The absence of a statistically significant difference between means was considered an indication of reproducibility. The intra-class correlation coefficient was 0.61.

Statistical analysis
For each evaluated parameter, normality was tested by Shapiro-Wilk test, and the appropriate statistical test was selected according to this assumption. Mean and standard deviation (SD) of body weight and Lee index were compared by Repeated Measurements one-way ANOVA followed by Bonferroni. Alveolar bone loss did not have symmetric distribution, therefore the data was evaluated by Kruskall Wallis followed by Dunn multiple comparison test and expressed in median and interquartile range. Aortic wall thickness was analyzed by one-way ANOVA, followed by Bonferroni's multiple comparison test. The level of significance was set at 0.05.

RESULTS
Obesity parameters
Body weight increased in all experimental groups (Fig 2). As from week 10, body weight was significantly higher in Groups OB and OB+PD than in Groups CT and PD.
Until week 12, the Lee index increased significantly in Groups OB and OB+PD (Fig 3). In addition, a significant interaction among groups and between time and groups ($P \leq 0.01$) for this index was demonstrated by Repeated Measurements ANOVA.

Alveolar bone loss

As expected, alveolar bone loss was significantly higher in groups with induction ligatures (PD and OB+PD) than in groups without induction (Fig.4). Interestingly, bone loss was 18% higher in Group OB+PD than in Group PD. ($p<0.001$).

Aortic wall thickness

Table 1 shows aortic wall thickness values. CAF diet induced significant increases, independently of the presence of periodontal disease. Statistically significant differences were found between OB and CT ($p=0.036$), OB+PD and PD ($p=0.004$), and OB and PD ($p=0.001$). No statistically significant difference was observed between CT and PD or between OB and OB+PD.

DISCUSSION

The present study evaluated aorta wall thickness and its relation to obesity and/or periodontal disease in Wistar rats. The rationale for such a study is that the inflammatory response of periodontal disease could be an additional factor in the pathogenesis of cardiovascular diseases, triggering atherosclerosis. The results demonstrated that obesity was able to increase aorta wall thickness; however, periodontal disease was not an additional modifier of wall thickness.

Cardiovascular diseases (CVD) have high morbidity and mortality rates worldwide[1]. It has been well established that atherosclerosis is the main factor in CVD etiopathogenesis. It is important to evaluate possible risk factors in addition to the established ones, and to ascertain whether they may worsen these health conditions. This would be helpful for establishing preventive and therapeutic strategies.

Table 1: Mean (±SD) of aortic wall thickness according to experimental group.

Group	Thickness (µm)
CT	2.18 ± 0.26 A
PD	2.14 ± 0.27 A
OB	2.33 ± 0.29 B
OB + PD	2.31 ± 0.28 B

Different letters in the column indicate statistically significant difference (One-way ANOVA- Bonferroni).

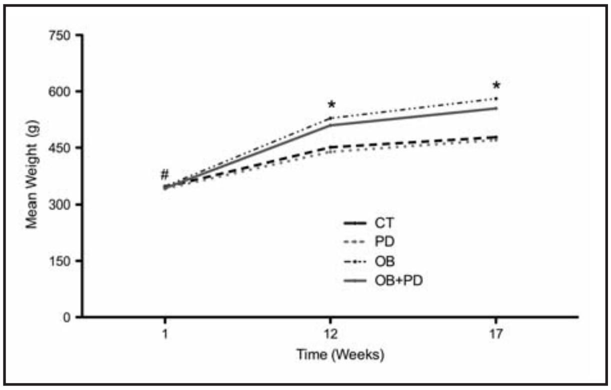

*Fig. 2: Mean body weight (g) according to experimental groups throughout the study. *Groups OB and OB+PD differed significantly from Groups CT and PD. #After baseline, mean weight increased in all groups (Repeated Measurements ANOVA – Bonferroni).*

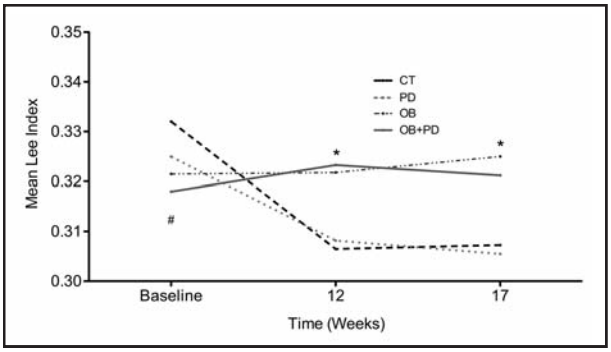

*Fig. 3: Mean Lee Index according to experimental groups throughout the study. *Groups OB and OB+PD differed significantly from Groups CT and PD. #After baseline, mean Lee Index increased in Groups OB and OB+PD and decreased in Groups CT and PD (Repeated Measurements ANOVA – Bonferroni).*

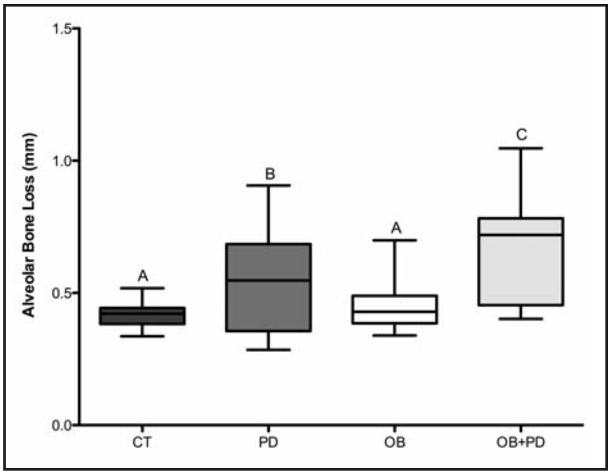

Fig. 4: Median and interquartile range (25th and 75th percentile) of alveolar bone loss according to experimental groups. Different letters indicate statistically significant difference among groups (Kruskall Wallis – Dunn).

Atherosclerosis involves inflammatory responses which have been related to other inflammatory conditions such as periodontal disease and cardiovascular risk factors, including obesity, smoking, sedentary lifestyle, dyslipidemia and diabetes[15], all of which could increase the inflammatory burden and change the arterial endothelial function, which is the first stage of atherosclerosis. It has also been suggested that overweight and obesity may be associated with periodontal disease[16]. However, the exact biological process underlying this relationship has not yet been established.

Clinical studies using true outcomes such as myocardial infarction or even death are scarce and subject to ethical restraints. Animal studies are thus warranted. This study used Wistar rats, which are widely used in both periodontal and cardiovascular studies. The experiment induced obesity by feeding rats on cafeteria diet, which mimics the western diet, with high carbohydrate and fat intake. This diet has been used in a previous study.[13] This kind of model has also been used in the study of pathogenesis of periodontitis and its association with metabolic abnormalities [17,18].

In the first 12 weeks, before ligature placement, Groups OB and OB+PD were fed cafeteria diet, which led to obesity[13,17,18]. Body weight and Lee index values were used as methods to infer obesity. At baseline, the rats weighed approximately 350g, with no statistically significant difference among groups. Body weight increased in all groups; however, as from week 10, the rats which received CAF diet had statistically significant higher body than Groups CT and PD. Additionally, the Lee index increased significantly in groups fed on cafeteria diet. Even though studies suggest that periodontitis may stimulate obesity by secretion of proinflammatory cytokines[18], no significant differences were observed between OB and OB+ PD with regard to body weight or Lee index in our study. Two parameters – body weight and Lee index – both showed that obesity occurred in the groups fed on CAF diet, thereby enabling the objectives of comparing how obesity and/or periodontal disease relates to potential negative cardiovascular outcomes.

Periodontal disease induction was effective, as shown by the mean values for alveolar bone loss which were considerably higher in the groups in which ligature was placed. Furthermore, alveolar bone loss was higher in Group OB+PD than in PD groups. These results corroborate previous findings reported by Verzeletti et al.[18]. These data suggested that in the presence of ligature, obesity acted as a synergistic factor in bone loss of inflammatory origin. A possible explanation for this synergism is that the continuous release of cytokines derived from the subclinical chronic inflammation which is characteristic of obesity may exacerbate other local inflammatory responses such as periodontal disease[19].

Aortic walls were thicker in Groups OB and OB+PD than in Groups CT and PD. This supports the concept that obesity increases aorta wall thickness, thereby increasing the chances of CVD. Induction of obesity by hypercaloric and hyperlipidic diet in Wistar rats produces major metabolic alterations, such as an increase in triglycerides. For this reason, the use of this model was suggested to investigate endothelial dysfunction[20], which is one of the factors which impact the pathogenesis of atherosclerosis. Our measurement method proved to be effective to show an increase in aortic thickness of the rats which received the CAF diet. Similarly, other authors also observed increased aortic wall thickness in rats exposed to a high-fat content diet different from CAF.[21].

In our model, periodontal disease did not modify the effect of obesity by either increasing or decreasing aortic wall thickness. There are several possible explanations for this, some of which would be worthwhile studying further. Firstly, the ligature was placed on only one tooth, which may produce a low inflammatory challenge compared to extensive periodontal disease. Secondly, aortic wall thickness is extremely variable but was only measured at one point. Nevertheless, the supporting literature is sufficient to warrant further studies of the relationship between periodontal diseases and CVD, in order to better guide preventive and therapeutic strategies. Furthermore, it should be highlighted that periodontal and cardiovascular diseases share risk factors, and should both be considered in the development of health policies.

In conclusion, obesity altered aortic wall thickness in Wistar rats. However, the presence of periodontal disease did not affect aortic wall thickness under the conditions of the present study.

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REFERENCES

1. World Health Organization. Obesity: preventing and managing the global epidemic. Report of a WHO consultation. World Health Organ Tech Rep Ser 2000; 894:1-253. URL: https://www.who.int/nutrition/publications/obesity/WHO_TRS_894/en/
2. Smith KB, Smith MS. Obesity Statistics. Prim Care 2016; 43:121-135.
3. Kappus RM, Fahs CA, Smith D, Horn GP, Agiovlasitis S, Rossow L, et al. Obesity and overweight associated with increased carotid diameter and decreased arterial function in young otherwise healthy men. Am J Hypertens 2014; 27:628-634.
4. Yatsuya H, Li Y, Hilawe EH, Ota A, Wang C, Chiang C, et al. Global trend in overweight and obesity and its association with cardiovascular disease incidence. Circ J 2014;78:2807-2818.
5. Kinane DF, Lowe GD. How periodontal disease may contribute to cardiovascular disease. Periodontol 2000 2000; 23:121-126.
6. Teng YT, Taylor GW, Scannapieco F, Kinane DF, Curtis M, Beck JD, et al. Periodontal health and systemic disorders. J Can Dent Assoc 2002; 68:188-192.
7. Pires JR, Dos Santos IP, de Camargo LF, Zuza EP, de Toledo BE, Monteiro SC. Framingham cardiovascular risk in patients with obesity and periodontitis. J Indian Soc Periodontol 2014; 18:14-18.
8. Chistiakov DA, Orekhov AN, Bobryshev YV. Links between atherosclerotic and periodontal disease. Exp Mol Pathol 2016; 100:220-235.
9. Ekuni D, Tomofuji T, Irie K, Kasuyama K, Umakoshi M, Azuma T, et al. Effects of periodontitis on aortic insulin resistance in an obese rat model. Lab Invest 2010;90:348-359.
10. Jain A, Batista EL, Jr., Serhan C, Stahl GL, Van Dyke TE. Role for periodontitis in the progression of lipid deposition in an animal model. Infect Immun 2003; 71:6012-6018.
11. Cavagni J, de Macedo IC, Gaio EJ, Souza A, de Molon RS, Cirelli JA, et al. Obesity and Hyperlipidemia Modulate Alveolar Bone Loss in Wistar Rats. J Periodontol 2016; 87:e9-17.
12. Kilkenny C, Browne WJ, Cuthill IC, Emerson M, Altman DG. Improving bioscience research reporting: the ARRIVE guidelines for reporting animal research. Osteoarthritis Cartilage 2012; 20:256-260.
13. Sampey BP, Vanhoose AM, Winfield HM, Freemerman AJ, Muehlbauer MJ, Fueger PT, et al. Cafeteria diet is a robust model of human metabolic syndrome with liver and adipose inflammation: comparison to high-fat diet. Obesity (Silver Spring) 2011; 19:1109-1117.
14. Bernardis LL, Patterson BD. Correlation between ‘Lee index’ and carcass fat content in weanling and adult female rats with hypothalamic lesions. J Endocrinol 1968 Apr; 40: 527-528.
15. Orlandi M, Suvan J, Petrie A, Donos N, Masi S, Hingorani A, et al. Association between periodontal disease and its treatment, flow-mediated dilatation and carotid intima-media thickness: a systematic review and meta-analysis. Atherosclerosis 2014; 236:39-46.
16. Keller A, Rohde JF, Raymond K, Heitmann BL. Association between periodontal disease and overweight and obesity: a systematic review. J Periodontol 2015; 86:766-776.
17. Cavagni J, Wagner TP, Gaio EJ, Rego RO, Torres IL, Rosing CK. Obesity may increase the occurrence of spontaneous periodontal disease in Wistar rats. Arch Oral Biol 2013; 58:1034-1039.
18. Verzeletti GN, Gaio EJ, Linhares DS, Rosing CK. Effect of obesity on alveolar bone loss in experimental periodontitis in Wistar rats. J Appl Oral Sci 2012; 20:218-221.
19. Pischon N, Heng N, Bernimoulin JP, Kleber BM, Willich SN, Pischon T. Obesity, inflammation, and periodontal disease. J Dent Res 2007; 86:400-409.
20. Rosini TC, Silva AS, Moraes C. Diet-induced obesity: rodent model for the study of obesity-related disorders. Rev Assoc Med Bras (1992) 2012; 58:383-387.
21. Turkay C, Saba R, Sahin N, Altunbas H, Ozbudak O, Akkaya B, Ozbilim G, Cölbasi I, et al. Effect of chronic Pseudomonas aeruginosa infection on the development of atherosclerosis in a rat model. Clin Microbiol Infect 2004; 10:705-708.

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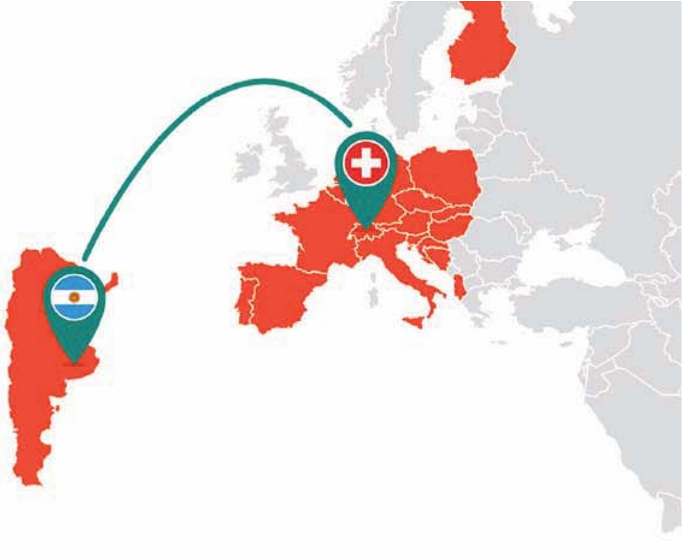

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