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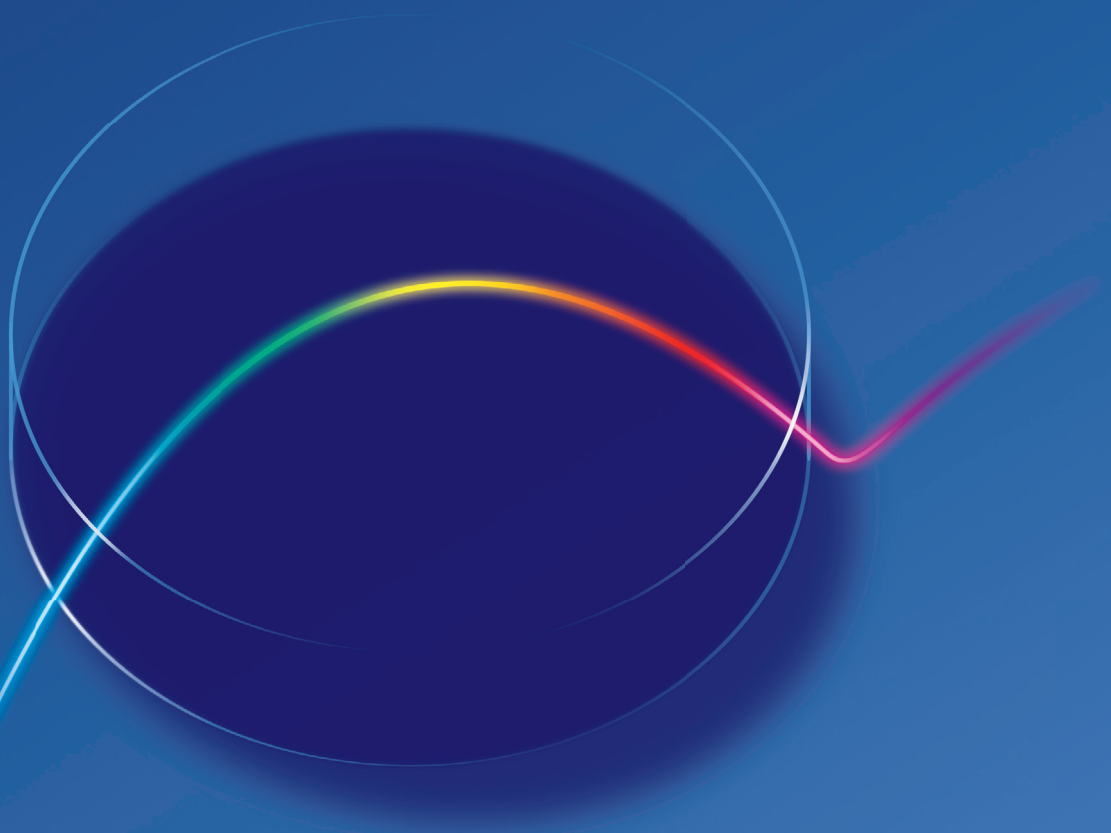

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Contents

PCR identification of endodontic pathogens and DNA quantification in samples from teeth with post-treatment apical periodontitis

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ABSTRACT | *Aim:* The aim of this clinical study was to quantify the concentration of DNA and to detect selected bacterial species from samples of infected root-filled teeth with post-treatment apical periodontitis after removal of gutta-percha (S1), after chemo-mechanical preparation at the first appointment (S2), 5 days after the canal was filled with sterile physiological solution (S3), after reinstrumentation at the second appointment (S4), and 14 days after an intracanal dressing was placed at the third appointment (S5). *Methods:* Fifteen root-filled teeth were selected. Removal of gutta-percha was performed using the crown-down technique. Chemo-mechanical preparation was performed with hand files associated with 2% chlorhexidine gel. An intracanal dressing based on $Ca(OH)_2$ was used. DNA was extracted from the samples and 14 endodontic 16S rDNA species-specific primers were tested. The concentration of DNA was quantified using a NanoDropTM 2000 spectrophotometer. *Results:* Bacteria were present in all cases at all sampling times, as revealed by a universal primer. DNA was isolated from all samples, with an average concentration of 4.24 ± 2.9 ng/µL (S1), 3.39 ± 1.54 ng/µL (S2), 4.0 ± 1.94 ng/µL (S3), 2.66 ± 0.98 ng/µL (S4) and 3.97 ± 2.32 ng/µL (S5). *Parvimonas micra* and *Enterococcus faecalis* (S1), *P. micra* (S2), *Porphyromonas endodontalis* and *E. faecalis* (S3), *E. faecalis* and *Prevotella nigrescens* (S4/S5) were the species most frequently deteced. DNA concentration reductions were detected between S3 and S4 (p = 0.0256), whereas an increase was found between S4 and S5. *Conclusion:* A wide variety of bacterial species was detected in root-filled teeth with post-treatment apical periodontitis. Moreover, the use of an intracanal dressing was unable to further reduce the concentration of bacterial DNA.

DESCRIPTORS | Endodontics; Treatment Failure; Periapical Periodontitis; Bacteria; Polymerase Chain Reaction; DNA; Chlorhexidine.

RESUMO | **Identificação de patógenos endodônticos por PCR e quantificação de DNA em amostras extraídas de dentes com periodontite apical pós-tratamento** • *Objetivo:* O objetivo deste estudo clínico foi quantificar a concentração de DNA e detectar algumas espécies bacterianas de amostras de dentes tratados endodonticamente com periodontite apical após a remoção da guta-percha (S1), após o preparo químico-mecânico na primeira sessão (S2), 5 dias após o preenchimento do canal com solução fisiológica estéril (S3), após reinstrumentação na segunda sessão (S4), e 14 dias após a inserção da medicação intracanal na terceira sessão (S5). *Métodos:* Quinze dentes tratados endodonticamente foram selecionados. A remoção da guta-percha foi realizada por meio da técnica coroa-ápice. Utilizaram-se limas manuais associadas à clorexidina gel a 2% durante o preparo químico-mecânico. A medicação intracanal selecionada foi à base de hidróxido de cálcio. DNA foi isolado das amostras e foram investigadas 14 espécies bacterianas (primer espécie-específico16S rDNA). A concentração de DNA foi quantificada utilizando o espectrofotômetro NanoDrop™ 2000. *Resultados:* Em todos os casos foram detectadas bactérias, como revelado por meio do primer universal. DNA foi isolado de todas as amostras, com uma concentração média de 4,24 ± 2,9 ng/µL (S1), 3,39 ± 1,54 ng/µL (S2), 4,0 ± 1,94 ng/µL (S3), 2,66 ± 0,98 ng/µL (S4) e 3,97 ± 2,32 ng/µL (S5). *Parvimonas micra* e *Enterococcus faecalis* (S1), *P. micra* (S2), *Porphyromonas endodontalis* e *E. faecalis* (S3), *E. faecalis* e *Prevotella nigrescens* (S4/S5) foram as espécies mais frequentemente detectadas. A concentração de DNA diminuiu entre S3 e S4 (p = 0,0256), ao passo que um aumento foi observado entre S4 e S5. *Conclusão:* Uma ampla variedade de espécies bacterianas foi detectada em canais radiculares de dentes tratados endodonticamente com periodontite apical. Além disso, o uso da medicação intracanal não potencializou a redução da concentração de DNA bacteriano.

DESCRITORES | Endodontia; Falha de Tratamento; Periodontite Periapical; Bactérias; Reação em Cadeia da Polimerase; DNA; Clorexidina.

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INTRODUCTION

Failure of endodontic treatment is acknowledged to be the continuing presence of bacteria within the root canal system, even in well-treated teeth.[1] Endodontic treatment failure that is attributable to remaining microorganisms will only occur if these microorganisms possess pathogenicity, reach sufficient numbers, and gain access to the periradicular tissues to induce or maintain periradicular disease.[1]

In most cases where endodontic treatment fails, failure is the result of treatment procedures not having met a satisfactory standard for control and elimination of infection.[2] Modern endodontic treatment procedures aim to eliminate microorganisms during root canal preparation and disinfection. Follow-up studies examining the outcome of endodontic therapy revealed a very high success rate when a negative bacterial culture was a prerequisite before root filling,[3] although there is clear evidence that a negative culture does not correlate with a bacteria-free root canal system.[4]

Most clinical trials evaluating the antibacterial effectiveness of intracanal procedures and the bacterial species persisting after treatment have been based on traditional culture-dependent methods.[5-7] Recent molecular biology studies have suggested that the microbiota in root-canal-treated teeth with apical periodontitis is more complex than previously shown by culture-dependent methods.[4,8] Bacteria have been detected in almost all treated canal associated with persistent disease and a higher mean number of taxa per case has been observed.[8,9]

Essentially, endodontic infections are treated by chemo-mechanical preparation supplemented or not by an interappointment intracanal medication. Although a substantial reduction in intracanal microbial communities is usually reached after chemo-mechanical procedures with antimicrobial irrigants, it has been shown that predictable disinfection in most cases can only be achieved after an interappointment intracanal medication.[6-7] Calcium hydroxide is arguably the most used substance between treatment sessions, but studies have shown inconsistent results as to its efficacy in significantly enhancing disinfection.[6-7,10] Then intracanal dressing was used to test if the concentration of DNA and bacterial species that might have survived after chemo-mechanical preparation would decrease.

The aim of this clinical study was to quantify the total concentration of DNA and to detect bacterial species from samples of infected root-filled teeth with post-treatment apical periodontitis after removal of the gutta-percha (S1), after chemo-mechanical preparation with 2% chlorhexidine (CHX) gel in the first appointment (S2), 5 days after the canal was filled with sterile physiological solution (S3), after reinstrumentation in the second appointment (S4), and 14 days after an intracanal dressing was placed in the third appointment (S5).

MATERIAL AND METHODS

Patient selection

Fifteen patients were selected from those who attended the Piracicaba Dental School, SP, Brazil, with a need for nonsurgical endodontic retreatment. The Human Volunteers Research and Ethics Committee of the Piracicaba Dental School approved a protocol describing the specimen collection for this investigation, and all patients signed an informed consent to participate. A detailed medical and dental history was obtained from each patient. Patients who had received antibiotic treatment during the last 3 months or had a general disease were excluded from the study. The age of the patients ranged from 19 to 65 years. All of the selected teeth were single-rooted. The teeth had been previously root-filled and showed radiographic evidence of apical periodontitis. Failure of the root canal treatment was determined on the basis of clinical and radiographic examinations. All teeth had been endodontically treated and filled

more than 2 years previously, and the patients were asymptomatic. All teeth had enough crown structure for adequate isolation with a rubber dam, and had no periodontal pockets deeper than 4 mm.

Microbial sampling

The teeth were isolated with a rubber dam. The crown and the surrounding rubber dam were disinfected with 30% H_2O_2 (v/v) for 30 s followed by 2.5% NaOCl for an additional 30 s. Subsequently, 5% sodium thiosulphate was used to inactivate the disinfectant agents.[11-12] A swab sample was taken from the surface and streaked on blood agar plates to test for disinfection. An access cavity was prepared with sterile high-speed diamond burs under irrigation with sterile physiological solution. Before entering the pulp chamber, the access cavity was disinfected following the same protocol as above, and sterility was checked again by taking a swab sample of the cavity surface and streaking it onto blood agar plates. Aseptic techniques were used throughout root canal treatment and sample acquisition. The samples (pre- and post-clinical procedures) were collected with three sterile paper points, which were consecutively placed into each canal to the total length calculated from the pre-operative radiograph, kept in place for 60 s and then pooled in a sterile tube containing 1 mL of VMGA III transport medium.[11] The samples were immediately frozen at -20°C.

Clinical procedures

The same endodontic specialist performed all retreatments, intracanal dressing and sampling procedures. The tooth was anesthetized and, after accessing the pulp chamber, the root filling materials were removed using the crown-down technique. No solvent was used at any time to avoid a negative effect on microbial viability. Buccolingual and mesiodistal radiographs of each tooth were taken to confirm gutta-percha removal.

First appointment

The root filling was removed using Gates-Glidden drills (Dentsply Maillefer, Ballaigues, Switzerland) of sizes 5 (1.3 mm), 4 (1.1 mm), 3 (0.9 mm) and 2 (0.7 mm) up to 6 mm shorter than the working length. Irrigation with sterile physiological solution was performed in order to remove any remaining materials and to moisten the canal prior to sample collection. A K-file of size #15 (Dentsply Maillefer, Ballaigues, Switzerland) was inserted to the full length of the root canal calculated from the pre-operative radiograph. This file was inserted to full length until canal patency was confirmed. Working length (at the apical foramen) was confirmed by an apical locator (Novapex, Forum Technologies, Rishon le-Zion, Israel). After removal of the gutta-percha with hand files, the first sample was taken with three paper points, which were then pooled in VMGA III. The apical preparation was performed using K-files ranging from size #40 to #45 followed by step-back instrumentation, which ended after the use of three files larger than the last file used for the apical preparation.

All root canals were irrigated with a syringe (27-gauge needle) containing 1 mL of the chemical auxiliary substance (2% CHX gel; Endogel, Itapetininga, SP, Brazil) before the use of each instrument and immediately rinsed afterwards with 4 mL of sterile physiological solution. The CHX gel consisted of a gel base (1% natrosol) and CHX gluconate at pH 7.0. Natrosol gel (hydroxyethyl cellulose) is a nonionic, highly inert and water-soluble agent. After the instrumentation, CHX activity was inactivated with 5 mL of a solution containing 5% Tween 80 and 0.07% (w/v) lecithin during a 1-minute period, which was removed with 5 mL of sterile physiological solution. Retreatment was deemed complete when the last file reached the working length, there was no filling material covering the instrument, and the canal walls were smooth and free of visible debris. Furthermore, a close inspection was conducted

with a dental operating microscope (DF Vasconcellos S/A, São Paulo, Brazil) under high magnification to confirm the complete removal of gutta-percha.

After the root canal preparation had been completed, the canal was irrigated for 3 minutes with 5 mL of 17% EDTA. Then, the root canal was rinsed with 5 mL of sterile physiological solution. Subsequently, the second (first chemo-mechanical) sample (S2) was taken with three paper points, which were pooled in VMGA III. The canal was then thoroughly rinsed with sterile physiological solution using a syringe (27-gauge needle). The access cavity was then temporized with cement to a thickness of at least 2 mm (Coltosol, Coltène/Whaledent Inc., Cuyahoga Falls, OH, USA) and a second layer of Filtek Z250® (3M ESPE, St. Paul, MN, USA) in combination with a single-bond adhesive (3M ESPE, St. Paul, MN, USA).

Second appointment

Rubber dam isolation and access cavity procedures were performed following the same protocol as described above. After access was obtained and coronal disinfection was performed, a third sample (S3) was taken with three paper points, which were pooled in VMGA III. Re-instrumentation was performed with the last file up to working length and following the same irrigation protocol. After this second chemo-mechanical preparation was performed, a fourth sample (S4) was taken with three paper points, pooled in VMGA III. An intracanal dressing with calcium hydroxide [$Ca(OH)_2$] associated with 2% CHX gel was placed over the entire length of the prepared canal using lentulo spiral fillers. The paste was packed at the level of the canal entrance and a radiograph was taken to check for adequate placement (homogeneous filling throughout the entire extent of the prepared canal). The access cavity was then temporized with temporary cement to a thickness of at least 2 mm (Coltosol, Coltène/Whaledent Inc., Cuyahoga Falls, OH, USA) and a second layer of Filtek Z250® (3M ESPE, St. Paul, MN, USA) in combination with a single-bond adhesive (3M ESPE, St. Paul, MN, USA).

Third appointment

The third appointment was scheduled for 14 days later. At this time, the tooth was isolated with a rubber dam, the operative field was disinfected, as previously described for the first visit, and a control bacteriological sample was obtained from the operating field. The composite restoration and temporary cement were removed with a sterile high-speed carbide bur and the canal was irrigated with sterile physiological solution. The canal walls were cleaned with a hand K-file one size greater than that of the master apical file, and irrigated with 5 mL of sterile physiological solution, dried with paper points, and irrigated with 5 mL of a solution containing 5% Tween 80 and 0.07% (w/v) lecithin during a 1-minute period to inactivate the CHX. After removal of the intracanal dressing, a fifth sample (S5) was taken with three paper points, which were pooled in VMGA III.

Finally, all teeth were filled using vertical and lateral compaction of the gutta-percha cones (Konne, Belo Horizonte, MG, Brazil) and with Endomethasone® sealer (Septodont, Saint-Maur-des-Fossés, France). The access cavities were restored with a 2 mm layer of Coltosol® (Coltène Whaledent, Cuyahoga Falls, OH, USA) and Filtek Z250® (3M Dental Products, St Paul, MN, USA).

DNA extraction

Microbial DNA from samples of all stages of endodontic retreatment (S1, S2, S3, S4, S5), from the control sample as well as from ATCC bacteria were extracted and purified by using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions.

DNA quantification

The extracted DNA samples were quantified by

using a NanoDrop spectrophotometer (Thermo Scientific NanoDrop™ 2000/2000c, Wilmington, DE, USA). A blank sample was established using AE buffer AE (Elution buffer; Qiagen, Hilden, Germany). Each sample (1.5 µL) was placed sequentially in the spectrophotometer. The DNA concentration was calculated from the 260 nm absorbance value for each replicate using the DNA-50 settings. The software automatically calculated the DNA concentration in ng/µL.

Bacterial detection (polymerase chain reaction - PCR 16S rDNA)

The reference bacteria strains used in this study were purchased from the American Type Culture Collection (ATCC) and are listed as follows:

- *Aggregatibacter actinomycetemcomitans* (ATCC 43718),
- *Enterococcus faecalis* (ATCC 4034),
- *Filifactor alocis* (ATCC 35896),
- *Fusobacterium nucleatum* (ATCC 25586),
- *Gemella morbillorum* (ATCC 27824),
- *Parvimonas micra* (ATCC 33270),
- *Porphyromonas endodontalis* (ATCC 35406),
- *Porphyromonas gingivalis* (ATCC 33277),
- *Prevotella intermedia* (ATCC 25611),
- *Prevotella nigrescens* (ATCC 33536),
- *Prevotella tannerae* (ATCC 51259),
- *Tannerella forsythia* (ATCC 43037),
- *Treponema denticola* (ATCC 35405) and
- *Treponema socranskii* (ATCC 35536).

PCR assay

The PCR reaction was performed in a thermocycler (My-Cycler; Bio-Rad, Hercules, CA, USA) with a total volume of 25 µL containing 2.5 µL of 10× Taq buffer (1×; MBI Fermentas, Mundolsheim, France), 0.5 µL of dNTP mix (25 µmol/L of each deoxyribonucleoside triphosphate – dATP, dCTP, dGTP, and dTTP; MBI Fermentas, Hanover, MD, USA), 1.25 µL of 25 mmol/L $MgCl_2$, 0.25 µL of forward and reversal universal primers (0.2 µmol/L; Invitrogen, Eugene, OR, USA), 1.5 µL of sample DNA (1 µg/50 µL), 1.5 µL of Taq DNA polymerase (1 U; MBI Fermentas), and 17.25 µL of nuclease-free water. The primer sequences and PCR cycling parameters were previously optimized[12] and are listed in Table 1.

Statistical analysis

The concentration of DNA from each treatment step was calculated based on quantitative data obtained from samples S1, S2, S3, S4 and S5. Quantitative data were statistically analyzed for differences by using the Mann-Whitney *U* test comparing pairs of groups. The significance level was always set at 5% ($p < 0.05$).

RESULTS

DNA quantification

DNA was isolated from all samples, with an average concentration of 4.24 ± 2.9 ng/µL (S1), 3.39 ± 1.54 ng/µL (S2), 4.0 ± 1.94 ng/µL (S3), 2.66 ± 0.98 ng/µL (S4) and 3.97 ± 2.32 ng/µL (S5; Table 2). The highest DNA amount was found in the initial samples (S1). No significant statistical difference was detected between the DNA concentration of samples S1 and S2 (Mann-Whitney test, $p = 0.3937$); S2 and S3 (Mann-Whitney test, $p = 0.1548$); and S1 and S5 (Mann-Whitney test, $p = 0.4017$). However, a statistically significant reduction in DNA concentration from S3 to S4 (Mann-Whitney test, $p = 0.0256$) and an increase from S4 to S5 (Mann-Whitney test, $p = 0.0445$) were detected.

Overview of microbial composition by 16S rDNA PCR

Bacteria were present in all samples (S1, S2, S3, S4, S5), confirming the infectious etiology of post-treatment disease after PCR amplification using broad-range 16S rDNA gene primers.

The bacteria recovered by species-specific

Table 1 | PCR primer pairs and cycling parameters used for detection of bacterial species in samples from root-filled teeth with post-treatment apical periodontitis.

Target bacteria	Primer pairs (5'–3')	Amplicon size	Cycles
Universal (16S rDNA)	Forward: TCC TAC GGG AGG CAG CAG T Reverse: GGA CTA CCA GGG TAT CTA ATC CTG TT	466 bp	Initial denaturation at 95°C for 10 min and 40 cycles of 95°C for 10 s, 60°C for 10 s, and a final extension step at 72°C for 25 s
Actinobacillus actinomycetemcomitans	Forward:AAA CCC ATC TCT GAG TTC TTC TTC Reverse: ATG CCA ACT TGA CGT TAA AT	557 bp	Initial denaturation at 94°C for 30 s and 36 cycles of 95°C for 30 s, 55°C for 1 min, 72°C for 2 min, and a final extension step at 72°C for 10 min
Prevotella intemedia	Forward: TTT GTT GGG GAG TAA AGC GGG Reverse: TCA ACA TCT CTG TAT CCT GCG T	575 bp	Initial denaturation at 95°C for 2 min and 36 cycles of 94°C for 30 s, 58°C for 1 min, 72°C for 2 min, and a final extension step at 72°C for 10 min
Prevotella nigrescens	Forward: ATG AAA CAA AGG TTT TCC GGT AAG Reverse: CCC ACG TCT CTG TGG GCT GCG A	804 bp	Initial denaturation at 95°C for 2 min and 36 cycles of 94°C for 30 s, 58°C for 1 min, 72°C for 2 min, and a final extension step at 72°C for 10 min
Tannerella forsythia	Forward: GCG TAT GTA ACC TGC CCG CA Reverse: TGC TTC AGT GTC AGT TAT ACC T	641 bp	Initial denaturation at 95°C for 1 min and 36 cycles of 95°C for 30 s, 60°C for 1 min, 72°C for 1 min, and a final extension step at 72°C for 2 min
Prevotella tannerae	Forward: CTT AGC TTG CTA AGT ATG CCG Reverse: CAG CTG ACT TAT ACT CCC G	550 bp	Initial denaturation at 95°C for 2 min and 36 cycles of 94°C for 30 s, 55°C for 1 min, 72°C for 2 min, and a final extension step at 72°C for 10 min
Treponema denticola	Forward: TAA TAC CGA ATG TGC TCA TTT ACA T Reverse: TCAAAGAAGCAT TCC CTC TTC TTC TTA	316 bp	Initial denaturation at 95°C for 2 min and 36 cycles of 94°C for 30 s, 60°C for 1 min, 72°C for 2 min, and a final extension step at 72°C for 10 min
Treponema socranskii	Forward: GAT CAC TGTATA CGGAAGGTAGACA Reverse: TAC ACT TAT TCC TCG GAC AG	288 bp	Initial denaturation at 95°C for 2 min and 36 cycles of 94°C for 30 s, 56°C for 1 min, 72°C for 2 min, and a final extension step at 72°C for 10 min
Enterococcus faecalis	F: CCG AGT GCT TGC ACT CAA TTG G R: CTC TTA TGC CAT GCG GCA TAA AC	138 bp	Initial denaturation at 95°C for 2 min and 36 cycles of 95°C for 1 min, 57°C for 1 min, 72°C for 1 min, and a final extension step at 72°C for 7 min
Filifactor alocis	F: CAG GTG GTT TAA CAA GTT AGT GG R: CTA AGT TGT CCT TAG CTG TCT CG	594 bp	Initial denaturation at 95°C for 2 min and 26 cycles of 95°C for 30 s, 58°C for 1 min, 72°C for 1 min, and a final extension step at 72°C for 2 min
Fusobacterium nucleatum	F: AGT AGC ACA AGG GAG ATG TAT G R: CAA GAA CTA CAA TAG AAC CTG A	1000 bp	Initial denaturation at 95°C for 5 min and 30 cycles of 94°C for 30 s, 40°C for 1 min, 72°C for 2 min, and a final extension step at 72°C for 10 min
Gemella morbillorum	F: GAC TAC CAG GGT ATC TAA TCC R: TAT GAG GTT GGC TGA CTC TCG	781 bp	Initial denaturation at 95°C for 2 min and 36 cycles of 94°C for 30 s, 52°C for 1 min, 72°C for 2 min, and a final extension step at 72°C for 10 min
Parvimonas micra	F: AGA GTT TGA TCC TGG CTC AG R: ATA TCA TGC GAT TCT GTG GTC TC	207 bp	Initial denaturation at 95°C for 2 min and 36 cycles of 94°C for 30 s, 60°C for 1 min, 72°C for 2 min, and a final extension step at 72°C for 10 min
Porphyromonas endodontalis	F: GCT GCA GCT CAA CTG TAG TC R: CCG CTT CAT GTC ACC ATG TC	672 bp	Initial denaturation at 95°C for 2 min and 36 cycles of 94°C for 30 s, 58°C for 1 min, 72°C for 2 min, and a final extension step at 72°C for 10 min
Porphyromonas gingivalis	F: AGG CAG CTT GCC ATA CTG CG R: ACT GTT AGC AAC TAC CGA TGT	404 bp	Initial denaturation at 95°C for 2 min and 36 cycles of 94°C for 30 s, 60°C for 1 min, 72°C for 2 min, and a final extension step at 72°C for 2 min

PCR: polymerase chain reaction

Table 2 | Bacterial frequency for 14 selected target species-specific primers and DNA concentration (ng/μL) in samples from root-filled teeth with apical periodontitis. Initial sample (S1), after first chemo-mechanical preparation (S2), 5 days after the canal was filled with sterile physiological solution (S3), after second chemo-mechanical preparation (S4), and 14 days after placement of intracanal dressing (S5).

	S1	S2	S3	S4	S5
Number of positive samples	31	15	31	20	32
DNA concentration (mean ± SD)	4.24 ± 2.9	3.39 ± 1.54	4.0 ± 1.94 a	2.66 ± 0.98 b,A	3.97 ± 2.32 B

Different lower letters (a,b) indicate a significant difference (Mann-Whitney test, $p < 0.05$). Different capital letters (A,B) indicate a significant difference (Mann-Whitney test, $p < 0.05$).

Table 3 | Frequency of positive samples for selected species in initial sample (S1), after first chemo-mechanical preparation (S2), 5 days after the canal was filled with sterile physiological solution (S3), after second chemo-mechanical preparation (S4), and 14 days after placement of intracanal dressing (S5).

Target species	Time of sample collection				
	S1	S2	S3	S4	S5
Fusobacterium nucleatum	1	0	4	3	4
Prevotella intermedia	2	0	0	2	2
Prevotella nigrescens	4	0	5	6	6
Prevotella tannerae	1	2	1	0	0
Enterococcus faecalis	6	2	5	6	7
Gemella morbillorum	3	0	4	1	6
Treponema denticola	1	0	0	0	0
Treponema socranskii	1	0	1	0	0
Porphyromonas endodontalis	0	2	8	1	3
Porphyromonas gingivalis	0	0	1	0	0
Filifactor alocis	0	0	1	0	0
Parvimonas micra	10	7	0	1	1
Tannerella forsythia	2	1	1	0	1
Actinobacillus actinomycetemcomitans	0	1	0	0	2
Total	31	15	31	20	32

primer 16S rDNA after removal of the gutta-percha (S1), after the first chemo-mechanical preparation (S2), 5 days after the canal was filled with sterile physiological solution (S3), after re-instrumentation (S4), and 14 days after the intracanal dressing was placed (S5) are shown in Table 3. At S1, the most prevalent taxon was *Parvimonas micra* (10 cases), followed by *Enterococcus faecalis* (6 cases) and *Prevotella nigrescens* (4 cases). After the first chemo-mechanical preparation (S2), the most prevalent taxon was *Parvimonas micra* (7 cases), followed by *Enterococcus faecalis* (2 cases). The most prevalent taxon at S3 was *Porphyromonas endodontalis* (8 cases) followed by *Enterococcus faecalis* (5 cases) and *Prevotella nigrescens* (5 cases). After re-instrumentation (S4), the most prevalent taxons were *Prevotela nigrescens* (6 cases) and *Enterococcus faecalis* (6 cases). At S5, the most prevalent taxon was *Enterococcus faecalis* (7 cases). The higher the concentration of DNA, the higher the number of bacterial species detected by 16S rDNA PCR in each step (Table 2).

DISCUSSION

The DNA concentration in samples from root-filled canals with periapical lesions was monitored at five time-points after endodontic procedures. Many studies have shown that microorganisms are the major causative agents of endodontic therapy failure; in contrast, to date no study has monitored the individual concentration of total DNA after gutta-percha removal, chemo-mechanical preparation and intracanal medication. Residual DNA may contribute to disease progression and/or maintenance. Furthermore, properties that enable bacteria to persist after therapy include resistance to disinfection by chemo-mechanical and intracanal medication procedures, and the ability to enter a viable but nonculturable (VBNC) state in response to stress.[13]

In the present study, DNA was measured using the NanoDrop system. It is a full-spectrum spec-

trophotometer for measuring the absorbance of DNA, RNA, protein, and dye, and it functions by combining fiber optic technology and natural surface tension properties for capture. The system employs shorter wavelengths, which result in a broad range of nucleic acid concentration measurements, essentially eliminating the need to perform dilutions. Moreover, molecular genetic studies hold the promise of identifying genetic factors that influence human disease susceptibility and outcome.[14] The accuracy and precision of DNA quantification are critical factors for efficient use of DNA samples in high-throughput genotype and sequence analyses.

Analysis of the endodontic microbiota is still focused on the detection and identification of bacteria using different methods, including culture[15] and PCR[4,8] techniques. PCR 16S rDNA assays represent the most sensitive method applied to the study of endodontic bacteria.[16] This technique can readily identify slow growing or uncultivable strains.[17] Among many potential amplification sites, 16S rDNA genes appear to be the most useful target of PCR 16S rDNA genes, are present in every bacterium and are highly conserved within a species.[18] It is noteworthy that all examined samples at the different timepoints contained bacterial DNA. These advantages of molecular methods also help to explain why bacteria were detected in all treated cases with post-treatment disease in this and other studies,[8-9] whereas culture studies have found bacteria in 44% to 85% of the cases.[8,15,19,20] For the present study, we used the PCR (16S rDNA) assay, which has the potential to offer more detailed insights into complex bacterial communities.[21] Furthermore, it seems interesting to evaluate the effects of endodontic procedures against these microorganisms, in case they are still present after root canal filling removal (S1), after the first chemo-mechanical preparation in the first appointment (S2), 5 days after the canal was filled with sterile physiological solution (S3), after re-instrumentation in the second appointment (S4), and 14 days after the intracanal dressing was placed in the third appointment (S5).

Our findings were confirmed by amplification with 16S rDNA universal primers, which generated the predicted amplicon for all samples (S1, S2, S3, S4, S5) from root-filled teeth with apical periodontitis. This assertion lends strong support to the claim that persistent intraradicular infection is the major factor associated with endodontic therapy failure.[3,8] On the other hand, it should be considered that not only the presence of the bacteria is an important factor for development or maintenance of disease, but also the population size.[22] Diagnostic methods using DNA-based tools allow the identification of viable but noncultivable cells that are metabolically active, but not dividing.[16] It is worth pointing out that the ability to detect DNA from dead cells poses a major problem when one is investigating the immediate effectiveness of antibacterial treatment because DNA from cells that have recently died can still be detected.[21] The possibility exists that DNA from dead cells may have been destroyed by the effects of the substances used during treatment.[23] Hydroxyl ions from calcium hydroxide also have oxidative damaging effects on DNA,[23] and may have contributed to the degradation of free DNA from dead cells. Failure to detect microorganisms does not necessarily mean that they are absent. Given the difficulties in taking samples from root-filled teeth, it is possible that many microorganisms can escape detection, particularly when they number below the detection rate of the identification method.[8] The PCR method used in this study only detects targeted microbial species. However, it is possible that species other than those studied could have been present in the examined teeth.

This molecular study also evaluated the reduction in bacterial DNA concentration promoted by intracanal disinfection procedures, and identified the 14 taxa persisting after each step. At S1, *Par-*

vimonas micra was the most frequent anaerobic species detected by PCR[24,25] followed by *Enterococcus faecalis*. The ecological niche of *Parvimonas micra* could be related to its wide range of peptidase activities, making amino acids and peptides available from serum glycoproteins.[26]

The present results also revealed the occurrence of some gram-negative bacteria after clinical procedures. Post-treatment apical periodontitis is almost always associated with intraradicular polymicrobial infection.[8,25] *Porphyromonas endodontalis* and *Parvimonas micra* are also considered as typical bacteria involved in endodontic infection.[25] *Tannerella forsythia*, *Porphyromonas gingivalis*, *Treponema denticola*,[27] *Fusobacterium nucleatum* and *P. gingivalis*[28] have also been described as common endodontic bacterial pathogens. These gram-negative bacteria, which are common members of primary intraradicular infections, are usually eliminated following treatment, though studies have reported that some anaerobic rods, such as *F. nucleatum* and *Prevotella* species, are among the most common species found in post-instrumentation samples.[3,4,10] At S1, many studies have detected gram-negative bacteria in root-filled teeth using PCR;[8,24,25] however, monitoring this species after clinical procedures is a necessity. The finding that several gram-negative species were found in samples (S2, S3, S4, and S5) might also indicate that bacterial persistence can be related to factors other than the intrinsic resistance to treatment procedures and substances by a specific taxon.

After endodontic procedures (S3, S4, and S5), *Enterococcus faecalis* was a species frequently detected. This species can colonize filled root canals and may be involved in endodontic failures.[8] It can survive in an environment in which there are scant available nutrients and in which commensality with other bacteria is minimal.[22] These bacteria, in particular *E. faecalis*, may also survive in the smear layer and other debris inside the root canal, and may be extremely difficult to remove by irrigation and instrumentation.[29] *E. faecalis* was found in a range of 0%–77% and is the predominant species in most of the studies of secondary infection.[8,15,24,30] In only three studies was *E. faecalis* not detected in persistent apical periodontitis.[4] Employing real-time PCR, *E. faecalis* was found to be three times more prevalent in refractory than in primary endodontic infections.[31] *E. faecalis* has already been previously found to endure endodontic treatment procedures in cases with necrotic pulp.[32] This finding is in line with studies showing that gram-positive bacteria might be more resistant to treatment procedures.[10] *E. faecalis* has been shown to have the ability to invade dentinal tubules[33] and adhere to collagen in the presence of human serum,[34] which can allow it to resist chemo-mechanical preparation. Clearly, more effective methodologies for disinfection must be established to eradicate this pathogen in the course of endodontic treatment.

The present study confirms that *E. faecalis* is resistant to endodontic procedures. At S2 and S4, this species could be detected after the chemo-mechanical preparation using 2% CHX gel, and, at S5, it could still be detected after using the combination of $Ca(OH)_2$ with 2% CHX gel for 14 days as intracanal dressing. Moreover, this species is resistant to calcium hydroxide,[35] a commonly used intracanal medicament. Combinations of $Ca(OH)_2$ with CHX[22] are used to enhance antimicrobial properties. All of these factors help to explain why *E. faecalis* is so prevalent in patients in whom endodontic treatment has failed.

When S3 and S4 were compared, there was a statistically significant reduction in DNA concentration after the second chemo-mechanical preparation. This corroborates the findings of several other studies[5,32] in which bacterial levels and the number of taxa were substantially reduced after chemo-mechanical preparation. During the treatment of infected root canals, two steps assume spe-

cial relevance with regard to bacterial elimination:

- the chemo-mechanical preparation and
- the interappointment dressing.

Studies have revealed that the chemo-mechanical preparation is not sufficient to predictably render root canals bacteria-free, with about 40%–50% of the prepared canals still containing cultivable bacteria.[5,6] To overcome the limitations of chemo-mechanical procedures in disinfecting the entire root canal system, the use of an inter-appointment medication has been advocated.[6,7] However, in the present study intracanal dressing was not able to eliminate or reduce the target species investigated and did not reduce DNA concentration when compared with the initial sample. The present results are in clear agreement with those of Blome *et al.*[25] They observed that a $Ca(OH)_2$ intracanal dressing placed for 14 days failed to achieve a further reduction of total bacterial counts when compared to the values observed immediately after chemo-mechanical preparation.[25] Moreover, this is also in agreement with the findings of Sakamoto *et al.*,[17] who found no significant difference between post-instrumentation samples and the samples collected after placement of the $Ca(OH)_2$ dressing, although these findings were associated with necrotic pulp tissue. The need for intracanal dressings is recognized especially in those cases where endodontic therapy cannot be successfully completed because of the presence of pain, constant exudation and lack of time.

CONCLUSION

A wide variety of bacterial species was detected in root-filled teeth with post-treatment apical periodontitis. Moreover, the use of an intracanal dressing was unable to further reduce the concentration of bacterial DNA.

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Avaliação, por meio da microtomografia computadorizada, do acúmulo de debris dentinários após o preparo do canal com um instrumento único reciprocante

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RESUMO | O objetivo do presente estudo foi avaliar e quantificar a presença de debris dentinários em canais curvos, após o preparo químico-cirúrgico com um instrumento único reciprocante, utilizando a microtomografia computadorizada (micro-CT). Vinte e quatro canais mesiais de molares inferiores foram submetidos a exames microtomográficos antes e após o preparo com instrumentos Reciproc R25, utilizando um microtomógrafo de raios X SkyScan 1176, a uma resolução de 17,42 µm. Após a reconstrução das imagens resultantes, o corregistro das mesmas foi realizado com o programa DataViewer. Os programas CTAn e CTvol foram utilizados para binarização dos objetos de interesse, análise volumétrica e reconstrução de modelos 3D. As análises de micro-CT revelaram debris dentinários acumulados no interior dos canais radiculares, ocupando uma porcentagem média de 3,4% em relação ao volume do canal. Concluiu-se que a micro-CT possibilitou identificar e quantificar debris dentinários produzidos após a instrumentação de canais mesiais de molares inferiores com um instrumento único reciprocante.

DESCRITORES | Microtomografia por Raio-X; Preparo de Canal Radicular; Camada de Esfregaço.

ABSTRACT | **Micro-computed tomography evaluation of hard-tissue debris accumulation after root canal preparation with a single-file reciprocating system** • The aim of the present study was to evaluate and quantify the presence of hard-tissue debris inside curved canals, after root canal preparation with a single-file reciprocating system, with the aid of microcomputed tomography (micro-CT). Twenty-four mesial canals of mandibular molars were subjected to microtomographic scanning before and after preparation with R25 Reciproc instruments, using the SkyScan 1176 X-ray microtomograph, at a resolution of 17.42 µm. After reconstruction of the resulting images, co-registration was accomplished with DataViewer software. The CTAn and CTvol softwares were used for binarization of the objects of interest, volumetric analysis and 3D model reconstruction. Analysis of the micro-CT scans revealed debris accumulated inside the root canal, occupying an average 3.4% of the canal's volume. It was concluded that micro-CT enabled identifying and quantifying the hard-tissue debris produced after instrumentation of mesial canals of mandibular molars with a single-file reciprocating system.

DESCRIPTORS | X-Ray Microtomography; Root Canal Preparation; Smear Layer.

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INTRODUÇÃO

O tratamento endodôntico tem por objetivo eliminar microorganismos, restos pulpares e necróticos, além de modelar o sistema de canais radiculares, a fim de facilitar os procedimentos de irrigação e obturação.[1]

Muitas vezes essa tarefa pode representar um desafio, em virtude da complexa anatomia do canal radicular, incluindo curvaturas, istmos, canais acessórios e ramificações apicais,[2,3] os quais facilitam o acúmulo de debris orgânicos e inorgânicos, formando o chamado magma dentinário.

O magma dentinário é uma consequência da instrumentação do canal, e sua permanência pode influenciar negativamente o tratamento, caso microorganismos remanescentes continuem a desenvolver-se em áreas não preenchidas pelo material obturador.[4]

Desde a introdução de técnicas de preparo do canal que utilizam instrumentos únicos, os sistemas com movimento reciprocante como Reciproc (VDW, Munique, Alemanha) tornaram-se populares.[5] Seu movimento consiste em uma rotação para a esquerda com o intuito de cortar dentina, e uma subsequente rotação horária impedindo que o instrumento se prenda às paredes do canal, o que aumentaria sua resistência à fadiga cíclica.[6] Em contrapartida, o movimento reciprocante parece permitir maior acúmulo de debris nas áreas mais retentivas do canal radicular.[7]

A irrigação com seringa e agulha ainda é o método mais tradicional utilizado para a irrigação dos canais radiculares, apesar de suas conhecidas limitações.[8] Diversos modelos experimentais são usados para avaliar a efetividade de técnicas de irrigação; contudo, a maioria envolve seções transversais ou longitudinais das raízes, fornecendo uma visão bidimensional, portanto limitada.[9,10] Além disso, alterações na localização dos debris podem ocorrer durante a manipulação dos espécimes.[11]

Superando essas dificuldades, a microtomografia computadorizada (micro-CT) apresenta-se como um método de pesquisa mais confiável e menos invasivo, que possibilita uma avaliação completa e detalhada do canal radicular, em todos os planos, e o registro de parâmetros morfológicos do interior de materiais sem destruí-los.[12,13]

Dessa forma, o objetivo do presente estudo foi avaliar e quantificar a presença de debris dentinários em canais mesiais de molares inferiores, após o preparo químico-cirúrgico com um instrumento único reciprocante, utilizando a microtomografia computadorizada.

MATERIAL E MÉTODOS

Após a aprovação do Comitê de Ética em Pesquisa da Faculdade de Odontologia da Universidade de São Paulo (FOUSP; protocolo n. 15598), vinte e quatro primeiros e segundos molares inferiores humanos cedidos pelo Banco de Dentes Permanentes Humanos da FOUSP foram selecionados.

Os dentes apresentavam câmara pulpar intacta, raízes mesiais com rizogênese completa, e ângulo de curvatura entre 25° e 35°, mensurados com o auxílio do programa Image J (National Institutes of Health, Maryland, EUA) e de acordo com o método de Schneider.[14] Foram excluídos os dentes com calcificações pulpares, reabsorções, tratamento endodôntico anterior e fraturas radiculares. Ainda, aqueles canais cuja patência não fosse conseguida após a cirurgia de acesso também foram excluídos do estudo.

O tamanho dos dentes foi padronizado em 17 mm, por meio do nivelamento de suas superfícies oclusais, utilizando um disco de diamante acoplado a um micrótomo de tecido duro (Extec Labcut 1010, Tóquio, Japão). Foi realizada a cirurgia de acesso convencional com brocas esféricas n. 1015 e Endo-Z (Maillefer, Ballaigues, Suiça) em alta rotação. Os canais foram explorados com um instrumento do tipo K #10 (Maillefer, Ballaigues, Suiça), e o comprimento de trabalho foi determinado quando a extremidade desse instrumento era vista através

do forame apical, com o auxílio de um microscópio operatório com aumento de oito vezes, recuando-se um milímetro. Nesse momento, também foram excluídos da amostra os canais em que o instrumento K #15 (Maillefer, Ballaigues, Suiça) estivesse folgado em seu interior.

Exames microtomográficos

Os exames microtomográficos foram realizados com um microtomógrafo de raios X SkyScan 1176 (SkyScan, Kontich, Bélgica). Cada espécime foi submetido a dois escaneamentos: um pré-operatório e outro pós-preparo. Os dentes foram incluídos em material de moldagem de alta precisão à base de vinil polisiloxano com as cavidades de acesso voltadas para baixo, com o intuito de obter um reposicionamento preciso do dente durante a aquisição das imagens pré e pós-operatórias. Em seguida, grupos de 7 dentes foram posicionados em um suporte para amostra (isopor tubular para modelo Skyscan 1176) e levados à cama de fibra de carbono do microtomógrafo. Os espécimes foram escaneados com uma potência de 90 kV e 278 μA, rotacionados a 360° com 0,5° de passo de rotação, produzindo uma imagem com tamanho de voxel de 17,42 μm. O filtro utilizado foi o de cobre + alumínio, e cada espécime levou em média 24 min e 40 s para completar o escaneamento.

Após a aquisição das imagens das projeções 2D, deu-se início à etapa de reconstrução das seções transversais, realizada no programa NRecon (SkyScan, Kontich, Bélgica).

Preparo químico-cirúrgico

Para a realização do preparo químico-cirúrgico, as raízes dos dentes foram cobertas com silicona de condensação com o objetivo de reter a solução irrigadora intracanal, evitando-se que a mesma extravasasse pelo forame apical.

Os canais mesiais foram pré-alargados com uma lima manual do tipo K #15. Em seguida os instrumentos Reciproc® R25 (VDW GmbH, Munique, Alemanha) foram utilizados, acionados com o motor elétrico VDW.Silver® (VDW GmbH, Munique, Alemanha), ajustado no movimento reciprocante. Os canais foram preparados por um único operador, de acordo com as instruções do fabricante: inserção lenta do instrumento no canal radicular em movimento de vai e vem e remoção após 3 avanços, com pouca pressão apical. Esse procedimento repetiu-se até o comprimento real de trabalho estabelecido.

A cada retirada do instrumento, a câmara pulpar era preenchida com gel de Endo PTC (Formula e Ação, São Paulo, SP, Brasil) e os canais eram irrigados com 2 mL de NaOCl a 1% (Formula e Ação, São Paulo, SP, Brasil). O comprimento de trabalho também era recapitulado nesse momento, com uma lima manual do tipo K #10. Foram utilizadas seringa descartável tipo luer lock (Ultradent, São Paulo, SP, Brasil) e agulha NaviTips tamanho 30 (Ultradent, São Paulo, SP, Brasil), posicionada o mais apicalmente possível sem que a mesma se prendesse às paredes do canal, sempre em movimentos de vai e vem.

Após o término do preparo químico-cirúrgico, foi realizada a irrigação final com 5 mL de NaOCl a 1%, seguida de 5 mL de EDTA a 17% (Formula e Ação, São Paulo, SP, Brasil) e mais 5 mL de NaOCl a 1%. A seguir, os canais foram aspirados com uma cânula suctora do tipo Capillary Tip (Ultradent, São Paulo, SP, Brasil) e secos com pontas de papel absorvente estéreis Reciproc R25.

A silicona de condensação foi então removida, e os dentes foram reposicionados no porta-amostra para a realização do escaneamento pós-preparo.

Metodologia de avaliação

Primeiramente, foi utilizado o programa DataViewer (SkyScan, Kontich, Bélgica) para o corregistro de dois conjuntos de imagens, um usado como referência (ref) e outro como alvo (tar), a fim de alinhar as imagens geometricamente (diff). O resultado foi um novo registro de dados das imagens

referência e alvo.

Em seguida, os conjuntos de imagens foram abertos no programa CTAn (SkyScan, Kontich, Bélgica) para o cálculo de parâmetros quantitativos e para a construção dos modelos visuais em 3D. Para cada espécime, a seção que mostrasse a separação total das entradas dos canais mesiais em relação ao canal distal, no nível da câmara pulpar, era escolhida como "top", e o primeiro corte apical em que fosse possível visualizar a raiz era selecionado como "bottom".

O novo volume de interesse era carregado novamente no programa CTAn, agora apenas com a raiz mesial, e o intervalo na escala de cinza necessário para reconhecer cada objeto a ser avaliado era determinado em um histograma de densidade, processo este denominado binarização ou segmentação. O resultado final foi uma imagem binária composta por pixels pretos que representam os espaços vazios (ar), ou brancos, que representam o objeto de interesse.

A seguir, a ferramenta *custom processing* era utilizada para um processamento personalizado das imagens, em que funções diversas e operações matemáticas eram executadas por meio da criação de *task lists*, ou listas de tarefas.

O resultado final foi a análise volumétrica e o modelo 3D de um determinado objeto apenas. Assim, foram criadas listas-padrão distintas para segmentar o canal radicular, a dentina e os debris dentinários.

O material com densidade similar à da dentina nas imagens pós-preparo, em regiões previamente ocupadas por ar no espaço do canal pré-operatório, foi identificado como debris e quantificado pela interseção entre as imagens antes e depois do preparo do canal (Figura 1).

Para o cálculo das porcentagens de debris, tomou-se como referência o volume do canal pós-preparo sem debris, obtido por meio da operação OR do plug-in *Bitwise Operations*. Assim, as imagens binárias do canal pré e pós-operatórios foram somadas, eliminado-se os debris.

A porcentagem de debris produzidos após o preparo foi calculada utilizando-se a seguinte fórmula:

$$\%\ debris\ pós\text{-}preparo = \frac{volume\ de\ debris\ pós\text{-}preparo \times 100}{volume\ do\ canal\ pós\text{-}preparo\ sem\ debris}$$

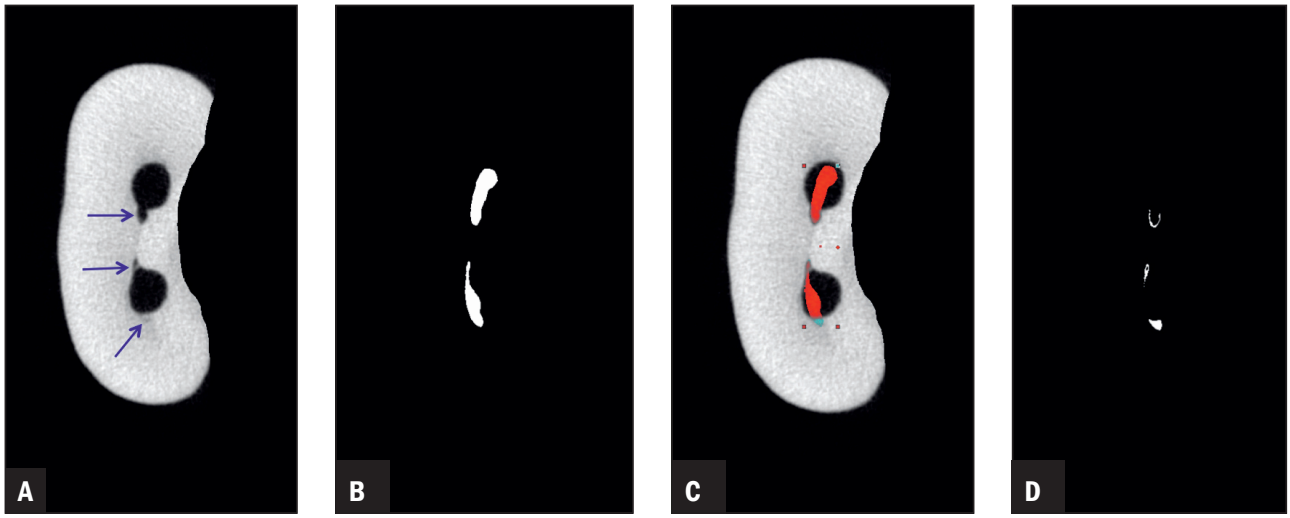

Figura 1 | Avaliação dos debris dentinários. **A:** Imagem original do canal pós-preparo; **B:** Imagem binária do canal no tempo pré-operatório; **C:** Sobreposição de A e B: intersecção em azul; **D:** Intersecção de A e B = debris binarizados: o material com densidade similar à da dentina nas imagens pós-preparo, em regiões previamente ocupadas por ar no espaço do canal no pré-operatório, foi identificado como sendo debris (setas azuis em A).

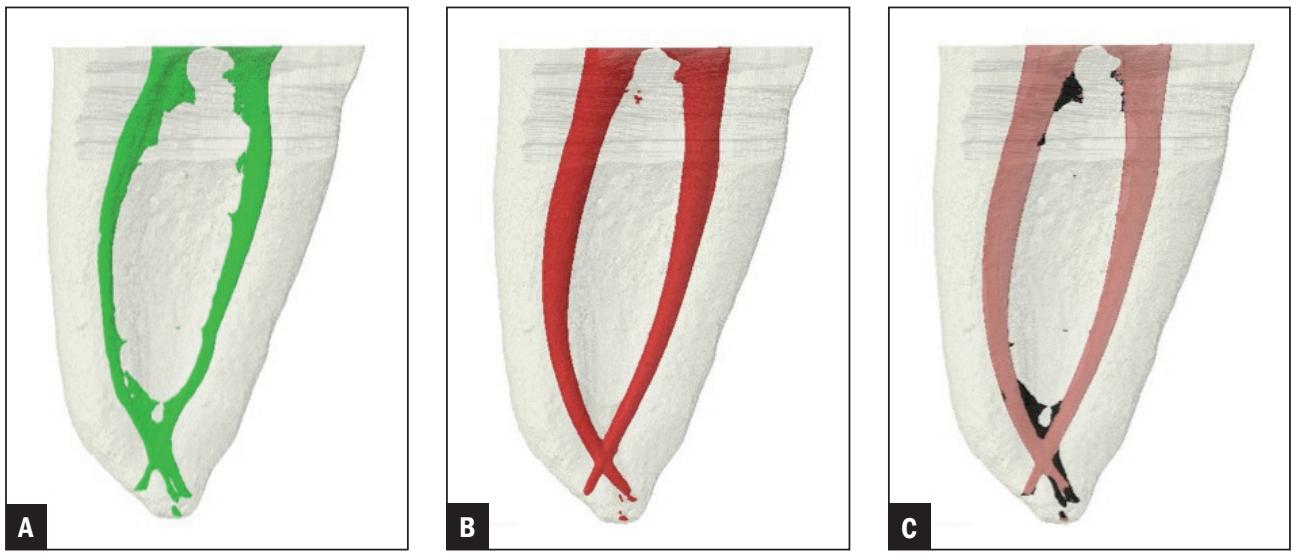

Figura 2 | Modelos 3D representativos. **A:** Canal radicular antes do preparo (verde); **B:** Canal radicular após o preparo (vermelho); **C:** Debris produzidos após o preparo (preto), sobrepostos à anatomia final do canal radicular (vermelho).

RESULTADOS

As análises de micro-CT revelaram ser possível identificar e mensurar o acúmulo de debris dentinários após o preparo de canais mesiais de molares inferiores. Os instrumentos Reciproc R25 produziram debris dentinários, os quais permaneceram no interior dos canais radiculares, ocupando, em média, 3,40% do volume do canal.

A avaliação qualitativa das reconstruções tridimensionais e secções transversais dos espécimes mostrou que a maioria dos debris acumulou-se nas áreas não instrumentadas, tais como istmos, projeções dos canais principais e ramificações.

A Figura 2 mostra o espaço do canal radicular antes do preparo (verde), após o preparo (vermelho) e os debris produzidos após o preparo do canal (preto). Na Figura 3, as linhas pontilhadas indicam a localização aproximada das secções transversais correspondentes.

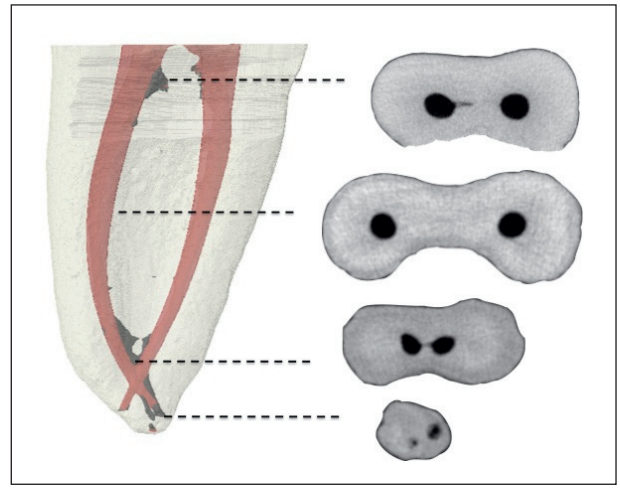

Figura 3 | Acúmulo de debris dentinários. As linhas pontilhadas indicam as secções transversais correspondentes.

DISCUSSÃO

A microtomografia computadorizada permitiu avaliar tridimensionalmente a anatomia do canal radicular original e o acúmulo de debris dentinários, mensurando o volume das regiões de interesse, sem qualquer manipulação dos espécimes. Talvez essa seja a maior vantagem desse método não-invasivo, que possibilita análises 3D de procedimentos sequenciais em um mesmo espécime, de modo quantitativo e qualitativo.

Essa tecnologia traz consigo ainda outros benefícios. Os dados armazenados podem ser trabalhados, gerando imagens de qualquer área de

interesse do dente para futuras análises comparativas.[15] Além disso, é considerada uma importante ferramenta educacional para o treinamento pré-clínico em endodontia.

Desde os primeiros relatos sobre a utilização da tomografia computadorizada para a visualização das imagens dos canais radiculares, avanços tecnológicos levando ao desenvolvimento de microtomógrafos, aliados a avanços nos softwares, aprimoraram a resolução das imagens, diminuindo também o tempo necessário para o escaneamento das amostras.[16]

Assim, a micro-CT abriu novas possibilidades e tornou possível aos pesquisadores mensurar parâmetros até então desconhecidos, como a alteração de volume do canal por diferentes protocolos de instrumentação,[15] detalhes da anatomia do canal radicular,[5] quantidade e distribuição das áreas da parede do canal não tocadas pelo instrumento,[17] volume de debris produzidos no canal radicular, e removidos dele, após o preparo,[12] volume de material obturador obtido por diversas técnicas de obturação,[18] volume remanescente de medicação de hidróxido de cálcio,[19] além de propiciar análises relativas a outros métodos de pesquisa.[20]

Os primeiros a validar um método para o estudo tridimensional do acúmulo de debris dentinários foram Paqué *et al.*[12] Eles identificaram como debris o espaço do canal original ocupado por material radiopaco após a instrumentação rotatória, encontrando um volume em torno de 29,1%. Em seguida, uma análise por espectroscopia de raios X por dispersão em energia (EDX) comparou a quantidade de cálcio e de fósforo desse material com a do tecido dentinário, confirmando que debris eram depositados no canal sob a forma de pequenas lascas de dentina. Vale salientar que, propositalmente, os autores não utilizaram nenhuma irrigação.

Nos anos seguintes, outros estudos foram realizados com micro-CT, comparando diferentes protocolos de irrigação.[7,21-25] Em todos eles, reconstruções tridimensionais de canais mesiais de molares inferiores mostraram o acúmulo de debris dentinários em regiões de istmos, ramificações e canais acessórios, assim como no presente estudo.

A literatura relata que a anatomia de molares inferiores é variada e que a incidência de istmos ocorre em todos os níveis da raiz mesial,[2] variando entre 17,25% e 50,25% nos 5 mm apicais.[3] Neste estudo, a análise da anatomia pré-operatória dos espécimes identificou irregularidades nas mais diversas posições ao longo do canal radicular.

Nas porções do canal onde a secção transversal é circular, normalmente os debris são levados coronalmente pelas lâminas do instrumento. Quando há espaço lateralmente, como no caso da presença de istmos, o instrumento pode empurrar os debris para essa região.[7]

Após a avaliação dos modelos 3D e das seções transversais das amostras, foi possível observar restos de tecido duro, não só nas áreas de istmos, mas também em irregularidades das paredes dentinárias ao longo do comprimento do canal, concentrando-se mais nos terços médio e apical. Nessas regiões, o espaço radiolúcido do canal se tornou rapiopaco, desaparecendo após a instrumentação.

Durante o preparo do canal, foi utilizado o protocolo de irrigação com NaOCl a 1%, gel de Endo-PTC e EDTA a 17%, resultando em uma média de debris acumulados na ordem de 3,4%. O uso dessas substâncias justifica-se pela capacidade de dissolução tecidual do NaOCl, combinado com um lubrificante que diminui o atrito do instrumento e facilita a suspensão dos detritos, e de um agente quelante que vai atuar na porção inorgânica do magma dentinário.[25]

Outros trabalhos que utilizaram a mesma metodologia de avaliação encontraram resultados com valores entre 3,8% e 11,35%.[21,23-25] No estudo de Robinson *et al.*,[7] no entanto, 19,5% de debris foram acumulados em canais preparados com instrumen-

to único reciprocante, um valor estatisticamente superior ao do grupo rotatório, em que se produziram 10,6% de debris. Todavia, eles não utilizaram nenhum agente quelante durante a irrigação final.

Por outro lado, Paqué *et al.*,[22] ao avaliarem um sistema de instrumentação que agrega irrigação simultânea por meio de um dispositivo acoplado no corpo do instrumento — a SAF (*self adjusting file*) —, obtiveram um volume menor de debris (1,7%). Paqué *et al.*[21] conseguiram uma redução em torno de 50% após a ativação da substância química com irrigação ultrassônica passiva.

Trabalhos utilizando diferentes metodologias concordam que, apesar de nenhuma técnica remover completamente os debris acumulados durante a instrumentação, os métodos que envolvem a ativação da substância química apresentam melhores resultados na remoção de restos de tecido orgânico e inorgânico.[9,10]

O principal motivo é atribuído à limitação do alcance da substância química para além da ponta da agulha,[3] e ao efeito "vapor lock".[26] Em uma situação clínica em que a raiz está delimitada pelo tecido perirradicular, o canal se comporta como um sistema fechado, causando esse efeito, o qual impede o irrigante de alcançar efetivamente o comprimento de trabalho. Portanto, para serem clinicamente relevantes, estudos que avaliam a irrigação devem simular um sistema fechado que previna a extrusão passiva de irrigantes. Essa foi a razão de se cobrirem as raízes dos dentes com silicona de condensação no presente estudo.

É consenso entre os autores que a não remoção do magma dentinário pode trazer consequências ao tratamento endodôntico. O acúmulo de debris dentinários possivelmente impede o fluxo de irrigantes, neutraliza a eficiência de medicamentos, interfere no preenchimento do canal radicular e mantém a sobrevivência microbiana dentro da complexa anatomia do canal radicular.[1,17,20]

Métodos utilizando equipamentos de última geração em microscopia óptica,[11] eletrônica ou confocal,[9,10] ainda que com validade indiscutível, possibilitam apenas a visualização de partes da superfície de uma amostra ou de cortes selecionados, não fornecendo, portanto, informações a respeito de volume.

Ainda, os procedimentos de seção dos espécimes podem resultar em deposição de magma,[3] levando a interpretações errôneas.[11,13] Também acabam limitando a utilização de dentes multirradiculares, por serem mais difíceis de manipular.

Avaliações tridimensionais por micro-CT indicam que nenhuma técnica é capaz de remover por completo restos de tecido duro acumulados. Assim sendo, resultados advindos de métodos bidimensionais que avaliam apenas uma parte do canal devem ser interpretados com cautela.

A micro-CT, como todo método de pesquisa, possui algumas limitações, identificadas neste trabalho e também por outros pesquisadores. A primeira é a impossibilidade de visualização de tecidos não mineralizados, por se tratar de uma tecnologia baseada em emissão de raios X. Especialmente para o estudo do magma dentinário, o ideal seria uma metodologia capaz de detectar também a matéria orgânica.

Estudos anteriores com micro-CT também reportaram dificuldade em distinguir debris dentinários e a dentina radicular, devido à radiopacidade similar.[12,25]

Acreditamos que essa dificuldade existe, mas pode ser superada seguindo todos os passos que envolvem a análise de imagens microtomográficas, de maneira criteriosa.

Ainda, a definição das imagens a serem estudadas depende da resolução do aparelho. No presente trabalho, algumas regiões de istmo muito estreitas, apesar de visualizadas nos cortes axiais, não puderam ser submetidas à análise e reconstrução 3D. Vale salientar que, quanto melhor a resolução, mais tempo será necessário para a aquisição das

imagens, reconstrução e análise, o que pode inviabilizar o estudo.

Pode-se citar também a necessidade de utilização de softwares de difícil entendimento e manipulação,[27] podendo levar a alguns erros processuais que se refletem nas medidas finais.[25] Ainda, tem-se o alto custo, dado o valor do microtomógrafo, dos pacotes de programas de computador e do treinamento, além de ser um processo trabalhoso, exigindo uma curva de aprendizado prolongada para obter os conhecimentos necessários.

Todos os canais utilizados foram instrumentados com instrumentos Reciproc R25, que operam em modo reciprocante e são indicados para canais estreitos e curvos. Eles possuem ponta de 25 mm e conicidade de 0,08 mm/mm nos últimos 3 mm, o que parece ser adequado para conseguir um fluxo hidrodinâmico dos irrigantes.[7]

Pesquisas mostram resultados favoráveis obtidos com o uso de instrumentos únicos em movimento recíprocante, salientando-se um menor tempo de trabalho,[5] um aumento da resistência à fadiga cíclica[6] e uma capacidade de modelagem similar a de uma sequência completa de instrumentos rotatórios.[20] No entanto, a qualidade da limpeza conseguida com esses instrumentos é controversa. Bürklein *et al.*[5] encontraram boa capacidade de limpeza em canais curvos. Por outro lado, Robinson *et al.*[7] reportaram mais acúmulo de debris em comparação com o sistema ProTaper.

Em conclusão, a instrumentação do canal radicular traz, como consequência evidente, o acúmulo de debris dentinários, independentemente da técnica ou instrumento utilizados. A micro-CT mostrou-se como uma excelente ferramenta metodológica para identificar e quantificar os debris remanescentes após o preparo do canal. Estudos futuros avaliando técnicas de limpeza e remoção de debris dentinários devem ser preferencialmente conduzidos com auxílio de métodos que permitam uma avaliação tridimensional como a micro-CT.

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Dimensional changes in lateral pterygoid muscles and disc position during mandibular movement using magnetic resonance imaging

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ABSTRACT | The lateral pterygoid muscle (LPM) has been the focus of numerous studies attempting to elucidate its possible role in temporomandibular disorders (TMDs). Disc displacement is widely accepted as a finding characteristic of this clinical condition. However, few studies have investigated the association between disc position and morphological alterations of the LPM. *Objectives:* to investigate the relationship between articular disc position and area measurements of the superior head (SH) and inferior head (IH) of the LPM using magnetic resonance (MR) imaging. *Methods:* The sample comprised 148 temporomandibular joints (TMJs) of 74 patients with complaints of pain and/or dysfunction in the TMJ area. Sagittal plane images were used for assessments of disc position and for tracings. Tracings of the areas of the heads were performed under 4 mandibular positions (at rest, and openings of 10 mm, 20 mm, and 30 mm) with the aid of image processing software. Data acquired was subjected to statistical analysis. *Results:* Statistical tests revealed changes in LPM head areas during mandibular opening movement, showing a reduction in total IH area and more heterogeneous changes in SH area. *Relevance:* The IH mean area was reduced in the positions assessed and showed no correlation with disc displacement. For the SH, reduced mean area was associated with anterior disc displacements without reduction, while increased mean areas were correlated with anterior disc displacement with reduction.

DESCRIPTORS | Magnetic Resonance Imaging; Temporomandibular Joint; Pterygoid Muscles.

RESUMO | **Avaliação por ressonância magnética da alteração dimensional do músculo pterigóideo e da posição do disco articular no movimento mandibular** • O músculo pterigóideo lateral (MPL) tem sido o foco de inúmeros estudos que tentam elucidar o seu possível papel na disfunção temporomandibular, condição na qual o deslocamento do disco é amplamente aceito como um possível aspecto clínico. No entanto, poucos estudos investigaram a associação entre a posição do disco e alterações morfológicas do MPL. *Objetivos:* investigar a relação entre a posição do disco articular e a área da porção superior e da porção inferior da músculo pterigóideo lateral usando ressonância magnética. *Métodos:* A amostra foi composta por 148 articulações temporomandibulares de 74 pacientes com queixa de dor e/ou disfunção articular. Imagens em plano sagital foram utilizadas para a avaliação da posição do disco e para traçados. Traçados das áreas do músculo foram realizados em 4 posições mandibulares (em repouso, e em aberturas de 10 mm, 20 mm e 30 mm) com a ajuda de software de processamento de imagem. Os dados adquiridos foram submetidos à análise estatística. *Resultados:* Os testes estatísticos revelaram mudanças nas áreas superior e inferior do músculo pterigóideo lateral durante o movimento de abertura mandibular, mostrando uma redução na área total e mudanças mais heterogêneos na área superior. *Relevância:* Observou-se que a área média da porção inferior muscular estava reduzida nas posições avaliadas e não mostrou correlação com deslocamento de disco. Para a porção superior, a redução da área média foi associada com o deslocamento anterior do disco sem redução, ao passo que o aumento da área média foi correlacionado com o deslocamento anterior do disco com redução.

DESCRITORES | Imagem por Ressonância Magnética; Articulação Temporomandibular; Músculos Pterigoides.

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INTRODUCTION

Studies on the anatomy and function of the masticatory muscles have had an important influence in dental clinical practice.[1] The lateral pterygoid muscle (LPM) is directly attached to the temporomandibular joint (TMJ), and is one of the key muscles in the physiology of mastication, participating in opening, closing, lateral and protrusive mandibular movements.[2]

The LPM consists of two parts, the superior head (SH) and inferior head (IH). Some fibers from the SH may insert into the anterior portion of the articular capsule and articular disc of the TMJ.[3] Although temporomandibular disorders (TMDs) are multifactorial, it is widely accepted that disturbances in lateral pterygoid muscle activity play an important role in the etiology of these disorders.[4] However, there is currently no rigorous scientific evidence correlating the two conditions.[1] Moreover, the role of LPM in normal functioning remains controversial.[1]

Magnetic resonance (MR) imaging is considered the gold standard for evaluating alterations in the TMJ and masticatory muscles. The technique is able to provide morphological and positional information of the articular disc through high resolution images of soft tissue without exposing patients to radiation.[2-5]

Although the LPM has been extensively studied in recent years, its anatomy and physiological and clinical implications are not fully understood.[4]

The aim of this study was to determine the relationship between articular disc position and area measurements of the SH and IH of the LPM using MR imaging.

MATERIALS AND METHODS

This study evaluated MR images of 148 TMJs, drawn from the files of 74 patients (51 female and 23 male, 19 to 78 years old) submitted to scans after indication by their physician and/or dentist. The inclusion criterion was the presence of at least one sign and/or symptom of TMD reported during clinical examination.

Scans were performed on a Sigma MR device (GE Medical Systems, Madison, WI, USA) at 1.5 tesla electromagnetic power using transverse relaxation time (T2) and proton density (PD)-weighted scan sequences with a 20 cm diameter dual surface coil (GE Medical Systems, Madison, WI, USA). Images were acquired on the sagittal plane using 3 mm-thick slices (7 slices per joint), a 0.3 mm reconstruction interval with 1500 ms relaxation time, and stored in Digital Imaging and Communication in Medicine (DICOM) format. The images obtained were assessed using a workstation running a high-resolution graphics processing program (Easy Vision, Philips Medical Systems, Best, The Netherlands).

The joints were assessed, bilaterally, in closed-mouth position and at openings of 10 mm, 20 mm and 30 mm. Mouth-opening positions were established with the aid of an intraoral plastic stabilizer (GE Medical Systems, Madison, WI, USA) bearing a scale in millimeters.

The sagittal PD slices chosen to trace SH and IH areas of the LPM and assess the disc positions were those offering the best visualization of the respective points of insertion and origin of the muscles and their relationship with the articular disc. These areas were measured independently by two examiners at different times, the first a physician and the second a dentist, both of whom were radiologists with at least ten years' experience. The areas of the LPM were traced as follows.

- SH: the boundaries between the fibers and the cortical bone of the infratemporal fossa and lateral perpendicular lamina of the sphenoid bone were outlined, descending tangentially to the fibrous borders of the upper part of the IH, following the fatty tissue or cord which separates the two heads up to the anterior segment of the articular disc;
- IH: the outermost boundaries of its fibers in re-

lation to the SH and to the fatty cord separating the two heads, projecting anteriorly up to the lateral and inferior bellies, were outlined, following the downward curve to the borders of its insertion into the pterygoid fovea of the condyle.

For enhanced visualization of the outline and borders of the effective head areas, color was introduced (Figure 1, A and B).

Disc status was classified as follows.

- Normal: the disc is located in the upper portion of the head of the mandible;
- Disc displacement with reduction (DDwR): the disc is displaced in the closed-mouth position and normal in the open-mouth position;
- Disc displacement without reduction (DDwoR): the disc is displaced in both open- and closed-mouth positions.

The study was approved by the Research Ethics committee, School of Dentistry, University of São Paulo, under protocol no. 80/04.

All data regarding the areas of SH and IH of the LPM and disc positions were treated with descriptive statistics and analysis of variance (ANOVA), adopting a 5% margin of error as the significance level. Statistically significant results were submitted to the Tukey test. Statistical analyses were performed using the Statistical Package for Social Sciences (SPSS for Windows, version 11.0, Chicago, IL, USA).

RESULTS

Analysis of the data showed that, regarding gender distribution of the 148 TMJs of 74 patients, 51 (68.9%) were female and 23 (31.1%), male.

With regard to gender and age interaction, the data showed a predominance of both genders in the 30–39 year age group and of female gender in the 40–49 year age group.

Regarding disc status, 94 TMJs (63.5%) were normal, 34 (23%) presented DDwoR, 19 (12.8%) presented DDwR, and 1 (0.7%) presented posterior disc displacement.

Analysis of the relationship between mean head areas (SH and IH) and articular disc position revealed that the highest means for the SH were observed in DDwR joints. A progressive increase was observed in these mean areas from at rest to maximum opening (30 mm) positions. The lowest mean areas of the SH were observed in DDwoR joints (Figure 2, A and B, and Table 1). For IH, the highest mean areas were observed in DDwR joints at

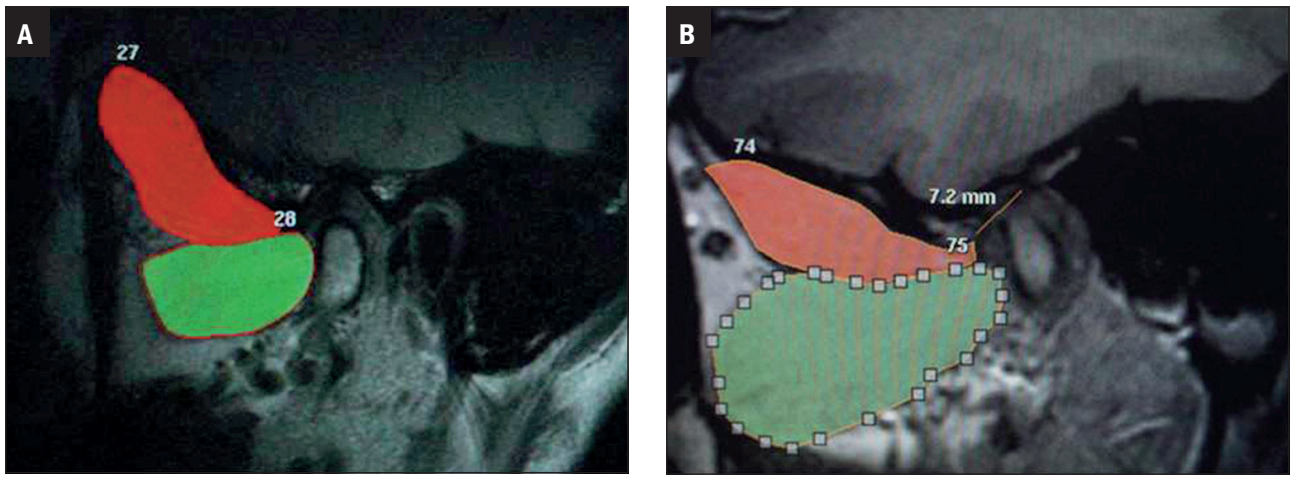

Figure 1 | Coloring of SH (orange) and IH (green) areas: delimitation and presentation of morphological characteristics of the pterygoid muscle and its relationship with articular disc and pterygoid fovea.

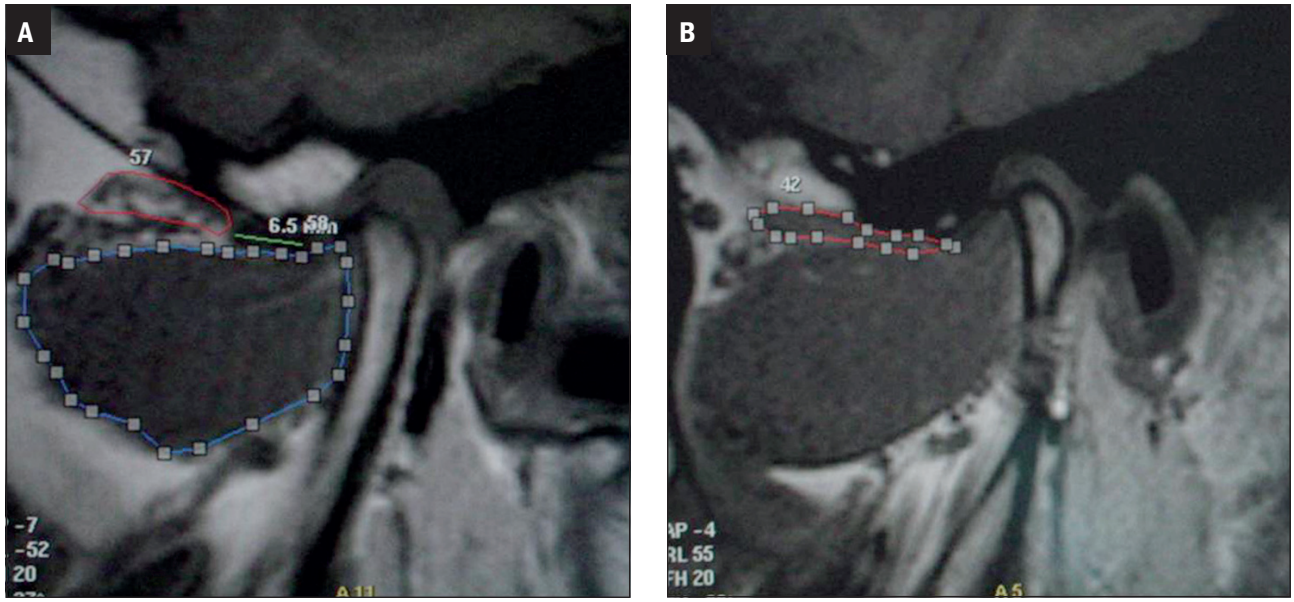

Figure 2 | LPM evidencing reduced SH area and changes in disc position.

Table 1 | Means and standard deviations for area (mm²) of the SH of the LPM.

Group	Position			
	At rest	10 mm	20 mm	30 mm
Normal	154.7 (61.0)	163.8 (63.1)	159.3 (61.5)	160.5 (64.1)
Anterior DDwR	165.8 (66.7)	168.0 (59.5)	172.4 (62.4)	188.5 (78.5)
Anterior DDwoR	133.2 (38.1)	130.3 (39.4)	134.1 (46.5)	135.4 (51.8)

Table 2 | Means and standard deviations for area (mm²) of the IH of the LPM.

Group	Position			
	At rest	10 mm	20 mm	30 mm
Normal	312.4 (109.5)	308.5 (102.8)	299.2 (96.2)	297.3 (100.5)
Anterior DDwR	364.7 (115.2)	385.1 (111.7)	334.4 (110.8)	328.5 (108.2)
Anterior DDwoR	335.5 (116.0)	320.6 (123.9)	313.6 (116.2)	311.0 (112.7)

rest and intermediate opening of 10 mm, while decreasing values were seen at openings of 20 mm and 30 mm. Decreasing mean areas were found among TMJs with normal disc and DDwoR positions, with the latter showing the highest mean values (Table 2).

DISCUSSION

There has been much speculation over the role of the LPM in TMD.[4] It is believed that this disorder may stem from LPM dysfunction or from poor co-ordination between the two heads.[1,6] However, there is currently no compelling scientific evidence that LPMs of TMD patients are functionally disturbed.[1]

The SH of the LPM has been the focus of numerous studies in a bid to explain the problems associated with the TMJ and anterior displacement of the disc.[3,6] Although electromyographic studies have

demonstrated a relationship between the insertion pattern of the LPM directly into the articular disc and DDwR, the role of LPM in TMJ dynamics remains unclear.[3,4,7] One reason for this incomplete understanding is that many studies on this muscle have been based on the dissection of human cadavers.[8,9]

The pattern of insertion of fibers of the SH of the LPM into the condyle-disc complex is highly variable in the literature. While some studies have found a greater volume of SH fibers attached to medial aspects of the articular capsule and anterior aspects of the disc,[10,11] others have observed SH fibers inserted directly into the pterygoid fovea or fused with IH fibers, and absence of muscle insertions directly into the discs.[8,12] In the present study, most LPMs had fibers inserted into the articular disc and anterior portion of the articular capsule, with some fibers inserted into the pterygoid fovea of the neck of the mandible. Both heads were clearly observed in all MR image sequences analyzed, showing that this diagnostic method is effective for assessing LPM, corroborating previous reports.[2,5]

There are few investigations involving dimensional studies of the masticatory muscles or assessing the relationship between dysfunction or instability of LPM heads and disc displacement, hampering comparison and discussion of results.

This study evaluated the mean areas of LPM heads measured during mandibular movements and examined their relationship with disc position. A reduction in IH area was noted during mouth opening, predominantly from the 20 mm to the 30 mm openings, irrespective of disc position, corroborating findings of other authors.[5,13] With regard to the SH, a variation in means was observed for the three types of disc position assessed (normal, DDwoR and DDwR), with the largest means being observed in DDwR joints. This increased area associated with DDwR may be indicative of muscle hyperactivity or some compensation overload. However, further morphological and quantitative studies are needed to clarify the relationship between these area changes and disc displacement, and to confirm whether this plays a role in TMD.

Based on our results, it can be concluded that the IH of the LPM exhibited reduced mean areas during the movement of mouth opening and showed no correlation with disc displacement status. Regarding the SH, reduced mean areas were associated with anterior disc displacement without reduction, while increased mean areas correlated with anterior disc displacement with reduction.

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CLRD in Clinical and Laboratorial Research in Dentistry | Oral Radiology

Prevalence of the developmental bone defect of the mandible in cone-beam computed tomography scans

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ABSTRACT | The developmental bone defect of the mandible is a bone cavity presenting as a well-defined, radiolucent lesion, located in the posterior region of the mandible, just below the inferior dental canal and above the mandibular base. It is asymptomatic, has a greater predilection for males, and a prevalence between 0.1% and 0.48%. The aim of this study was to conduct a review of the literature on the prevalence of this bone defect and compare the literature data to that of an assessment conducted of routine cone-beam computerized tomography (CBCT) scans from a radiological clinic. The use of diagnostic resources such as cone-beam volumetric tomography was also highlighted. CBCT routine scans taken from July 1st, 2012 to September 27, 2012 were retrieved from the digital archives of a private dental radiology clinic and evaluated, for a total of 1,344 CBCT images. Stafne's cavity was observed In 22 cases (0.16%). Among the 19 male cases, 15 were Type I, 3 were Type II and 1 was Type III, according to Ariji's classification.[5] All of the 3 female cases (the male-to-female ratio was 6.33:1) were Ariji Type I. The findings of this study were consistent with those from the literature consulted, in that the highest prevalence rates were observed for unilateral, Ariji Type I lesions and in the male gender.

DESCRIPTORS | Odontogenic Cysts; Cone-Beam Computed Tomography; Prevalence.

RESUMO | **Prevalência do defeito ósseo de desenvolvimento da mandíbula em exames de tomografia computadorizada por feixe cônico** • O defeito ósseo de desenvolvimento da mandíbula (DODM) é uma cavidade óssea que se apresenta como uma lesão radiolúcida de limites definidos e corticalizados. É assintomático, apresenta uma maior predileção pelo gênero masculino, e tem uma prevalência de 0.1% a 0.48%. O objetivo deste estudo foi realizar uma revisão de literatura com o intuito de ampliar o conhecimento a respeito da prevalência desse defeito ósseo e comparar os dados da literatura com os obtidos por meio de uma avaliação de 1344 exames de rotina realizados por meio da tomografia computadorizada de feixe cônico (TCFC) recuperados dos arquivos de uma clínica privada de radiologia odontológica. As imagens foram analisadas no período de 1º de julho de 2012 a 27 de setembro de 2012. Foram observados 22 casos de DODM (0,16%), sendo que 19 casos eram do gênero masculino, totalizando 15 casos do Tipo I, 3 casos do Tipo II e 1 caso do Tipo III de Ariji.[5] Foram também encontrados 3 casos do gênero feminino, todos classificados como Tipo I de Ariji, equivalendo a uma proporção entre os gêneros masculino e feminino de 6,33:1. Os resultados encontrados no presente estudo foram compatíveis com os relatados na literatura consultada. Constatou-se uma maior prevalência de lesões unilaterais, Tipo I de Ariji e envolvendo o gênero masculino.

DESCRITORES | Cistos Odontogênicos; Tomografia Computadorizada de Feixe Cônico; Prevalência.

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INTRODUCTION

The developmental bone defect of the mandible (DBDM), or Stafne's bone cyst, is seen radiographically as a radiolucent and circular or oval image, with defined and corticalized limits, very similar to those of an odontogenic cyst. However, the limits of the DBDM are more dense and thick, as first described by Stafne.[1] He observed 35 bone cavities located bilaterally near the angle of the mandible, between the canal and the base of the mandible. This condition is usually seen in patients who are in their 5th or 6th decade of life, and the prevalence of this radiographic finding is in the 0.10%–0.48% range. Patients are usually asymptomatic and the defect is often diagnosed by routine radiography.[2,3] Male patients aged 50–70 years are most affected.[2,4]

Ariji *et al.*[5] analyzed CT scans of 15 patients (16 cavities) with Stafne's bone cyst and classified the bone cavities according to their margins, relationship with the buccal cortical bone, and internal content. In the images, the deepest portion of the cavities either did not reach the buccal cortical bone (Type I), reached the buccal cortical bone, but not causing their expansion (Type II), or did so causing their expansion (Type III). Regarding their internal content, three classifications were also made according to tissue characteristics:

- fat density (F type),
- soft tissue density (S type), and
- gland within the cavity or close to it (G type).

Cone-beam computed tomography (CBCT) is a useful diagnostic tool and allows to confirm the initial radiographic findings obtained by conventional radiography, e.g. extraoral panoramic radiography.[3,6,7]

OBJECTIVES

In this context, the aim of this study was to conduct a literature review to allow a better understanding of the etiology and epidemiology of this bone defect and to assess its prevalence among 1,344 CBCT scans retrieved from the files of a private dental radiography clinic.

METHOD

Images from 1,344 routine CT scans obtained with an iCat cone-beam CT scanner (Imaging Sciences, Hartfield, USA) and retrieved from the digital files of a private dental radiography clinic were analyzed to search for the presence of Stafne's bone defect.

The images were analyzed from July 1st to September 27, 2012. No criteria for inclusion or exclusion in the sample were applied in this observational study.

RESULTS

Among the digital files analyzed in this study (1,344 CBCT scans), 22 (0.16%) cases of DBDM were observed in male (19) and female (3) patients, with a male:female ratio of 6.3:1 (86.36% versus 13.64%). Images of DBDM were observed on the right (14) and left (8) sides. Among male patients, the defect was observed on the right (12) and left (7) sides, distributed into Ariji[5] Type I (with a right:left ratio of 9:6), Type II (2:1), and Type III (1:0). Among female patients, the defect was observed on the right (2) and left (1) sides, and was Ariji[5] Type I (2:1) in all cases (Table 1 and Figure 1).

DISCUSSION

The incidence of the posterior variant of the developmental bone defect of the mandible (DBDM) is in the 0.10%–0.48% range.[8-13] This large incidence rate variation found in the literature has been attributed to difficulty in radiographically identifying this entity.[10] In the present study, 1,344 CBCT scans were analyzed. Among them, 22 cases of Stafne's bone cyst (0.16%) were found, in agreement with previously reported findings. Sisman *et al.*[10] analyzed 34,221 panoramic radiographs and found 29 Stafne cysts (0.08%), a rate slightly lower than that

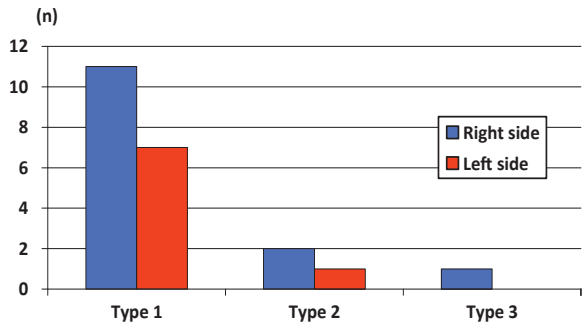

Figure 1 | Distribution of CBDM types into right and left sides.

Table 1 | Distribution of CBDM cases into genders and types.

Genders	Sides		Total
	Right	Left	
Male	12	7	19
Type 1	9	6	15
Type 2	2	1	3
Type 3	1	0	1
Female	2	1	3
Type 1	2	1	3
Type 2	0	0	0
Type 3	0	0	0
Total	14	8	22

previously reported.

Stafne's bone cysts occur more commonly in males. Philipsen *et al.*[9] reviewed the literature extensively and found a male:female ratio of 6:1. Quesada-Gómez *et al.*[8] reported 11 cases of Stafne's bone cyst, of which 8 were from male patients. Schneider *et al.*[4] found a male:female ratio of 14:7, and all cavities were in the posterior region of the mandible. This study reports 19 male and only 3 female patients. The male:female proportion (6.3:1) is in agreement with that reported in previous studies showing a higher prevalence in males. Regarding age range, most studies reported that cavities were found in patients over 20 years of age, with the average located in the 50–60 year range.

The etiology of this cavity remains unclear.[4] Various theories have been proposed, and Stafne *et al.*[1] suggested a possible failure in the process of ossification of Meckel's cartilage. This theory is controversial,[14] and the presence of this bone cavity in patients younger than 10 years old has not been reported so far. Most studies suggest the presence of normal or inflamed glandular histological tissue[5,8,10,15,16] within the cavity. Minowa *et al.*[14] analyzed the bone cavities of 4 patients using CT and correlated these results with those of histopathology. The study revealed the presence of adipose tissue and many dilated vessels inside the 4 cavities, but no tissue related to submandibular glands, indicating its vascular origin. Another 10 cases were analyzed[17] using CT and MRI, and no glandular tissue was found inside the bone cavity, only blood vessels and other soft tissues. Sisman *et al.*[10] suggest that the variety of tissues found in bone cavities may be due to the removal of surrounding soft tissues. Barker[18] used sialography and reported a case in which the bone cavity was closely related with salivary and parotid gland tissues.

Since the DBDM is an anatomical depression and not a pathological cavity, it requires no treatment, just radiographic control,[8-10,15] as described in the study by Dereci and Duran[19] and Herranz-Aparicio *et al.*[3] According to these authors, CBCT is the most suitable diagnostic examination because it is noninvasive and allows visualization of the lingual cavity. Our study showed that use of CBCT allows both the obtaining of more detailed images and the effective observation of the DBDM. The cavity may not be perceived with the use of panoramic radiography when it is out of the image layer or its size is reduced.

CONCLUSION

The use of CBCT allowed locating the DBDM, detailing its characteristics, and determining its classification according to the types described by Ariji.[5] Our findings are in agreement with those of

the literature, in that the male gender, Ariji[5] Type I and unilateral presentation were the most prevalent occurrences. New studies on the DBDM must be conducted with larger samples and in other geographic regions (multicenter studies) to confirm its prevalence in different populations.

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CLRD in Clinical and Laboratorial Research in Dentistry | Oral Radiology

Influence of voxel size on the accuracy of linear measurements taken in CBCT images

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ABSTRACT | *Objectives:* Cone-beam computed tomography (CBCT) has brought innovation to imaging examination methods, and has shown great potential in the maxillofacial area. The aim of this study was to evaluate the influence of different resolution values on the accuracy of linear measurements performed using a CBCT system. *Methods:* Seven pig mandibles were used in this study. Measurements were taken of the mesiodistal distance of the right and left first molars. The mandibles were scanned using an iCAT CBCT apparatus and different voxel sizes, namely 0.125 and 0.25 mm. The images thus obtained were visualized using Xoran CT software, and the linear measurements were taken at different times by two examiners who used the software's electronic ruler. A digital caliper was used to perform the measurements on the anatomical specimens. The data were analyzed statistically. *Results:* The results indicated no statistically significant difference between the measurements obtained by both examiners. As for the methods used in this study, no statistically significant difference was observed between the measurements obtained with the digital caliper and those obtained using CBCT. Similarly, no significant difference was observed between the measurements obtained using the different CBCT protocols. *Conclusion:* There is no significant difference between linear measurements obtained from CBCT images with 0.125 or 0.25 mm voxel sizes.

DESCRIPTORS | Radiology; Cone-Beam Computed Tomography; Voxel.

RESUMO | **Influência do tamanho do voxel na acurácia de medidas lineares obtidas em imagens de TCFC** • *Objetivo:* A tomografia computadorizada de feixe cônico (TCFC) introduziu uma inovação nos métodos de exame por imagem e tem apresentado grande potencial na área maxilofacial. O objetivo deste estudo foi avaliar a influência de diferentes resoluções (voxels) sobre medidas lineares obtidas utilizando-se a TCFC. *Método*: Foram utilizadas 7 mandíbulas suínas neste estudo. Foram obtidas medidas da distância mésio-distal dos primeiros molares direito e esquerdo. As mandíbulas suínas foram escaneadas num aparelho iCAT com voxel de 0,125 e 0,25 mm. As imagens obtidas foram visualizadas no software Xoran CT, e as medidas lineares foram realizadas por dois examinadores em tempos distintos com o auxílio da régua eletrônica do software. Os mesmos utilizaram um paquímetro digital para a medição realizada nas peças anatômicas. Os dados foram submetidos à avaliação estatística. *Resultados:* Os resultados apontam não haver diferença estatisticamente significativa entre as medidas obtidas por ambos os avaliadores. Com relação aos métodos empregados, não se observou diferença estatisticamente significativa entre as medidas obtidas com o paquímetro digital e com a TCFC. Da mesma forma, não se observou diferença significativa entre as medidas obtidas utilizando-se os diferentes protocolos de TCFC. *Conclusão:* Não há diferença significativa entre medidas lineares de imagens de TCFC obtidas com tamanho de voxel de 0,125 e 0,25 mm.

DESCRITORES | Radiologia; Tomografia Computadorizada de Feixe Cônico; Voxel.

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INTRODUCTION

Cone-beam computed tomography (CBCT) has brought innovation to imaging examination methods. This technique has shown a great potential in the maxillofacial area, with faster acquisition of volumetric images and lower radiation doses compared to those used in helical computed tomography (CT).[1-3]

CBCT can be applied in several fields of dentistry, including implant dentistry, surgery, and oral diagnosis.[1] However, further evaluation of the accuracy of linear measurement data is necessary to expand its application.[1-3] Diagnosis and treatment of facial asymmetries require precise measurements of various anatomical sites. Therefore, conventional radiographs are not recommended due to the effects of geometric projection and horizontal and vertical magnification.[2,4]

Image accuracy has been confirmed for several different CBCT devices. In addition, it was observed that the accuracy of images may be influenced by factors such as voxel resolution.[3,5] In CBCT images, volume is represented by voxels, and each voxel is assigned a value in the gray scale, which, in turn, represents the radiographic density of the corresponding structure. A reduction in voxel resolution can result in a low-quality image, with more artifacts and less detailed anatomical information than that of the real structure.[3,5]

Therefore, the aim of this study was to evaluate the influence of different voxel resolutions on the accuracy of linear measurements taken in CBCT images.

MATERIALS AND METHODS

This study was approved by the Ethics Committee for Animal Use (CEUA, in Portuguese), Institute of Biomedical Sciences, University of São Paulo (protocol No. 052/2012).

Seven dry pig mandibles were used, and all animals came from the same slaughterhouse. Age, gender, and date of slaughter of the animals were not considered. Measurements were taken of the mesiodistal distance of the right and left first molars (two measurements per mandible), for a total of 14 measurements.

The mandibles were scanned in a CBCT unit (i-CAT Vision CB500; Gendex Dental Systems, Hatfield, PA, USA) using the following parameters for the X-ray source:

- 120 kV;
- variable current (mA; regulated by the equipment according to the exposure necessary to achieve the volume to be analyzed);
- 360° rotation;
- focal area of 0.5 mm;
- Flat Panel-type sensor (amorphous silicon; 13 × 13 cm);
- 14-bit grayscale;
- FOV of 8 × 8 cm;
- voxel sizes of 0.25 and 0.125 mm; and
- scanning time of 23 s.

The mandibles were positioned following the reference lines of the equipment: the reference line was centered on the mandibular symphysis (Figure 1) and positioned on the most eminent mental foramen (Figure 2).

The images thus obtained were visualized using Xoran CT software. Two examiners (AV1 and AV2) performed the linear measurements (sagittal sections; 200% magnification) at different times, using the software's electronic ruler (Figures 3 and 4).

The same examiners used a digital caliper (Coolant Proof Absolute; Mitutoyo Sul Americana Ltda, São Paulo, SP, Brazil) for measuring the same distances described above on the anatomical specimens.

The examiners' procedures were standardized for both software tool and digital caliper.

All values were transferred to an electronic spreadsheet (Microsoft Excel, Microsoft Office,

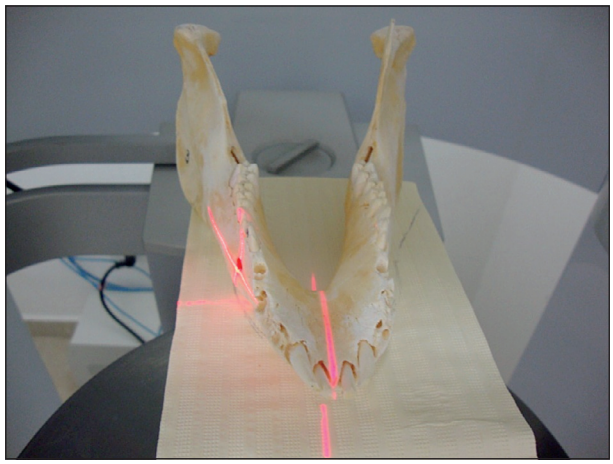

Figure 1 | Positioning of the pig mandible in the cone-beam computed tomography unit: the reference line was centered on the mandibular symphysis.

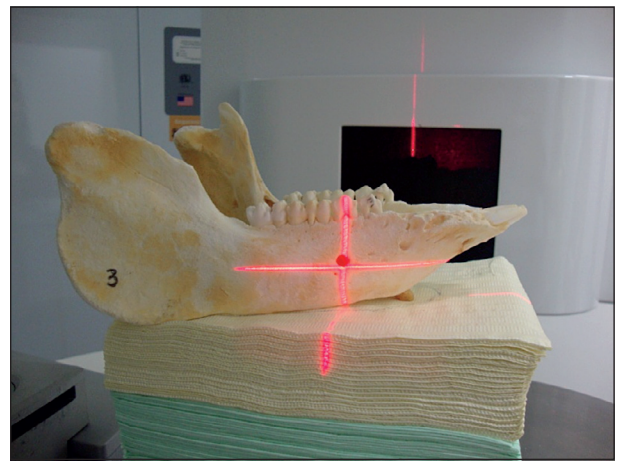

Figure 2 | Positioning of the pig mandible in the cone-beam computed tomography unit: the reference line was positioned on the most eminent mental foramen.

Figure 3 | Image visualized using Xoran computed tomography software.

Microsoft Corporation, Redmond, WA, USA) and statistically analyzed (ANOVA and Mann-Whitney) using Origin software (version 8,7.5; OriginLab Corporation, Northampton, MA, USA). The significance level was set for a p-value < 0.05. The Bonferroni and Tukey corrections were applied for significant differences.

RESULTS

Initially, we sought to identify differences in all measurements between the right and left sides (Table 1). The results showed no difference between the data for molars on both sides. Therefore, the total sample (14 measurements) was utilized.

According to the statistical evaluation, there

was no significant difference between the measurements performed by examiners AV1 and AV2 using the three methods (Table 2). This confirmed the standardization of the examiners' procedures and the appropriateness of the methodology.

Table 3 shows an analysis of the data obtained using the different methods. The results indicate no statistically significant difference between the measurements obtained with the caliper and those obtained using CBCT. Similarly, no significant difference was observed between the measurements obtained using the different CBCT voxel sizes.

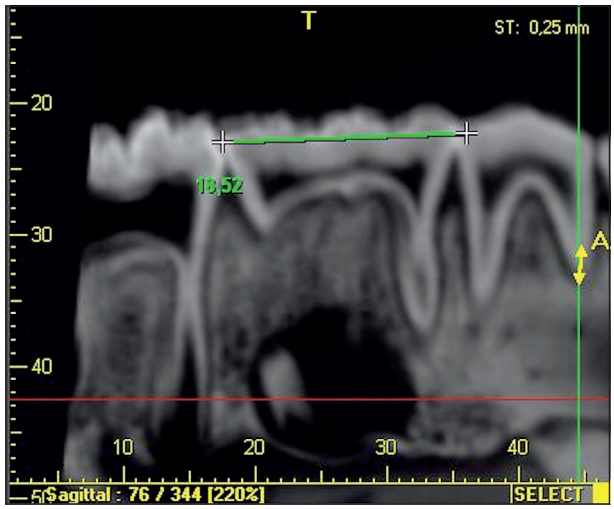

Figure 4 | Use of the software's electronic ruler.

DISCUSSION

Three-dimensional information has become an important input for surgical diagnosis and planning in the oral and maxillofacial regions. In recent years, CBCT has achieved a great acceptance among clinicians in the dental field, and has replaced helical CT in various tasks given its low scanning time and low dose of radiation.[1-3,5]

Investigators have worked with linear measurements in CBCT images and found CBCT measurements to be similar to those obtained *in vivo*, thus validating measurement and planning protocols for anatomical sites for implant placement and orthognathic surgery. Furthermore, CBCT eliminates the difficulty associated with positioning the patient's head and with possible uncertainties in measurements derived from asymmetry or malposition of the patient.[1,2] In the present study, we obtained images of pig mandibles using a CBCT device. Given that positioning of the skull does not interfere with

Table 1 | Linear measurements taken by the two examiners of the mesiodistal distance of the right (R) and left (L) mandibular first molars, using the direct and indirect methods.

Methods	Examiners	Sides	Mean values	Standard deviations	*P* values*
Caliper	AV1	R	18.49	0.978	0.369
		L	18.5	0.998	0.377
	AV2	R	18.42	0.943	0.356
		L	18.53	0.910	0.344
CBCT* (voxel: 0.125 mm)	AV1	R	18.37	0.936	0.354
		L	18.07	0.830	0.313
	AV2	R	18.11	0.806	0.304
		L	18.07	0.888	0.335
CBCT (voxel: 0.25 mm)	AV1	R	18.11	1.133	0.428
		L	17.78	1.103	0.416
	AV2	R	18.14	0.946	0.357
		L	18.10	0.944	0.357

CBCT: cone-beam computed tomography; *significant for $p < 0.05$.

Table 2 | Comparison of data obtained by the two examiners according to the methods used to obtain linear measurements of the mesiodistal distance in the total sample (N = 14) of pig mandibles.

Methods	Examiners	Mean values	Standard deviations	*P* values*
Caliper	AV1	18.49	0.949	0.253
	AV2	18.482	0.892	0.238
CBCT* (voxel: 0.125 mm)	AV1	18.225	0.865	0.231
	AV2	18.091	0.815	0.217
CBCT (voxel: 0.25 mm)	AV1	17.949	1.087	0.290
	AV2	18.125	0.908	0.242

CBCT: cone-beam computed tomography; * significant for p < 0.05.

Table 3 | Comparison of data obtained by each examiners according to the methods used to obtain linear measurements of the mesiodistal distance in the total sample (N = 14) of pig mandibles.

Examiners	Methods	Mean values	Standard deviations	*P* values*
AV1	Caliper	18.496	0.949	0.253
	CBCT* (voxel: 0.125 mm)	18.225	0.865	0.231
	CBCT (voxel: 0.25 mm)	17.949	1.087	0.290
AV2	Caliper	18.482	0.892	0.238
	CBCT (voxel: 0.125 mm)	18.091	0.815	0.217
	CBCT (voxel: 0.25 mm)	18.125	0.908	0.242

CBCT: cone-beam computed tomography; * significant for p < 0.05.

the measurement of interest, and that pigs have a pattern of growth and embryological development similar to that of humans[6] (being also similar in their anatomy and pathophysiology),[7] we considered the protocol valid.

In clinical practice, CBCT image quality can be influenced by many factors such as field of view, tube voltage and amperage of the apparatus, as well as spatial resolution as defined by voxel size. Images with an increased spatial resolution have less metal artifacts and an increased signal-to-noise ratio. However, there is an increase in the scanning time and a risk of patient movement, with a consequent increase in the radiation dose received by the patient.[3,5] Therefore, although CBCT has improved in terms of unnecessary exposure to radiation, the relationship between radiation dose and image quality should be part of the protocol choice process, since the radiation emitted has ionizing properties.[5]

Previous studies on the relevance of voxel size in linear measurements found no difference between the resolutions considered.[3,5,8,9] Damstra *et al.*[3] compared the data obtained using CBCT (voxels of 0.25 and 0.4 mm) with that obtained using a digital caliper (regarded as the gold standard). There was no statistically significant difference between the measurements obtained on CT images and the actual anatomical measurements. Torres *et al.*[8] compared four CBCT protocols (voxels of 0.2, 0.25, 0.3, and 0.4 mm) and all of them showed to be effective, without any significant difference between them for vertical and horizontal measurements. Patcas *et al.*[9] evaluated bone height and thickness and found that measurements obtained on images with a 0.4 mm voxel size were as accu-

rate as those obtained on images with a 0.125 mm voxel size. Similarly to that reported in the literature, this study found no significant difference between measurements obtained on images with voxels of 0.125 and 0.25 mm (Table 3). In addition, we highlight that the increase in voxel resolution did not result either in greater image accuracy or greater diagnostic accuracy. Nevertheless, the literature emphasizes that images with lower voxel values have specific indications for the evaluation of root fracture and resorption because these require more detail.[8] Thus, we emphasize that the influence of other aspects on the accuracy of linear measurements should be investigated.

In conclusion, there is no significant difference between linear measurements obtained from CBCT images with voxel sizes of 0.125 or 0.25 mm. Further studies on variables that may interfere with the accuracy of linear measurements are warranted.

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eLRD in Clinical and Laboratorial Research in Dentistry | Public Health

Translation and validation of the Brazilian version of the Dentist Satisfaction Survey

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ABSTRACT | *Objectives*: The aims of this study were to evaluate the level of overall job satisfaction among a group of Brazilian dentists and to test the validity of a cross-cultural translation to Brazilian Portuguese of the modified version of the Dentist Satisfaction Survey (DSS). Methods: This cross-sectional study had the participation of 605 dentists practicing in public dental care services in São Paulo, SP, Brazil. Job satisfaction was measured by a modified version of the DSS, which is based on responses to a 29-item self-administrated questionnaire, using a 5-point Likert scale. The items were translated into Brazilian Portuguese, back-translated and compared with the original English version. Measures for internal consistency and concurrent validity were calculated, respectively, by Cronbach's α coefficient and Spearman's correlation coefficient. Results: The level of overall job satisfaction among the sample was 2.86 (± 0.20) out of 5 on the Likert scale. Cronbach's α (0.83) showed a good internal consistency and homogeneity between items. Item-scale reliability correlations were good. Conclusion: The level of overall job satisfaction was neutral. Further actions and policies have been developed to increase the level of job satisfaction among Brazilian dentists working in the public sector. The Portuguese version of the modified DSS showed satisfactory psychometric properties regarding its reliability, reproducibility and validity for the Brazilian culture.

DESCRIPTORS | Job Satisfaction; Dentistry; Translations; Validation Studies.

RESUMO | **Tradução e validação da versão brasileira da pesquisa de satisfação de cirurgiões-dentistas** • *Objetivos*: avaliar o nível de satisfação geral com o trabalho de um grupo de dentistas brasileiros e testar a validade de uma tradução transcultural para o português do Brasil da versão modificada do Dentist Satisfaction Survey (DSS). *Métodos*: Participaram deste estudo transversal 605 dentistas que prestavam à época serviços públicos de atendimento odontológico em São Paulo, SP, Brasil. A satisfação no trabalho foi medida por meio de uma versão modificada do DSS, que é baseado em respostas a 29 itens de um questionário auto-administrado, usando a escala de Likert de 5 pontos. Os itens foram traduzidos para o Português, retraduzidos e comparados com os itens da versão original. Foram calculadas as medidas de consistência interna e validade externa, respectivamente, por meio do coeficiente de Cronbach e do coeficiente de correlação de Spearman. *Resultados*: O nível de satisfação geral com o trabalho foi de 2,86 (± 0,20) em 5 na escala de Likert. O α de Cronbach (0,83) mostrou uma boa consistência interna e homogeneidade entre os itens. As correlações de confiabilidade entre item e escala foram boas. *Conclusão*: O nível de satisfação geral com o trabalho foi neutro. Outras ações e políticas foram desenvolvidas para aumentar o nível de satisfação com o trabalho entre os dentistas brasileiros que trabalham no setor público. A versão em português do DSS modificado apresentou propriedades psicométricas satisfatórias quanto à sua confiabilidade, reprodutibilidade e validade para o Brasil.

DESCRITORES | Satisfação no Emprego; Odontologia; Traduções; Estudos de Validação.

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INTRODUCTION

Job satisfaction has been defined as a pleasurable or positive emotional state resulting from the appraisal of one's job or job experiences.[1] The practice of Dentistry has been considered to be the most stressful of all healthcare professions,[2] with more dentists in general practice experiencing more physical and mental ill health in comparison with other healthcare workers.[3]

The stress of dental practice has been related to time-related pressures, fearful patients, high case loads, financial worries, problems with staff, equipment breakdowns, defective materials, poor working conditions, and the routine and boring nature of the job.[4-6] However, many of these perceived causes of occupational stress, indentified as stressors, may be within the control of the dentist. Occupational stress may potentiate the onset of burnout, leading to a gradual loss of interest and decrease in job satisfaction. Burnout occurs in all health professionals at some time in their professional lives, with the constant and daily repetition of stressful events being a potentiating factor. By recognizing the potential sources of occupational stress, dentists can prevent burnout.[2]

One of the most widely reported and comprehensive instruments used to evaluate job satisfaction in dentistry is the Dentist Satisfaction Survey (DSS).[1,7-14] The DSS is an instrument used to measure dentist overall job satisfaction and dentist evaluation of various facets of the job. The original version of the DSS contains 54 statements with a 5-point Likert-type response format ranging from "strongly disagree" (scale = 1) to "strongly agree" (scale = 5). These items represent 11 facet scales and one overall satisfaction scale.[7-8] Validation of this instrument was tested in English language in others studies,[7-11] and in a Lithuanian language study,[13] showing acceptable levels of validity and reliability, with a good internal consistency. Modified versions of this instrument were tested and validated in an English language study[1] and in a Korean language study.[12] A translation into Brazilian Portuguese of the original DSS or its modified version was not yet available.

Knowledge of modifiable potential stressors related to job satisfaction could lead to a strategy and policy to provide a better work environment for dentists and dental team, preventing job burnout and positively influencing job engagement. This environmental and motivational improvement could aid in the improvement of patient care. As a result, the entire dental care system would benefit.[2,4,7,12,14] The characteristics of the work performed in public dental care services are different from those of the work performed in private offices.[2,12,15,16] Within a busy dental practice, it is essential that dentists be able to assess the degree of occupational stress being experienced by their staff and themselves.[16]

Issues concerning cross-cultural adaptation of self-reported instruments have been studied.[17-18] It is important that an adopted instrument be culturally relevant and valid for the local population while also demonstrating acceptable psychometric properties.[19-21]

The aims of this study were to evaluate the level of overall job satisfaction among a group of Brazilian dentists and to test the validity of a cross-cultural translation into Brazilian Portuguese of the modified version of the Dentist Satisfaction Survey.

MATERIAL AND METHODS

Study Design

The protocol for this study was approved by the Research Ethics Committee of the School of Dentistry, University of Sao Paulo, São Paulo, SP, Brazil. This cross-sectional study was conducted in three phases:

1. Cross-cultural translation process: a translation into Portuguese was performed of the modified version of the DSS.

2. Test of reliability: a pilot study was conducted to assess the reliability of the survey instrument. A pilot group (n = 50) completed the survey twice at a two-week interval.
3. Reliability and validity of the final version: the assessed instrument was tested and retested by two different raters with the subjects of the study at a two-week interval.

Survey instrument

Job satisfaction was measured by a Portuguese translation of the modified version of the Dentist Satisfaction Survey,[10] originally published in English language. This version was developed based on the original version of the DSS.[7,8] Of the 12 facet scales (domains), originally containing 54 items distributed among 11 scales related to dental practice characteristics (potential stressors) and one overall dental job satisfaction scale, five domains were not applicable. The final version of this instrument contained 29 items:

- 22 items related to 6 facet scales to measure job characteristics, and
- 7 items to measure overall job satisfaction.

The job characteristics included perception of income, personal time, professional time, staff, patient relations and delivery of care. All items were measured by a 5-point Likert scale:

- 1 = strongly disagree,
- 2 = disagree,
- 3 = neutral,
- 4 = agree, and
- 5 = strongly agree.

The total score of job satisfaction was classified in dissatisfied (1.0–2.5), neutral (> 2.5 but < 3.5), and satisfied (3.5–5.0).[7,8,12]

The questionnaire also gathered information about the personal and professional characteristics of the subjects including gender, age, educational level, specialty training, years of practice, working hours per week, type of work contract in public dental care services (part-time or full-time), part-time work in a private practice, number of treated patients in the scheduled work period (four hours / day), number of dental auxiliaries and annual income from clinical practice.

Cross-cultural translation

The translation of the instrument into Portuguese followed international guidelines for instrument linguistic validation,[17-18] and the culture and parlance differences of both countries—USA and Brazil—were considered to proceed with DSS adaptation and translation. The original English-language modified version of the DSS[12] was translated into Portuguese by two bilingual translators whose first language was Brazilian Portuguese, and by two bilingual translators whose first language was European Portuguese. Both Portuguese versions were back-translated from Portuguese into English by two bilingual translators whose first language was English in order to verify the semantic equivalence between the original English and back-translated versions and among the Portuguese versions. The structure of the instrument, instructions and mode of administration were similar to those of the original modified version of the DSS.

Functional equivalence (the combined effect of assessing conceptual, item, semantic, operational and measurement equivalence) was assessed by a panel of 12 experts (comprising health professionals, policy makers and community oral health professors) with regard to the performance of the instrument and the possibility of comparisons with studies conducted in different cultures.

Content validity according to the degree of importance of each item rated by the panel of experts on a defined scale (agreed without modifications / agreed with modifications / disagreed) was

assessed. The Portuguese version was then pilot-tested in 50 practicing dentists. Minor revisions were made based on their feedback. As a result, the final version was developed. This cross-sectional study had the participation of 605 dentists practicing in public dental care services in São Paulo, SP, Brazil.

Statistical analysis

The survey data were analysed using STATA 12.0™ (Statacorp Lp, College Station, TX, USA) and a confidence interval of 95% was used for all tests. Internal consistency was examined by Cronbach's α coefficient. In order to find the correlation between each question and the overall outcome of the test, Spearman's correlation coefficient (SCC) was calculated, using data from the first interview (main test).

RESULTS

The sample was composed by 605 dentists practicing in public dental care services in the city of São Paulo, SP, Brazil. The mean age of the participants was 43.33 years (SD = 8.85), of which 70.74% were female. Participants had practiced dentistry for a mean period of 19.49 years (SD = 8.75) and worked a mean of 37.3 hours per week.

About 63% of the studied sample also worked part-time in a private practice. All of the respondents to this survey worked with only one dental auxiliary, they treated a mean of 8.38 (SD = 5.35) patients / period, and received an annual mean income of 32,540.01 US dollars (US$) from clinical practice (Table 1).

The translation and cross-cultural adaptation of the Brazilian Portuguese version of the DSS is presented in Table 2. Its psychometric proprietiers are presented in Table 3.

The total score of job satisfaction among the studied group of Brazilian dentists was 2.86 (SD = 0.20) out of 5 on the Lickert scale. This level of job satisfaction was classified as neutral (> 2.5 but < 3.5).[5,6,10] The least satisfying facet scales were overall job satisfaction (2.94 ± 0.33), perception of income (2.77 ± 0.33), personal time (2.82 ± 0.46), professional time (2.78 ± 0.35), staff (2.76 ± 0.43), patient relations (2.79 ± 0.30) and delivery of care (3.20 ± 0.46; Table 4).

Table 1 | Distribution of personal and professional characteristics of the sample.

Characteristics	Mean	SD	Range
Gender	(a)	(a)	(a)
Age (in years)	43.33	8.85	25–67
Educational level			
• Graduate course (Master's or PhD)	(b)	(b)	(b)
• Specialty training	(c)	(c)	(c)
Years of practice	19.49	8.75	1–45
Hours worked / week	37.3	14.95	5–80
Work contract	(d)	(d)	(d)
Working part-time in private office	(e)	(e)	(e)
Treated patients / period (four hours)	8.38	5.35	3–6
Number of dental auxiliaries	(f)	(f)	(f)
Annual income from clinical practice (g)	32,540.01	29,674.06	7,405.56–555,416.66

(a) 428 (70.74%) female, 177 (29.26%) male; (b) n = 32; Master's = 29 (4.79%), PhD = 3 (0.50%); (c) n = 259 (42.81%); (d) Part-time (4 hours) = 381 (62.98%); Full-time (8 hours) = 224 (37.02%); (e) Yes = 381 (62.98%); No = 224 (37.02%); (f) All subjects worked together with one dental assistant; (g) Estimated in US dollars (1 Real = 1.8 US dollar). In Reals (local currency): Mean = 58,572.02; SD = 53,413.31; Range = 13,330–999,750

Table 2 | Portuguese translation and cross-cultural adaptation of the modified version of the Dentist Satisfaction Survey (DSS).

Block	Id	Questions	Portuguese version
Overall job satisfaction	05	Dentistry fulfills my current career aspiration	*A odontologia(*) preenche a minha atual aspiração de carreira profissional*
	06	I wish I could drop my job to do something else	*Eu gostaria de deixar meu trabalho para fazer outra coisa*
	09	I appear more satisfied with my job than I really am	*Eu pareço estar mais satisfeito(a) com meu trabalho do que realmente estou*
	12	Knowing what I know now, I would make the same decision to go into dentistry again	*Sabendo o que eu sei atualmente, eu tomaria a mesma decisão de escolher a odontologia como profissão outra vez*
	14	Dentistry is the place where I can make my best contribution	*A odontologia é o lugar onde posso dar a minha melhor contribuição*
	17	Overall, I am extremely satisfied with my career	*De modo geral, estou muito satisfeito(a) com a minha carreira*
	20	I feel trapped in my current position	*Eu me sinto aprisionado(a) em meu atual trabalho*
Perception of income	02	My income allows me to provide very well for my family	*Meu rendimento financeiro permite que eu sustente minha família muito bem*
	10	Compared to other dentists my total earnings are much lower than I desired	*Comparado com outros cirurgiões-dentistas(*), meus ganhos totais são muito menores do que eu desejaria*
	21	The income that I receive from my practice is most satisfactory for my needs	*O rendimento financeiro que recebo de minhas atividades clínicas é, na maior parte das vezes, satisfatório para as minhas necessidades*
	23	My income is not nearly as high as that of other dentists	*Meu rendimento financeiro é baixo em relação a outros cirurgiões-dentistas*
	25	My income compares favorably to that of other dentists	*Meu rendimento é comparável ao de outros cirurgiões-dentistas*
Personal time	07	I have enough time available for my personal life	*Eu tenho bastante tempo disponível para a minha vida pessoal*
	15	I have sufficient time available for leisure activity	*Eu tenho tempo disponível suficiente para atividades de lazer*
	29	I have too little time available for leisure	*Eu tenho muito pouco tempo disponível para lazer*
Professional time	01	I have very little time to keep abreast of advances in the field of dentistry	*Eu tenho muito pouco tempo para acompanhar os avanços na área da odontologia*
	04	I have enough time to improve my clinical skills	*Eu tenho bastante tempo para aprimorar minhas habilidades clínicas*
	22	I have sufficient time for professional contracts with colleagues	*Eu tenho tempo suficiente para contatos profissionais com colegas*
	26	I have very limited opportunity to discuss difficult cases with colleagues	*Eu tenho oportunidades muito limitadas para discutir casos difíceis com colegas*
Staff	08	The quality of my auxiliary personnel is lacking	*A qualidade do meu pessoal auxiliar é inadequada*
	18	The work performance of my auxiliaries is outstanding	*O desempenho do trabalho de meus auxiliares é excelente*
	19	The office staff works well together	*Os funcionários do consultório trabalham bem em conjunto*
Patient relations	11	Relating to patients is very frustrating for me	*O relacionamento com os pacientes é muito frustrante para mim*
	16	I do not enjoy interacting with my patients	*Eu não tenho prazer interagindo com os meus pacientes*
	24	The quality of interpersonal care I provide is very high	*A qualidade da assistência interpessoal que eu ofereço é muito alta*
	27	I enjoy helping patients	*Eu tenho prazer em ajudar os pacientes*
Delivery of care	03	I am skilled at dealing with my patients' dental problems	*Eu estou apto a tratar de meus pacientes com problemas dentais*
	13	I lack opportunities to provide quality care	*Eu não tenho oportunidades de prestar atendimento de qualidade*
	28	I am extremely pleased with the technical quality of my work	*Eu estou extremamente satisfeito com a qualidade técnica do meu trabalho*

(*) The word dentistry is usually translated in Brazil as "odontologia" (odontology) and in Portugal as "medicina dentária" (dental medicine). The dental career denomination in Brazil is "cirurgião-dentista" (dental surgeon) and in Portugal, Angola, Cape Verde, Guinea-Bissau, Mozambique, Sao Tome and Principe, and East Timor is "medico dentista" (dental physician). The term dentista (dentist) is also used in popular language in Brazil.

Table 3 | Reability and validity of the Portuguese version of the Dentist Satisfaction Survey (DSS).

Domain	Id	Reliability			Validity
		Correlation between the item and the overall result (*)	Correlation between the item and the domain result (*)	Cronbach's α excluding the item	Correlation between overall job satisfaction and domains (#)
Overall job satisfaction	05	0.987	0.765	0.554	0.765
	06	0.897	0.884	0.543	0.884
	09	0.789	0.997	0.528	0.997
	12	0.834	0.863	0.574	0.863
	14	0.654	0.622	0.617	0.622
	17	0.765	0.875	0.611	0.875
	20	0.894	0.875	0.632	0.875
	Cronbach's α of the domain = 0.692				
Perception of income	02	0.887	0.984	0.765	0.987
	10	0.765	0.876	0.748	0.987
	21	0.733	0.765	0.643	0.987
	23	0.875	0.765	0.777	0.965
	25	0.881	0.885	0.843	0.754
	Cronbach's α of the domain = 0.827				
Personal time	07	0.993	0.888	0.822	0.678
	15	0.876	0,843	0.812	0.789
	29	0.834	0.844	0.822	0.876
	Cronbach's α of the domain = 0.863				
Professional time	01	0.765	0.876	0.556	0.765
	04	0.755	0.865	0.522	0.678
	22	0.876	0.754	0.513	0.789
	26	0.754	0.894	0.599	0.789
	Cronbach's α of the domain = 0.674				
Staff	08	0.765	0.855	0.765	0.665
	18	0.743	0.829	0.723	0.747
	19	0.897	0.818	0.711	0.878
	Cronbach's α of the domain = 0.800				
Patient relations	11	0.888	0.997	0.621	0.888
	16	0.753	0.876	0.623	0.897
	24	0.845	0.777	0.601	0.897
	27	0.875	0.865	0.599	0.789
	Cronbach's α of the domain = 0.627				
Delivery of care	03	0.890	0.920	0.799	0.789
	13	0.897	0.987	0.744	0.789
	28	0.841	0.885	0.723	0.895
	Cronbach's α of the domain = 0.560				
Total	Cronbach's α of all domains = 0.834				

(*) Spearman's correlation coefficient; (#) interclass correlation coefficient (ICC).

Table 4 | Job satisfaction among a group of Brazilian dentists.

Facet scales	Mean	SD	Range
Overall job satisfaction	2.94	0.33	2.29–3.57
Perception of income	2.77	0.43	1.16–3.60
Personal time	2.82	0.46	2.00–4.00
Professional time	2.78	0.35	2.00–3.50
Staff	2.76	0.43	1.33–3.66
Patient relations	2.79	0.30	2.25–3.50
Delivery of care	3.20	0.46	2.33–4.00
Total	2.86	0.20	2.34–3.41

DISCUSSION

Satisfaction is a highly subjective concept that can be viewed as a dynamic process linked to lifestyle, expectations, personal experience and individual, social or cultural values. An instrument related to measuring satisfaction used in one cultural group may not be sufficient for use in another cultural group. In addition, the terminology or descriptions of a test in different languages may bring literal and idiomatic differences.

Cross-cultural adaptation procedures are a critical component of the validation process of an instrument for research. Direct translations may present linguistic problems because some words and phrases have no direct translation, and questions conceived in the context of one language may not be understood in the same way in the other language. Further, languages exist within social and cultural frameworks that are frequently unique, and some questions therefore may become different or meaningless in a different culture and location.[17,22,23]

In the present study, the translation process from English into Portuguese was straightforward, and the comparison between the original DSS questionnaire and the back-translated English version did not reveal conceptual content differences. The equivalent words needed to translate the questions were not difficult to find, and the grammar structure of the sentences was not difficult to build during the translation process.

The first goal of the present study was to assess the level of job satisfaction among a group of Brazilian dentists working in the public sector. The mean overall job satisfaction score of these Brazilian dentists was 2.8 out of 5, which is considered neutral (> 2.5 but < 3.5).[5,6,10] This result was similar to the mean score of overall job satisfaction, measured by the original or modified DSS versions, reported for American (mean = 63 of 100),[6] South Korean[10] and British[24] (neutral category = 73.6%) general practitioners, but was much lower than that reported for Canadian orthodontists[1] (mean = 4.0 of 5) and Lithuanian[13] general dental practitioners (mean = 4.0 of 5).

The difference among these overall job satisfaction scores can be explained due to the diversity of samples, which included subjects with various backgrounds and nationalities. In addition, although these studies evaluated the same profession, the cross-cultural differences influenced the responses to the self-reported questionnaires about job satisfaction. These facts indicate that the results of the present study must be interpreted within certain limitations. The participants in this study were practicing general dentists from a single Brazilian city. It is possible that dentists from other geographical areas would have reacted differently to the survey instrument used in this research. Another characteristic of the sample possibly influencing this result is the type of practice. Differently from other cited studies, all dentists evaluated in this study worked in public community dental care services. Career insecurity and lack of opportunity may lead to feelings of dissatisfaction with one's career, thereby increasing the likelihood of burnout.[4,16,21,23] When the level of career satisfaction among dentists was compared with that of other dental healthcare professionals, the dentists showed lower levels of job satisfaction.[24] Some fac-

tors intrinsic to a job in community dental care services, such as the nature of Dentistry, the working environment, and time scheduling were related with these lower levels of job satisfaction among dentists.[16] The self-contained nature of Dentistry leads to a perception of lack of freedom and control by the dentists, leading, in turn, to a desire to leave the community practice. Years of experience, freedom of professional judgment, altruistic motivation, importance placed on loan repayment and amount of administrative time allowed were associated significantly with career change intentions among community dental practitioners.[24]

Salaries and annual income from clinical practice were not associated with lower levels of job satisfaction among community dental practitioners.[24] These factors were not evaluated in the present study; however, the studied sample showed a mean annual income of US$ 32,540.01 from clinical practice (Table 1). Despite the economic differences between countries, this value is lower than the mean annual salary found in another study conducted among community dental practitioners (mean = US$ 81,603).[21] The city where the present study was developed, São Paulo, is the most populous city in Brazil, the sixth most populous city in the world, and capital of the most economically developed state in Brazil, with an estimated population of 11,037,593 residents. The dentist/habitant rate in this city was 1:445, indicating a high concentration of dentists. The mean annual income from clinical practice previously reported for this region was US$ 34,540.00.[13] This income is similar to that found in the present study (Table 1).

Some other limitations must be considered when interpreting the findings of the present study. First, this survey was based on a cross-sectional design that did not allow determination of causality for any of the associations identified. Thus, prospective and longitudinal studies are strongly needed to examine the influence of changing work environment factors on job satisfaction.

Furthermore, potential stressors in dental practice may be classified according to factors intrinsic to the job (the nature of dentistry, the working environment, time scheduling, the introduction of new technologies and the resulting need to keep up to date, risks and dangers), factors related to relationships at work (the role of the dentist in the dental team, working relationships) and factors related to the lack of career development (career insecurity and lack of opportunity may lead to feelings of dissatisfaction with one's career).[2-4] In addition to the recognition of occupational stressors, job satisfaction in dentistry can also be determined by other factors related to the worker's attributes (demographic characteristics, emotional well-being and personality factors) and to non-work factors (social interaction, family life and general life satisfaction).[5] The present study was limited to determining the level of job satisfaction among dentists. Identification of potential stressors related to job satisfaction is strongly needed, and should be the object of future research among Brazilian dentists.

Knowledge of modifiable potential stressors related to job satisfaction could lead to a strategy and policy to provide a better work environment for dentists and dental team, thus preventing job burnout and positively influencing job engagement. This environmental and motivational improvement could aid in the improvement of patient care. As a result, the entire dental care system would benefit. Therefore it is important to understand dentists' job satisfaction and how to work with the impact of environmental factors.[2,4,7,12,15]

The second goal of this study was to test the validity of a cross-cultural translation into Portuguese of the modified version of the DSS among a group of Brazilian dentists. The Portuguese version of the DSS showed acceptable validity and reliability, in agreement with studies conducted with the original version in English[7-11] and the Lithuanian

language[13] or the modified version in English[1] and the South Korean language.[12]

The overall results for internal consistency (Cronbach's α coefficient = 0.834) were considered satisfactory (good). Satisfactory Cronbach's α coefficients for some of the scales (income, personal time, and staff) confirm the internal consistency of this instrument. The internal consistency of the other facet scales assessed (overall job satisfaction, professional time, patient relations, and delivery of care) were considered low, indicating a need to redesign the test items[23-25] (Table 3). However, Nunnally and Bernstein recommended a Cronbach's α coefficient equal to 0.60 as a minimum reliability criterion.[26] future studies are suggested to develop new survey items that could form more internally consistent scores for these facet scales.

In conclusion, we found that the translated DSS demonstrated acceptable reliability and validity, and that it could be used as a valuable instrument for measuring job satisfaction among the Brazilian dentist population. For further research, the reliability and validity of the translated Portuguese version of this instrument should be tested in other dentist populations living in other Portuguese-speaking countries.

Job satisfaction is an important issue because satisfaction at work affects the productivity of a working person and contributes to the quality of his or her life.[14] The findings of the present study can help policy makers devise programs to increase the level of job satisfaction and to prevent the overall occupational stress among Brazilian dentists working in public dental care services. In addition, regarding the application of this data, further studies on job satisfaction are necessary to improve the working environment for dentists in Portuguese-speaking countries, and, specifically, in Brazil.

This Portuguese version of the the DSS showed adequate psychometric properties to evaluate job satisfaction among Brazilian dentists.

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CLRD in Clinical and Laboratorial Research in Dentistry | Restorative Dentistry

Influência do ângulo cavo-superficial no selamento marginal de restaurações de Classe II em resina composta

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RESUMO | *Objetivo:* Avaliar *in vitro* a influência do formato do ângulo cavo-superficial no selamento marginal de restaurações diretas de Classe II em resina composta. *Métodos:* Quinze terceiros molares humanos inclusos, recém-extraídos, foram seccionados paralelamente ao seu longo eixo no sentido vestíbulo-lingual. As metades obtidas tiveram suas raízes inclusas em resina acrílica e em cada uma delas foi confeccionada uma cavidade do tipo *slot* proximal. Os 30 espécimes foram subdivididos aleatoriamente em três grupos experimentais (n = 10), de acordo com o preparo do ângulo cavo-superficial: G1, bisel reto; G2, bisel chanfrado; G3, sem bisel. As cavidades foram restauradas por meio da técnica incremental com resina composta (Z350 XT). Após as ciclagens térmica (5000 ciclos, 5°C a 55°C) e mecânica (50000 ciclos, 50 N), os espécimes foram isolados, deixando-se apenas 1 mm ao redor das restaurações, imersos em solução de azul de metileno a 2% por 4 horas a 37°C, e seccionados. Os fragmentos foram analisados microscopicamente e atribuíram-se *scores* às imagens obtidas. A análise estatística foi realizada por meio dos testes de Kruskal-Wallis e Student-Newman-Keuls ($\alpha = 0.05$). *Resultados:* O grupo com término em bisel reto (G1) apresentou microinfiltração significativamente menor do que o grupo com bisel chanfrado (G2; p = 0,0158) e do que o grupo sem bisel (G3; p = 0,0448). Não foram verificadas diferenças estatisticamente significativas entre G2 e G3 (p = 0,6844). *Relevância:* Os resultados deste estudo *in vitro* sugerem que a confecção de bisel reto no ângulo cavo-superficial de preparos de Classe II é capaz de melhorar o selamento marginal das restaurações em resina composta.

DESCRITORES | Infiltração Dentária; Preparo do Dente; Resinas Compostas; Esmalte Dentário.

ABSTRACT | **Influence of the cavosurface angle on the marginal leakage of Class II composite resin restorations** • *Objective:* The aim of this study was to evaluate, *in vitro*, the influence of the cavosurface angle preparation on the leakage of Class II composite-resin restorations. *Methods:* Fifteen sound human third molars were sectioned parallel to their long axis through the secondary groove in the buccolingual direction. The roots of the tooth halves thus obtained were embedded in acrylic resin, and Class II proximal slot cavities were prepared. The thirty specimens were then randomly divided into three groups, as follows: G1, bevel preparation; G2, chamfer preparation; G3, butt joint (no preparation on the cavity margin). Composite resin (Z350 XT) was incrementally placed in all cavities. After thermal (5000 cycles, 5°C to 55°C) and mechanical cycling (50000 cycles, 50 N), the specimens were sealed, infiltrated with 2% methylene blue (4 h, 37°C) and sectioned in halves, which were microscopically analyzed and scored based on a scale. Statistical analyses were performed using the Kruskal-Wallis and Student-Newman-Keuls tests ($\alpha = 0.05$). *Results:* G1 (bevel) presented significantly lower microleakage than G2 (chamfer; p = 0.0158) and G3 (butt joint; p = 0.0448). There was no statistically significant difference between G2 and G3 (p = 0.6844). *Relevance:* The results of this *in vitro* study suggest that a bevel preparation is able to decrease microleakage in Class II composite-resin restorations.

DESCRIPTORS | Dental Leakage; Tooth Preparation; Composite Resins; Dental Enamel.

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INTRODUÇÃO

Apesar de melhoras significativas dos sistemas adesivos e das resinas compostas, a formação de *gaps* marginais[1] permanece um problema para os clínicos. Uma das causas para esse problema é a contração de polimerização das resinas compostas, por afetar a integridade da interface adesiva dente/restauração, criando um ponto de origem para defeitos marginais,[2] cuja consequência é a microinfiltração, que pode gerar a descoloração marginal e a formação de cáries secundárias.[3]

A contração de polimerização pode afetar a interface dente/restauração em diferentes níveis, dependendo da configuração do preparo; quanto maior for o fator C (razão entre a área aderida e a área não aderida) de uma cavidade, maiores serão as tensões geradas, contrárias às forças de adesão.[4] Algumas técnicas foram desenvolvidas para prevenir ou reduzir falhas marginais. Como exemplos, podem-se citar a técnica incremental de inserção das resinas compostas, que diminui a contração de polimerização,[5] e o biselamento do ângulo cavo-superficial do preparo.[6]

Enquanto a adesão em esmalte é mais estável,[7] a adesão em dentina é mais complexa. A dentina é um substrato úmido, podendo apresentar-se de diferentes formas e com túbulos dentinários em orientações diversas.[8,9] O biselamento do ângulo cavo-superficial das cavidades aumenta a área de esmalte disponível, expondo um substrato mais favorável à adesão formado por esmalte prismático, uma vez que, com o desgaste, são removidos a camada aprismática e eventuais defeitos superficiais que poderiam comprometer a adesão.[10] Como consequência, criam-se condições para aumentar a estabilidade da interface adesiva e diminuir a formação de *gaps* marginais,[11] evitando-se a microinfiltração e a formação de cáries secundárias.[6] Além disso, com o biselamento de preparos cavitários em dentes posteriores, são removidos manchamentos marginais que poderiam ser erroneamente confundidos com cáries secundárias, principalmente no ângulo cavo-superficial proximal, evitando-se assim substituições desnecessárias de restaurações.[12]

A remoção de estrutura dental durante o preparo cavitário apresenta uma relação direta com a diminuição de sua resistência à fratura.[13] Entretanto, quando a restauração dental é realizada com materiais adesivos, pode existir uma recuperação da resistência à fratura, dependendo do tipo de sistema adesivo e da técnica restauradora empregados.[14] Estudos apontam que o uso do bisel em restaurações de Classe II em resina composta confere aos dentes restaurados o benefício do aumento de sua resistência à fratura.[6,15]

Quanto ao formato, os biséis podem ser classificados em retos e chanfrados. O bisel reto é obtido por meio de uma ponta diamantada cônica posicionada de modo que o ângulo cavo-superficial resultante seja de 45°. Já para o bisel chanfrado, utiliza-se uma ponta diamantada em forma de chama para que todo o ângulo cavo-superficial fique arredondado. Entretanto, não há consenso na literatura a respeito do formato de bisel a ser confeccionado no ângulo cavo-superficial para melhorar o desempenho das restaurações em resina composta. Se, por um lado, alguns estudos demonstraram que não há diferenças significativas entre os dois tipos de bisel,[16,17] Coelho-de-Souza *et al.*[18] concluíram que o bisel reto foi o preparo mais efetivo em aumentar a resistência à fratura quando associado a adesivos dentinários com condicionamento ácido prévio; entretanto, o selamento marginal de cada tipo de preparo não foi avaliado, nem foi realizado o envelhecimento dos espécimes.

O envelhecimento clínico das restaurações adesivas reduz seu desempenho em longo prazo por degradação da interface adesiva, principalmente em dentina.[6] Metodologias simulando os estresses térmico e mecânico que normalmente ocorrem na cavidade oral têm sido aplicadas em estudos *in vitro*.[19,20] De acordo com de Paula *et al.*,[20] as ciclagens

térmica e mecânica influenciam a adaptação marginal de restaurações em resina composta, aumentando o percentual de *gaps*.

Assim, buscou-se por meio deste estudo demonstrar, *in vitro*, qual formato do ângulo cavo-superficial em restaurações diretas de Classe II em resina composta é capaz de melhorar o selamento marginal após o envelhecimento dos espécimes pelas ciclagens térmica e mecânica.

MATERIAIS E MÉTODOS

O projeto deste estudo foi aprovado pelo Comitê de Ética em Pesquisa da Faculdade de Odontologia da Universidade de São Paulo (parecer número 317.624).

Foram utilizados 15 terceiros molares humanos inclusos, livres de trincas e/ou defeitos de esmalte, obtidos no Banco de Dentes da Faculdade de Odontologia da Universidade de São Paulo. Foram realizadas a limpeza dos dentes, com a utilização de curetas periodontais (Duflex, SS White, Rio de Janeiro, RJ, Brasil), e a limpeza com pedra-pomes (SS White, Rio de Janeiro, RJ, Brasil) e água, com o auxílio de escovas do tipo pincel (KG Sorensen, Barueri, SP, Brasil). Em seguida, a amostra foi armazenada em água destilada a 4°C até o momento do uso.

Cada elemento dental foi seccionado paralelamente ao seu longo eixo no sentido vestíbulo-lingual, utilizando-se um disco diamantado dupla face (n. 11-4224, série 15 HC; Buehler UK Ltd., Lake Bluff, IL, EUA) acoplado a uma máquina de corte de alta precisão (Isomet 1000; Buehler UK Ltd., Lake Bluff, IL, EUA), sob refrigeração à água.

Cada uma das trinta metades obtidas após o corte teve sua raiz incluída em resina acrílica (Artigos Odontológicos Clássico, São Paulo, SP, Brasil) até 1 mm abaixo da junção amelo-cementária, utilizando-se uma matriz de PVC cilíndrica.

Em cada espécime foi confeccionada uma cavidade do tipo *slot* proximal utilizando-se uma ponta diamantada n. 2135 (KG Sorensen). A cada cinco preparos, uma nova ponta foi utilizada para garantir o seu poder de corte. As paredes cervicais localizaram-se em esmalte, e cada cavidade apresentou 3 mm de profundidade no sentido ocluso-cervical, 2 mm de extensão no sentido vestíbulo-lingual, e 2 mm de extensão no sentido mésio-distal. Uma sonda milimetrada (Duflex) foi utilizada para aferir as dimensões dos preparos cavitários.

Tabela 1 | Grupos experimentais subdivididos de acordo com o preparo do ângulo cavo-superficial.

Grupos	Configuração da cavidade	Ponta diamantada
G1	Bisel reto	n. 1190
G2	Bisel chanfrado	n. 3118F
G3	Sem bisel	-

Os trinta espécimes foram subdivididos aleatoriamente em três grupos experimentais (n = 10; Tabela 1). No grupo G1, foi utilizada uma ponta diamantada cônica n. 1190 (KG Sorensen), posicionada de modo que o ângulo cavo-superficial resultante fosse de 45°. Já no grupo G2, foi utilizada uma ponta diamantada em forma de chama n. 3118F (KG Sorensen) para produzir uma angulação arredondada na região cavo-superficial. Em ambos os casos, a porção média da ponta diamantada foi utilizada para o desgaste.

Após o preparo, foi realizada a limpeza cavitária com pedra-pomes (SS White) e água, com o auxílio de escovas do tipo pincel (KG Sorensen), seguida da aplicação de detergente aniônico fraco (lauril sulfato de sódio; Fórmula e Ação, São Paulo, SP, Brasil), friccionado nas paredes cavitárias com penso de algodão, e lavagem.

Realizou-se o condicionamento com ácido fosfórico a 35% (3M ESPE, St. Paul, MN, EUA) por 15 s em esmalte e 10 s em dentina, seguido de lavagem com água por 15 s. A secagem foi realizada colocando-se um penso de algodão na cavidade e aplicando-se um jato de ar, impedindo, assim, a desidrata-

ção da dentina. O adesivo Adper Single Bond (3M ESPE) foi aplicado por 20 s, evaporando-se o solvente com um jato de ar por 5 s a uma distância de 5 cm. Repetiu-se o processo, e foi, então, realizada a fotoativação (Elipar Freelight 2; 3M ESPE) com a aplicação de 1200 kW/cm² por 30 s.

Matrizes de metal do tipo Tofflemire (TDV Dental Ltda.; Pomerode, SC, Brasil) foram utilizadas para restabelecer a parede proximal dos *slots*. As cavidades foram restauradas com a resina composta Z350 XT (3M ESPE), inserida no *slot* proximal por meio da técnica incremental, i.e. em três incrementos. Foi realizada a fotoativação (Elipar Freelight 2; 3M ESPE) com a aplicação de 1200 kW/cm² por 20 s para cada incremento de resina. A intensidade da fonte de luz foi medida por meio de um radiômetro (Curing Radiometer Model 100; Kerr/Demetron, Danbury, CT, EUA) antes da restauração de cada cavidade. O acabamento das restaurações foi realizado com pontas siliconizadas (Enhance; Dentsply Maillefer, Ballaigues, Vaud, Suiça).

Após sua armazenagem por 24 h, em ambiente 100% úmido e a 37°C, os espécimes foram submetidos à ciclagem térmica, que consistiu em 5000 ciclos que alternaram banhos de 5°C e 55°C, sendo 1 min o tempo de imersão de cada banho e 15 s o tempo de transferência entre as temperaturas. O aparato utilizado para esse processo pertence ao Departamento de Dentística da Faculdade de Odontologia da USP.

Terminada a ciclagem térmica, os espécimes foram submetidos à ciclagem mecânica. O aparato utilizado para essa ciclagem pertence ao Departamento de Biomateriais e Bioquímica Oral da Faculdade de Odontologia da USP, e consiste em dez pistões de aço inoxidável com término esférico com 2 mm de diâmetro. Essas esferas foram colocadas em contato com a crista marginal de cada restauração. Foram realizados 50000 ciclos com força axial de 50 N e frequência de 2 Hz. Os espécimes foram mantidos hidratados durante esse processo.

Após as ciclagens, os espécimes foram protegidos por duas camadas de verniz ácido-resistente (Risqué; São Paulo, SP, Brasil) exceto sobre as restaurações e 1 mm ao redor das mesmas. Para o teste de microinfiltração, os espécimes foram imersos em solução de azul de metileno a 2% (Merck; Darmstadt, Alemanha) por 4 h a 37°C, seguindo-se o enxágue em água corrente durante 15 min para eliminar o corante de sua superfície.[21] Os espécimes foram seccionados verticalmente no sentido mésio-distal e horizontalmente no sentido vestíbulo-lingual utilizando-se um disco diamantado de dupla face (n. 11-4224, série 15 HC; Buehler UK Ltd., Lake Bluff, IL, EUA), em baixa rotação, acoplado a uma máquina de corte de alta precisão (Isomet 1000; Buehler UK Ltd., Lake Bluff, IL, EUA), sob refrigeração à água. Dessa maneira, foi possível observar as paredes oclusal e gengival das restaurações nos fragmentos. Fotografias coloridas dos fragmentos foram tiradas, todas com distância, resolução e iluminação padronizadas, utilizando-se um microscópio óptico com 40× de aumento (Miview USB Digital Microscope Video Camera; Cosview Technologies Co., Ltd, Longgang District, Shenzhen, China).

A penetração do corante foi analisada por dois examinadores independentes e calibrados (Kappa interexaminadores e intraexaminador iguais a 0.90 e 0.95, respectivamente). Foram atribuídos *scores* às imagens obtidas de acordo com o seguinte critério:

- 0 = sem penetração;
- 1 = penetração parcial nas paredes oclusal e gengival;
- 2 = penetração total nas paredes oclusal e gengival; e
- 3 = penetração total, incluindo a parede axial.

Os dados foram analisados por meio do programa Bioestat 5.0 (Institute for Sustainable Development Mamirauá, Belém, AM, Brasil) e submetidos ao teste não-paramétrico de Kruskal-Wallis, segui-

do da comparação entre grupos por meio do teste de Student-Newman-Keuls ($\alpha = 0.05$).

RESULTADOS

A frequência de distribuição dos *scores* e os resultados estatísticos apresentam-se na Tabela 2. O teste de Kruskal-Wallis indicou que existe uma diferença estatisticamente significativa entre os grupos testados (p = 0.0272). As figuras de 1 a 4 são representativas de cada um dos *scores* determinados.

Com o objetivo de verificar quais grupos apresentavam diferenças estatisticamente significativas entre si, foi utilizado o teste *post-hoc* de Student-Newman-Keuls. De acordo com esse teste, o grupo com término em bisel reto (G1) apresentou microinfiltração significativamente menor do que os grupos com bisel chanfrado (G2; p = 0,0158) e sem bisel (G3; p = 0,0448), e não foram verificadas diferenças estatisticamente significativas entre G2 e G3 (p = 0,6844).

Tabela 2 | Distribuição de frequências dos *scores* em cada grupo testado e resultados estatísticos.

	Score 0	*Score* 1	*Score* 2	*Score* 3	Significância (*post-hoc*)
G1	5	2	3	0	A
G2	0	4	2	4	B
G3	0	5	2	3	B

Letras diferentes indicam diferenças estatisticamente significativas entre os grupos de acordo com o teste *post-hoc* de Student-Newman-Keuls (p < 0.05).

DISCUSSÃO

Os resultados deste estudo demonstraram que, em relação ao selamento marginal das restaurações com resina, o bisel reto foi mais eficaz em comparação com o bisel chanfrado e com a ausência de bisel. A diferença obtida entre os biséis reto e chanfrado pode ser atribuída à extensão e profundidade do desgaste de esmalte obtido em cada uma das modalidades. No caso do bisel chanfrado, há desgaste de uma espessura maior de esmalte, podendo-se removê-lo totalmente em alguns pontos. O objetivo do biselamento é a remoção apenas do esmalte aprismático a fim de expor um substrato mais favorável à adesão.[10] No caso da remoção total do esmalte no ângulo cavo-superficial, haverá exposição de dentina, um substrato úmido e com adesão menos estável.[8] Consequentemente, poderá haver uma maior formação de *gaps* marginais responsáveis pela microinfiltração.[6] Logo, o desgaste do esmalte durante a confecção do bisel deve ser mínimo, sem exposição de dentina, o que pode ser obtido com uma maior facilidade técnica por meio do bisel reto.

Já o fato de os preparos sem bisel terem apresentado menor selamento marginal do que aqueles com bisel reto provavelmente se deve à menor área de esmalte disponível e à presença da camada aprismática, desfavorável ao condicionamento seletivo e à criação adequada das microrretenções.[10,18] O excesso de resina composta além do ângulo cavo-superficial não biselado, pobremente aderida ao esmalte, ficará mais susceptível a fraturas sob cargas mastigatórias, favorecendo a microinfiltração marginal. Sendo assim, nas restaurações sem bisel, recomenda-se que os excessos marginais sejam completamente removidos, o que torna a técnica mais complexa, principalmente na face oclusal devido aos acidentes anatômicos.

Em relação ao selamento marginal, Peixoto *et al.*[16] demonstraram que não há diferença estatisticamente significativa entre os biséis reto e chanfrado. Já o estudo de Gandhi e Nandlal[17] encontrou resultados semelhantes para os preparos com bisel reto e chanfrado em relação à retenção das restaurações em dentes anteriores. No que se refere à resistência à fratura, Coelho-de-Souza *et al.*[18] estudaram pré-molares superiores, que são elementos dentais bastante sujeitos à fratura de cúspides devido ao seu posicionamento no arco dentário e sua

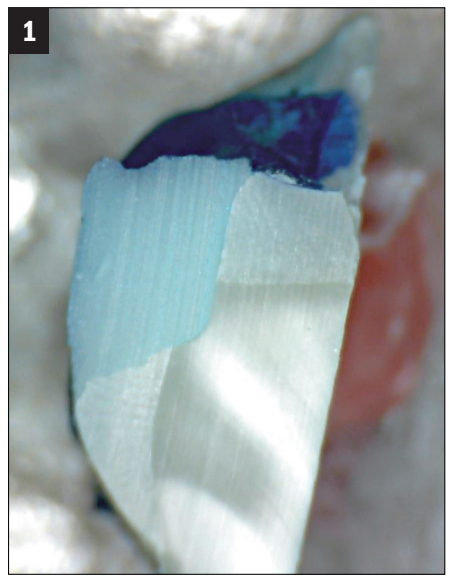

Figura 1 | Imagem representativa do score 0 (sem penetração do corante).

Figura 2 | Imagem representativa do score 1.

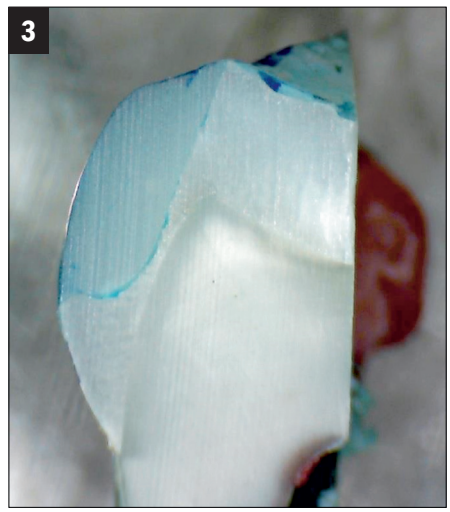

Figura 3 | Imagem representativa do score 2.

Figura 4 | Imagem representativa do score 3.

função mastigatória. Foram realizados diferentes preparos do ângulo cavo-superficial, sendo os elementos dentais restaurados com resina composta e diferentes sistemas adesivos. Ressalta-se que não foi realizado o envelhecimento dos espécimes. Concluiu-se que o bisel reto associado a adesivos dentinários com condicionamento ácido prévio foi capaz de aumentar a resistência à fratura dos prémolares restaurados. Portanto, o resultado de Coelho-de-Souza *et al.*[18] fortalece ainda mais os achados do presente estudo, sugerindo que o bisel reto não somente reforça a estrutura dentária restaurada como também melhora o selamento marginal da restauração.

Ao se substituirem restaurações de amálgama por restaurações de resina composta, a confecção de um bisel cavo-superficial permite a remoção de eventuais manchamentos marginais do esmalte sem ampliar a extensão do preparo cavitário, com menor desgaste da estrutura dental. Outros estudos longitudinais, preferencialmente clínicos, são necessários para avaliar o desempenho do bisel

reto realizado no ângulo cavo-superficial de preparos restaurados com resina composta em dentes posteriores a fim de validar esse procedimento como um protocolo clínico para melhorar o desempenho dessas restaurações, cada vez mais executadas nos consultórios dentários por razões estéticas, e tendo em vista a possibilidade do banimento do amálgama dental pelas organizações mundiais.[22]

Logo, concluiu-se que a confecção de um bisel reto no ângulo cavo-superficial de preparos de Classe II foi capaz de melhorar o selamento marginal das restaurações em resina composta.

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eLRD in Clinical and Laboratorial Research in Dentistry | Oral Pathology

A systematic review of the long-term effects of dental development disturbances after hematopoietic stem-cell transplantation in pediatric patients

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ABSTRACT | The purpose of this systematic review was to evaluate published data and to update our current knowledge about the impact on dental development of childhood hematopoietic stem-cell transplantation (HSCT), as well as the late effects of preparative regimens, for the treatment of onco-hematological malignancies. A systematic literature research was conducted to assess articles published since January 1980 until the present day that fitted the predetermined inclusion/exclusion criteria. Data compilation was divided into qualitative and quantitative dental development disturbances. Demographic records were also gathered. First and second premolars and second molars were significantly more affected in HSCT children. There was a positive correlation between age at the time of anticancer therapy administration and qualitative and quantitative dental development disturbances. The association of total body or head and neck radiation mieloablative treatments was shown to enhance the magnitude of dental development disturbances. Dental development disturbances due to childhood HSCT are commonly seen in long-term survivors. The knowledge of these alterations may help improve dental care and elevate the quality of life of these patients. Further studies are needed to understand the long-term effects of dental development disturbances in this group of patients.

DESCRIPTORS | Bone Marrow Transplantation; Hematopoietic Stem Cell Transplantation; Child; Drug Therapy; Radiotherapy.

RESUMO | **Revisão sistemática dos efeitos tardios de distúrbios de desenvolvimento dentário em pacientes submetidos a transplante de células-tronco hematopoiéticas durante a infância** • O objetivo desta pesquisa foi revisar sistematicamente as publicações referentes aos efeitos tardios decorrentes das alterações do desenvolvimento dentário em pacientes submetidos ao transplante de células-tronco hematopoiéticas durante a infância. A pesquisa sistemática da literatura avaliou os artigos publicados desde janeiro de 1980 até a presente data que se enquadraram nos critérios de inclusão/exclusão pré-determinados. A compilação dos dados foi dividida em distúrbios de desenvolvimento dentário qualitativos e quantitativos. Além disso, os registros demográficos foram contabilizados. Os primeiros e segundos pré-molares e os segundos molares foram os dentes significativamente mais afetados nas crianças submetidas ao transplante de células-tronco hematopoiéticas. Houve uma correlação positiva entre a idade em que as terapias antineoplásicas foram administradas e os distúrbios de desenvolvimento dentário qualitativos e quantitativos. A associação do tratamento mieloablativo por meio de radioterapia total ou em região de cabeça e pescoço apresentou correlação positiva com os distúrbios de desenvolvimento dentário. Essas alterações podem ser comumente vistas em pacientes que conseguem sobreviver ao transplante de células-tronco hematopoiéticas durante a infância. O conhecimento dessas alterações pode ajudar a melhorar o atendimento odontológico e elevar a qualidade de vida desses pacientes. Mais estudos são necessários para entender os efeitos de longo prazo dos distúrbios de desenvolvimento dentário para esse grupo de pacientes.

DESCRITORES | Transplante de Médula Óssea; Transplante de Células-Tronco Hematopoéticas; Criança; Quimioterapia; Radioterapia.

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INTRODUCTION

The treatment of childhood onco-hematological malignancies through hematopoietic stem-cell transplantation (HSCT) combined with surgery, multi-agent chemotherapy (CT) and/or radiotherapy (RT) has been successfully employed in more than 30,000 patients per year worldwide with successful results.[1,2] Currently, a 5-year survival rate for the most common hematologic neoplasia—acute lymphoblastic leukemia—has exceeded 80%. In some groups, this rate may achieve 95%.[3,4]

However, at least more than two-thirds of these children may present several long-term complications due to HSCT and its preparative regimen.[5,6] It is estimated that between 62% and 95% of the children surviving bone-marrow transplantation (BMT) will experience a late effect resulting from treatment and 27%–40% present serious consequences with elevated risk of death.[7] The most commonly reported systemic complications involve the endocrine system, heart, lung, kidneys, bones and neurological structures, and may present as osteopenia, growth deficiency, changes in anatomical proportions, hypogonadism, premature menopause/andropause and disorders in the development of secondary sexual characteristics.[8-10]

Dental development disturbances from HSCT have also been described and may involve tooth agenesis, microdontia, shortened roots, changes in tooth proportions (namely in the crown/root ratio), root stunting and V-shaped apex.[11-15] Chemotherapy-induced tooth formation anomalies have been reported to emerge mostly in the first and second premolars, and the second molar.[15-17] These complications have been mostly associated with patients' younger age at the time of transplantation but great controversy remains about:

1. the real impact of radiation therapies,
2. the extent of dental development disturbances and
3. patients' quality of life impairment due to these complications.[18]

However, in spite of these relevant publications, a conclusive data compilation is still lacking about dentofacial development disturbances in long-term survivors of childhood HSCT. In this paper we present a systematic review of all articles published in English about the dentofacial abnormalities observed in onco-hematologic children submitted to HSCT. The results include summarized demographic data about the patients, and the most commonly observed dental development disturbances findings in these long-term survivals.

METHODOLOGY

Search strategies and criteria for selecting studies

We performed a systematic review of published manuscripts that discussed the dental development disturbances and their subsequent late effects among survivors of HSCT for the treatment of childhood onco-hematological cancer, using the *Pubmed* and *Google Scholar* search engines. The following keyword combinations were employed in this review:

- hematopoietic stem cell transplantation;
- dental abnormalities;
- children;
- tooth development;
- bone marrow transplantation;
- dental development.

All of the article abstracts derived from this search were thoroughly reviewed for relevance. The inclusion criteria encompassed all of the full-articles published in the English language regarding the long-term dental follow-up of childhood-HSCT patients. The exclusion criteria comprised papers that failed to thoroughly present a comprehensive

description of the chemo- and/or radiotherapy involved; studies describing a follow-up period of less than 2 years; case reports or case-series; studies on adult-HSCT; and studies on HSCT for the treatment of solid tumors or congenital diseases.

Data collection

Two authors independently evaluated the methodological quality of selected studies. Study quality was determined using four criteria:

- comparability of subjects,
- clear definition of exposure or intervention,
- standard outcomes measurement and
- appropriate statistical analysis.

Any discrepancy in the validity assessment was resolved by discussion between authors. The relevant data about sample size, age at diagnosis, type of diagnosis, follow-up period, type and details of treatment, late dental effects, and main endpoints from the selected studies were extracted by researchers using a standardized form.

RESULTS

Data compilation

Our initial research retrieved a total of 117 titles and abstracts. After thorough examination of these abstracts and full-text articles, 105 papers were discarded for not matching the inclusion criteria. Ten papers fulfilled all of the previous established inclusion criteria and were considered in this review. Six of them were cross-sectional studies, two were case-controls, one was a retrospective study and one was a cohort study (Figure 1).

All together, the studies evaluated a total of 423 patients with a mean age of 3 years (range: 4.7–25.9 years). Patients' median age at dental evaluation was 9 years, and, at HSCT, it was 3 years. Follow-up median time was 5 years. The most common onco-hematological malignancy was acute lymphoblastic leukemia (ALL) comprising 45% of the diagnoses (n = 190), followed by acute myeloblastic leukemia (AML; 11%, n = 44), and neuroblastoma (NBL; 10%, n = 43). Other diagnoses comprised 45% of the study population (n = 144; Figure 2).

Total body radiation therapy (TBR) was administered to 25.9% (n = 88) of the patients with a mean dose of 11.5 Gy (range: 6–22 Gy), mainly for ALL treatment (76%; n = 72; Figures 3 and 4). When the authors provided a full description of the chemotherapeutic protocols involved, the most

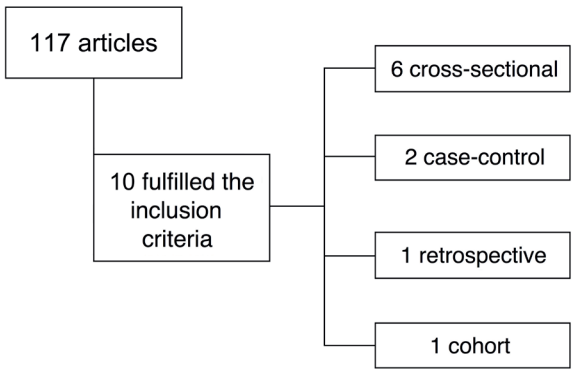

Figure 1 | Paper selection algorithm. The keyword search returned 117 papers. After thorough review, only 10 fulfilled the inclusion criteria. Of these, 6 were cross-sectional studies; 2 were case-control studies; 1 was a retrospective study and 1 was a cohort study.

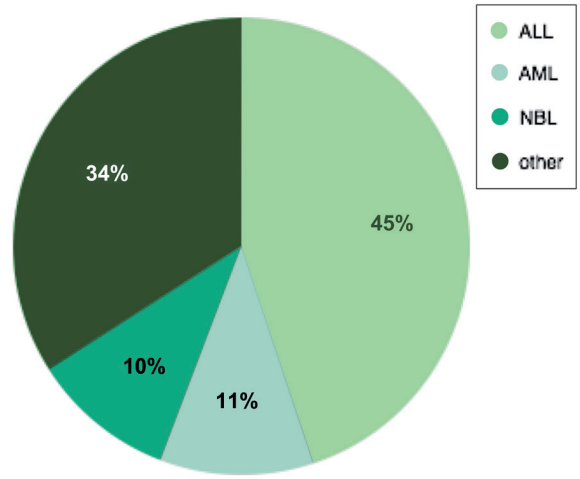

Figure 2 | Descriptive diagnosis distribution. ALL: Acute lymphoblastic leukemia; AML: Acute myeloblastic leukemia; NBL: Neuroblastoma; Other: other onco-hematological malignancies.

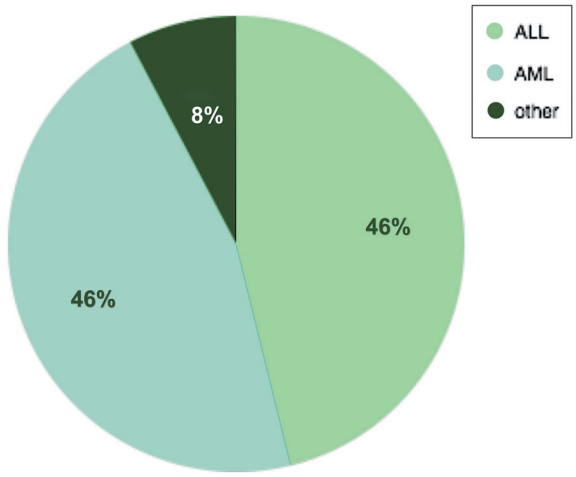

Figure 3 | Total body irradiation (TBI) versus focal radiation therapy (non-TBI; %) disk chart distribution. ALL: Acute lymphoblastic leukemia; AML: Acute myeloblastic leukemia; Other: other onco-hematological malignancies.

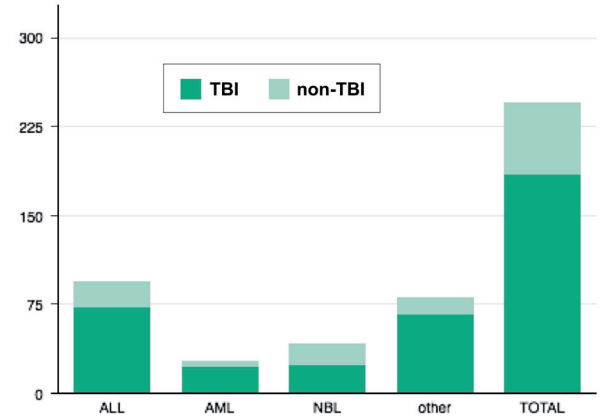

Figure 4 | Total body irradiation (TBI) versus focal radiation therapy (non-TBI; %) column chart comparison. ALL: Acute lymphoblastic leukemia; AML: Acute myeloblastic leukemia; NBL: Neuroblastoma; Other: other onco-hematological malignancies.

common antineoplastic agent was a combination of cyclophosphamide (Cy) or busulfan (Bu) with vincristine (Vin) in association with methotrexate (MTX).

Qualitative comparisons of dental development disturbances

A qualitative evaluation of dental developmental disturbances revealed that the most commonly described dental changes were root stunting, shortened or V-shaped roots, and premature apical closure. The authors also extensively described dental agenesis and microdontia. These alterations were seen in a variable number of patients (33.3%–100%), but most articles concluded that nearly all of the childhood cancer patients submitted to HSCT presented at least one dental development disturbance. The most affected teeth were the first and second premolars and the second molar, with the first molars or anterior teeth rarely being involved. A significant dental development disturbance was found in those patients submitted to radiation therapy as a preparative regimen for HSCT ($p = 0.01$). The studies that presented statistical comparisons between different chemotherapeutic preparative regimens revealed poor statistical significance for differences in chemotherapeutic regimens. Age at HSCT was found to be more relevant in dental development disturbances.

Quantitative comparisons of dental development disturbances

The papers published more recently used panoramic radiography to assess and compare dental developmental disturbances, and these evaluations involved performing tooth-area or crown-root (C:R) ratio measurements with imaging software and then performing statistical comparisons. The authors quantitatively evaluated crown-root proportion alterations and correlated them with administered chemotherapeutic agents, focal or total body radiation therapy, age at HSCT and, in one study, alveolar bone growth and facial development.[5]

The evaluation of tooth area or C:R proportion revealed that nearly 80% (± 3.7) of the HSCT patients showed smaller teeth or C:R alterations with shortened roots. When tooth area or C:R proportions of HSCT patients were compared with matched age and sex pairs, statistically significant

differences were found (p = 0.002). In addition, the most affected teeth were the first and second premolars and the second molars.

Correlation of age with dental development disturbances

There were strong correlations between age at HSCT and risk of developing dental disturbances. The patients that were submitted to HSCT before the age of 4 years showed a significantly higher prevalence of qualitative and quantitative developmental dental disturbances compared to normative data for first and second premolars in both the maxilla and mandible, as well as for second molars in the mandible ($p < 0.001$). In addition, the childhood cancer patients revealed to be at significantly higher risk of presenting precocious apical closure and advanced dental age in comparison with non-HSCT children with matched age and sex ($p < 0.020$). Childhood HSCT radiation therapy appeared to increase the risk of the occurrence of dental development disturbances, although, due to different preparative regimens, no statistical test could be performed to evaluate this risk increase. Different chemotherapeutic agents did not change the risk of developing dental developmental disturbances.

Review considerations

Our reviewers identified several faults in the published articles. First, not all of the studies presented detailed information about the chemotherapeutic preparative regimens used, failing to fully describe the dosages and administration protocols of antineoplastic agents. In addition, poor descriptions were found of the total body radiation therapy, whenever it was employed. Since we believe that dental development is a highly sensitive continuous process, detailed descriptions would seem to be important for identifying, preventing and managing the late effects of dental alterations induced by the chemo-radiotherapy administered for HSCT. The reviewers also identified an inaccurate description of the follow-up time among the published papers. This must be due to the difficulty involved in accompanying childhood-HSCT long-term survivors. Since the survival rates are continually rising, as is the number of patients likely to be included in study groups, we believe that this time issue will be surpassed as further investigations are conducted about the late systemic and local effects of HSCT.

Conclusions and future directions

This systematic review summarizes the literature on the long-term effects of treatment-related dental development disturbances in survivors of childhood onco-hematological cancer. Only ten published papers fulfilled the inclusion/exclusion criteria and were evaluated for:

- type of study,
- number of individuals (n),
- preparative regimen therapies,
- qualitative and quantitative dental development disturbances,
- follow-up time, and
- age at HSCT.

In addition, data on the purpose, results, and main conclusions were gathered.

Data assembling suggested that a younger age at transplantation and preparative regimens seemed to be related to a higher risk of developing dental alterations, such as agenesis, dental hypoplasia, root stunting, crown-root proportion alterations, and microdontia. Additionally, total body radiation therapy and high-dose chemotherapy increased the risk of developing dental development disturbances in HSCT children. There was not sufficient evidence to indicate that different chemotherapeutic mieloablative agents can have increased effects on dental development.

There was a positive correlation between pa-

tient age at HSCT and an increased risk of developing dental developmental disturbances. This finding seems reasonable, since tooth formation is a continuous, highly sensitive and complex process led by several cytokines and an intricate interaction between buccal epithelium and underlying ectomesenchyme.[19,20] Tooth development begins just after birth, lasts all childhood, and follows subsequential stages:

- initiation stage,
- bud stage,
- cap stage,
- bell stage, and finally
- maturation.[21]

These physiological processes occur at different moments of life, and any disturbance may interfere with tooth development. At the age of 4 years, a child has already formed the first molars and anterior teeth, while premolars and second molars are developing.[22,23] This chronology may explain why these teeth showed the highest qualitative and quantitative dental alterations.

The limitations of the studies included in this systematic review should be considered. First, the cross-sectional observations of childhood cancer may have resulted in selection bias. It is possible that children with adverse dental outcomes were more likely to return for evaluation and care, which could have inflated report estimates. On the other hand, those patients who did not survive long enough to be included in the study groups may have biased the reported dental outcomes toward the null. Another study shortcoming was related to the small number of survivors, especially when there was no non-HSCT control group with matched age and sex, which may have compromised the data evaluation and led to non-reliable results. Furthermore, some authors failed to present accurate descriptions of the chemical agents, radiation mieloablative therapy and preparative regimens used, whereas others failed to report specific information about the demographic characteristics of the study population and mean follow-up time.[14,15,24] To offset this lack of data, further investigations are warranted to provide more accurate information and help patients and dental care providers to improve the long-term quality of life of HSCT patients, focusing on the late effects involved.

In conclusion, although HSCT has improved the survival rates of childhood cancer patients, this improvement has been accompanied by the occurrence of long-term treatment-related complications such as secondary neoplasms, organ dysfunction and psychosocial and cognitive disturbances[25] that may entail the perception of a poorer quality of life.[18] The dental alterations induced by HSCT include aesthetic and functional impairment, and periodontal bone resorption, resulting in impairment of chewing ability and a greater risk of early tooth loss, thus jeopardizing the long-term maintenance of oral health.[24] In addition, it has been recently suggested that these dental complications could increase the cardiovascular morbidity and mortality rates associated with dental care intervention among survivors. Further studies are required to outline the late dental effects of HSCT among survivors of childhood cancer treatment, contributing with new information about the extension of dental alterations, and with useful evidence to help predict the location of enamel defects. This information would help the dental team to provide improved dental care for these children and more accurate information to patients and parents on the possible long-term sequelae they might experience.

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