The influence of cone-beam computed tomography and periapical radiographic evaluation on the assessment of periapical bone destruction in dog’s teeth

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Objective. The aim of this study was to determine the influence of periapical radiographs, cone beam computed tomography (CBCT) sections, and cone beam volumetric data on the determination of periapical bone destruction in endodontically treated distal root canals of premolar canine teeth. Nontreated mesial roots were used as controls.

Study design. Enterococcus faecalis strain (ATCC 29212) was inoculated into 30 root canals of 2 mongrel dogs to induce apical periodontitis. After 60 days, the root canals of the distal roots of the 11 mandibular and 4 maxillary premolars were endodontically treated (n = 15). The mesial root canals were used as controls (no treatment). The bone destruction was evaluated after 6 months by 5 evaluators using periapical radiographs and by CBCT (coronal and sagittal sections). After the experimental period, the area of the lesions in periapical radiographs and CBCT sections were measured in mm² using the ImageTool software. A single evaluator measured the volumetric data using the OsiriX software. The comparison between the diagnosis methods in treated root canals and controls was performed using parametric and nonparametric criteria. The Pearson correlation coefficient was computed between radiographic values and CBCT volumetric data in treated root canals and controls.

Results. The results showed the presence of chronic apical periodontitis in every inoculated tooth. After 6 months, periapical radiographs, coronal CBCT sections, and volumetric data showed lower bone destruction in endodontically treated teeth in comparison with the control group (P < .05). The 5 evaluators found no differences between the apical periodontitis area of treated teeth and controls when CBCT sagittal sections were used (P > .05). No correlation was found between x-ray and CBCT volumetric values in treated root canals.

Conclusions. Although selected CBCT sagittal sections showed similar values of bone destruction in endodontically and nontreated root canals, volumetric CBCT data showed that periapical lesions of endodontically treated root canals had half of the volume of periapical lesions in nontreated root canals. No relationship could be found between the periapical values of bone destruction and volumetric data found in CBCT of treated root canals. (Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2011;112:272-279)
detected by radiograph.\textsuperscript{8} Barthel et al.\textsuperscript{12} and Tanomaru-Filho et al.\textsuperscript{13} suggested that in some cases, histologic signs of inflammation can be present in periapical tissues without suggestive image of apical periodontitis in periapical radiographs.

The use of cone beam computed tomography (CBCT) has shown promissory results in recent years in the diagnosis of apical periodontitis in comparison with periapical radiographs.\textsuperscript{14,17} An advantage of CBCT is the ability to show reconstructed slices avoiding the cortical bone through the use of specific software.\textsuperscript{17} Wu et al.\textsuperscript{18} discussed the impact of CBCT for future clinical research involving the success/failure of endodontic treatment because limited bone destruction shown in periapical destruction could not correspond to the real status of the periapical tissues. Despite the clear advantages of CBCT, current literature shows that it still is necessary to establish qualitative and quantitative parameters for the evaluation of teeth with endodontic treatment using this technology. The presence of periapical lesions in CBCT images can be determined measuring the area of the lesion on tomographic sections,\textsuperscript{19} using scores\textsuperscript{20,21} or volumetric data.\textsuperscript{22} This last method has the advantage of taking the 3D information into consideration. The aim of this study was to determine the influence of periapical radiographs, CBCT sagittal and coronal sections, and CBCT volumetric data on the determination of periapical bone destruction in endodontically treated distal root canals of premolar canine teeth. Nontreated mesial roots were used as controls.

**MATERIAL AND METHODS**

This study was performed in accordance with the Ethical Committee for Animal Experiments (CEPA 08/2007). A total of 11 mandibular premolars and 4 maxillary premolars of 2, 1-year-old mongrel dogs were selected for treatment (30 roots). The animals were intramuscularly and intravenously anesthetized using tiletamine-zolazepam (zoletil 100, Virbac, São Paulo, Brazil) at a dose of 0.10 mL/kg body weight; the dose was supplemented when necessary. Local anesthesia was also induced using lidocaine. The same anesthetic protocol was repeated for each study procedure. Periapical radiographs of the selected teeth were taken using a custom-made film holder. After rubber dam placement and decontamination procedures using hydrogen peroxide and 4% tincture iodine, access cavities were made on the occlusal surface using high-speed burs (KG Sorensen, São Paulo, Brazil).

Mechanical disruption of the pulp tissue was performed using a 25-size Hedstrom file and the root canals were contaminated with 100 μL of an overnight culture of brain heart infusion (BHI) *Enterococcus faecalis* (ATCC 29212).\textsuperscript{5} The access cavities were sealed with glass ionomer cement (Resiglass R, Biodinâmica, Ibiporã, PR, Brazil) and standard periapical radiographs were taken after 60 days to monitor the development of radiolucent periapical areas. Heliodent x-ray unit (Siemens, Malvern, PA) was set at 60 kV(p), 10 mA, and 0.4-second exposure.

After the induction period, the temporary material was removed. Then, the pulp chamber was irrigated with 2.5% sodium hypochlorite and the root canals of the distal roots were endodontically treated (n = 15). Initially, the distal canals were negotiated using size 15 and 20 K-files (Dentsply Maillefer, Ballaigues, Switzerland), 2 to 3 mm short of the radiographic length. Then, RaCe rotary instruments 35.08 and 40.10 (FKG, La Chaux-de-Fonds, Switzerland) were used at 500 rpm in a crown-down motion 2 mm short of the radiographic length. Next, the working length (WL) was established radiographically and the 40.10 instruments were used at the WL, and apical preparation was completed using 45.02 and 50.02 K-files; 2 mL of 2.5% sodium hypochlorite was used continuously after the use of each manual or rotary instrument. Root canals were then irrigated with 2 mL of 17% EDTA (Biodinâmica, Ibiporã, PR, Brazil) and a final flush of 2 mL sodium hypochlorite was used. After that, the canals were immediately dried using paper cones and filled with gutta-percha and Sealer 26 (Dentsply, Rio de Janeiro, Brazil) using the lateral compaction technique. The pulp chamber and the access cavity were filled with glass ionomer. Mesial canals were not endodontically treated and served as controls (n = 15). After the follow-up period of 6 months, the animals were killed using an anesthetic overdose and the maxillaries were dissected and fixed in formalin buffer solution. Then, radiographic and tomographic images were acquired according to the following procedures that were performed on the same day.

**Method of radiographic and tomographic analysis**

A Heliodent x-ray unit (Siemens) and 0.4-second exposure time was used. Periapical radiographs (Ektaspeed, Kodak, Rochester, NY, USA) of the dissected specimens were taken using a film holder that was parallel to the studied teeth to minimize size distortion. The radiographs were developed and digitized using a scanner (HP ScanJet G4050, Wilmington, DE, USA) and the images were saved as TIFF files with 600-dpi resolution using Adobe Photoshop software (Adobe Systems Inc., San Jose, CA).

For tomographic evaluation, the maxilla and mandible specimens were left in occlusion and submitted to tomographic cone beam scanning (I-CAT tomograph, Imaging Sciences International, Hatfield, PA, USA). The parameters for scanning procedure were field of view 13
cm; exposure time 40 seconds; and 0.2 voxel. The DICOM data of each dog was recorded on a CD and the data were transferred to an Apple MacBook computer. To compare the periapical images with the tomographic pictures, sagittal sections of each hemiarcade were produced by using the multiplanar reconstruction tool (MPR) using OsiriX 3.6.1 software (Open-source DICOM viewer, http://www.osirix-viewer.com) running under Mac OSX 10.5.8 software (Snow Leopard, Cupertino, CA, USA). Coronal tomographic sections corresponding to the major axis of the treated and nontreated root canals were also created. See Fig. 1. A scale of 1 cm (10 mm) was outlined in the tomographic images using the software to assist posterior analysis.

Digital periapical and tomographic images were imported into the Image Tool software, version 1.2 (University of Texas Health Science Center at San Antonio [UTHSCSA], TX). Periapical images presented to the evaluators were restricted to the evaluation of the apical area of each root being the cervical third and the pulp chamber of the root canals covered.

The Image Tool software was calibrated using the dimensional known values of the periapical radiographs 30 × 40 mm or by using the 10-mm scale in the tomographic images. Five postgraduate students (4 endodontists and 1 oral radiologist) evaluated the periapical and CBCT images. Images of periapical tissues of unaffected teeth were shown to the evaluators to recognize the normal periapical anatomy in dogs. The contour of the radiolucent images suggestive of periapical lesions in the periapical radiographs and tomographic images were outlined on the computer monitor with the cursor and the values obtained were automatically converted to square millimeters (mm²). Blind evaluation of the treated and nontreated root canals was not possible because identification of the control group was evident in periapical radiographs by the absence of filling material.

A single evaluator (endodontist) with experience in the use of OsiriX software performed the volumetric analysis using the sagittal slices. The periapical lesion of each root was outlined using the polygonal tool in all the sections in which the periapical lesion was present (region of interest). After the selection of all of the regions of interest, OsiriX was used to calculate the volume of the selected areas in cubic millimeters (see Fig. 2). The measurements were performed twice to ensure reproducibility.

**Statistical analysis**
The measurements of the 5 evaluators in periapical or tomographic evaluations were submitted to statistical analysis. The absence of normal distribution of the data was confirmed in preliminary tests. To evaluate the effect of endodontic treatment, the Mann-Whitney U test was performed in periapical images of the radiolu-
cent areas of the control and treated root canals and also in coronal sections and sagittal sections. To determine the reliability of the evaluators’ measurements, the Friedman test and intraclass correlation coefficient were calculated. For volumetric data, the paired $t$ test was used to measure differences of the 2 evaluations performed by the evaluators. An independent $t$ test was used to show differences between treated root canals and controls. Normal distribution of volumetric data were confirmed in preliminary analysis. The Pearson correlation coefficient was also computed between radiographic values and CBCT volumetric data in treated root canals and controls. The analysis of all the tests was performed using the GraphPad Prism (GraphPad, La Jolla, CA) and MedCalc software (MedCalc Software, Mariakerke, Belgium).

Fig. 2. A, Representative pictures of a CBCT “stack” of a maxillary premolar. The region of interest was segmented in each slice using the OsiriX software. The 3D reconstruction of the segmented lesion can be seen in (B) and (C).
Table I. Median and range in mm$^2$ of the area of periapical lesions in endodontically treated root canals and controls measured by 5 evaluators on periapical radiographs and CBCT sections

<table>
<thead>
<tr>
<th>Evaluators</th>
<th>Periapical radiographs</th>
<th>CBCT coronal sections</th>
<th>CBCT sagittal sections</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Treatment</td>
<td>P</td>
</tr>
<tr>
<td>1</td>
<td>4.26 (2.18-11.00)$^{ab}$</td>
<td>2.43 (0.61-6.10)$^a$</td>
<td>.020</td>
</tr>
<tr>
<td>2</td>
<td>4.73 (0.00-18.31)$^{ab}$</td>
<td>2.60 (0.70-7.00)$^a$</td>
<td>.012</td>
</tr>
<tr>
<td>3</td>
<td>5.63 (0.00-15.08)$^{ab}$</td>
<td>3.45 (0.13-6.00)$^a$</td>
<td>.021</td>
</tr>
<tr>
<td>4</td>
<td>3.81 (1.76-7.80)$^{ab}$</td>
<td>1.12 (0.52-7.55)$^a$</td>
<td>.001</td>
</tr>
<tr>
<td>5</td>
<td>3.71 (0.59-15.50)$^a$</td>
<td>1.34 (1.25-12.98)$^a$</td>
<td>.046</td>
</tr>
<tr>
<td>ICC</td>
<td>0.73 (0.45-0.89)</td>
<td>0.87 (0.73-0.95)</td>
<td>.95 (0.84-0.98)</td>
</tr>
</tbody>
</table>

The comparison between treated root canals and controls was performed using the Mann-Whitney U test ($P$ value). CBCT, cone beam computed tomography; ICC, intraclass correlation coefficient is also indicated.

$^{a,b}$Different letters in each column indicate significant difference among the evaluators. Friedman test ($P < .05$).

RESULTS

Preoperative periapical radiographs showed that the lamina dura was present in all experimental teeth before the experimental procedures. After 60 days of apical periodontitis induction, all the evaluated teeth showed absence of lamina dura or showed periapical lesions in radiographic images. The median and range of the experimental values of each evaluator are shown in Table I. After 6 months, Mann-Whitney U test showed lower areas of bone destruction in endodontically treated teeth in comparison with the control group when periapical radiographs and CBCT coronal sections were evaluated ($P < .05$). No statistical differences were found when the periapical area of the lesions of endodontically treated roots was compared with controls when sagittal sections were used ($P > .05$).

Friedman test results for interobserver variation and intraclass correlation coefficient (ICC) are shown in Table I. Friedman test restricted to evaluators 1, 2, and 4 showed no statistical differences among the measurements using periapical x-ray and CBCT images in treated and untreated teeth ($P > .05$). The third and the fifth evaluator had a tendency to measure the higher and lower area values in all the analyzed images. Although significant differences in measurement were seen in control teeth using periapical radiographs and in CBCT images, the means of these differences were less than 1.92 mm$^2$, 2.01 mm$^2$, and 1.28 mm$^2$ in untreated teeth using periapical radiographs and CBCT images of control and endodontically treated teeth, respectively. The volume of periapical lesions in mm$^3$ of the evaluated groups is shown in Table II. Paired Student t test showed no statistical differences between the 2 measurements performed by the evaluator. Overall, the lesions in untreated teeth doubled the volume in comparison with the volume of treated teeth, which was a significant difference ($P < .05$). Pearson correlation coefficient was computed using the mean of radiographic values of the 1, 2, and 4 evaluators because they showed the highest agreement among their measurements and the mean of CBCT volumetric data. No correlation was found between these parameters in treated root canals ($r = 0.48$). A positive correlation was found in controls ($r = 0.73$). Fig. 3 shows representative images of the evaluated sections.

DISCUSSION

In the past 3 years, there has been an increase in the number of studies aimed at showing the limitations of periapical radiographs for the diagnosis of apical periodontitis.10,19,25,26 The present study confirms this information and shows that larger resorption areas can be seen using selected CBCT sections in comparison with the areas found in periapical radiographs showing the complexity to delimitate the extension of radiolucent images in periapical radiographs, thus in agreement with previous studies.10,14,15 The limitations of periapical radiographs is a topic being regularly discussed; this fact was shown by Bender and Seltzer in 1961 when they showed that cancellous bone lesions with absence of cortical resorption cannot be observed using intraoral radiographs. This fact was confirmed in the present study by the absence of correlation between the periapical images and volumetric data found in CBCT.
slices of treated root canals. Therefore, bone destruction can be present and no compatible image can be detected in periapical radiographs.26

The median of the lesion area in the untreated roots found in the periapical radiographs was in the range of 3.71 to 5.63 mm²; similar values were found in other studies, 4.35 mm² (Tanomaru-Filho et al.13) and 4.00 mm² (De Rossi et al.25) after 60 days of contamination.

In the endodontically treated teeth using a 1-appointment procedure, the results of the evaluators showed medians of 5.16 to 6.44 mm² and 1.12 to 3.43 mm², respectively, when the CBCT sagittal and periapical radiographs were used. These results are similar to the values found in another study19 that found values of 7 mm² when the treatment was performed in a single appointment protocol and 5 mm² when calcium hydroxide was used as dressing.

The increased measured areas in tomographic sections in comparison with periapical radiographs can be explained by the possibility of exploring the CBCT data using specific software (as I-CAT Vision or OsiriX). In this way, the periapical region of the evaluated teeth can be explored from the buccal to the lingual aspect at intervals of 0.2 mm, which is impossible to be performed using periapical radiographs in which the final image represents the sum of several structures, including healthy cortical and cancellous bone.27 In addition, this study confirms that sagittal sections of the hemi-arch give a more reliable determination of the major diameter of the periapical lesion extension.19 Ideally, the area of the periapical lesions of the studied root canals should be compared with tomographic baseline images taken before and after the induction procedure.27 However, technical limitations for positioning the head of the animals in the I-CAT device and potential movement with subsequent distortion during the scanning discarded this analysis.

Regarding the low success rate found in the endodontically treated teeth, the results could be explained by the poor microbial control of the selected protocol that includes the lack of intracanal medication. Leonardo et al.28 showed that even intentional enlargement of the apical foramen of infected root canals treated in a single appointment was not followed by a higher degree of repair in comparison with root canals treated with calcium hydroxide. Borlina et al.29 showed that for

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Fig. 3. A to F, Representative periapical radiographs and CBCT sagittal sections. In all the cases a marked increased area of periapical bone destruction can be seen in CBCT sections.
Sealer 26 and endomethasone sealers, the widening of the apical foramen favored healing after the use of calcium hydroxide. Interestingly, in this work, the area of periapical destruction was restricted to 1.12 to 3.43 mm$^2$ when periapical radiographs were used, showing that endodontically treated tooth lesions could be more restricted to the narrow bone in comparison with the nontreated roots. These results are in agreement with restricted to the narrow bone in comparison with the that endodontically treated tooth lesions could be more restricted to the narrow bone in comparison with the nontreated roots. These results are in agreement with previous studies and were confirmed using volumetric data. In fact, the goal of endodontic treatment is not only to eliminate bacteria, but also their toxins. Previous studies in animals have shown the ability of calcium hydroxide to neutralize endotoxins in necrotic root canals. Clinically, it appears that endodontic treatment still should be limited to protocols with well-documented histologic success. The void created by new diagnosis technology (CBCT) emphasizes the necessity of well-controlled clinical studies so as to establish the success rate and limitations of nonsurgical endodontic treatment. The same can be applied to the use of intracanal medication when the diagnosis of necrotic pulp and chronic apical periodontitis is present.

Despite the advantages of sagittal sections to evidence apical periodontitis, the selection of the slice with the largest periapical bone destruction in this study can overestimate the actual bone loss because it did not take the 3-dimensional change into consideration. As with other studied techniques, subjectivity of the evaluator may confound the data obtained because tracing of the periapical lesion on the slices using OsiriX software is still dependent on the accuracy of the examiner. However, OsiriX software is a free open-source software that can help to encourage future CBCT volumetric studies. The training and use of multiple evaluators to measure volumetric data and the improvement of the voxel size of future CBCT devices could lead to more accurate results. In addition, the use of microcomputed tomography that reaches resolutions of 9 to 18 μm is also interesting for the understanding of experimental CBCT-based volumetric studies.

CONCLUSIONS

Although selected CBCT sagittal sections showed similar values of bone destruction in endodontically treated and nontreated root canals, volumetric CBCT data showed that periapical lesions of endodontically treated root canals had half of the volume in comparison with nontreated root canals. No relationship could be found between the periapical values found in x-ray and CBCT volumetric data of treated root canals.

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