Validation of micro-computed tomography for occlusal caries detection: an in vitro study

Abstract: Conventional methods of caries detection, including the gold standard of histological examination, have certain disadvantages that must be addressed prior to validating any other diagnostic technique—current or new. Here we evaluated the validity of micro-computed tomography (micro-CT) as an alternative gold-standard technique for caries detection. Sixty teeth with suspected occlusal caries were chosen from a pool of teeth extracted for orthodontic, periodontal, or surgical reasons. Identical reference points were marked on photographs taken for teeth and were used to evaluate each method. Dimensions of caries were assessed by two calibrated examiners using the ICDAS-II visual examination system, bitewing radiographs, and micro-CT. The teeth included in the study were selected randomly from solution before all measurements. For micro-CT, the device was set to 50 kV, 800 µA, pixel size 15 µm (at 1024 × 1024 resolution), and 1° rotation step. NRecon software (SkyScan) was used to obtain reconstructed images. For each diagnostic method, results were compared with histology results using the McNemar test. Receiver operator characteristic (ROC) analysis was also performed for each method (Z-test; p < 0.05). Besides showing a high correlation with histology results, micro-CT yielded the greatest values at the D3 threshold; moreover, accuracy and area under the ROC curve (AUC) values were greatest at the D1 threshold. Our results indicate that micro-CT performs as well as or better than histological examination for the purpose of comparing methods for caries detection.

Keywords: Dental Caries; Microtomography; Occlusal Caries.

Introduction

Radiographic and visual examinations are usually insufficient for detecting occlusal caries lesions. Therefore, research has been undertaken to improve these methods, and new techniques have been designed to achieve an ideal method for caries detection.1,2,3 An ideal method of caries detection must comprise the highest sensitivity, specificity, and accuracy with the greatest area under the receiver operator characteristic (ROC) curve (AUC).

Histological sectioning is the most-preferred method for validating caries depth. During histological sectioning, however, the sections can be damaged or the area of interest may be compromised because of sample...
loss owing to saw blade thickness. Briefly, problems encountered during sectioning and the limited ability of microscopes to visualize the affected tissue are the main disadvantages of histological validation. To overcome such obstacles, a non-destructive visualization method, namely micro-computed tomography (micro-CT), has begun to be used to examine caries.4,5,6,7

High-resolution micro-CT is an innovative version of medical CT, which can be used to obtain images of dental structures in three dimensions with cubic voxels and isotropic resolution. Micro-CT has been used to measure the dimensions of teeth, to assess root canal morphology, to monitor root canal preparation procedures of teeth, and to evaluate cortical bone dimensions and implantology procedures in dentistry.7,8,9,10 Although dental tissues can be scanned at high resolution with micro-CT, this technique cannot be adapted in routine clinical practice because it entails a high dose of radiation.11 Other limitations of micro-CT are its high cost, long scanning and reconstruction times, and the expense of software needed to manipulate images and obtain the desired measurements.12 Still, in addition to its high image quality, micro-CT allows three-dimensional reconstruction of images and permits evaluation of tooth sections without superimposition.9,10,13,14 Owing to these attractive features, micro-CT can be used to detect the depth of a lesion, although there is still no consensus regarding the validity of micro-CT for this purpose. Therefore, we sought to validate the use of micro-CT as an alternative to histological examination for determining the depth of caries in vitro.

Methodology

After receiving approval from the Ethics Committee of Hacettepe University (registration number: LUT 11/38), written informed consent was obtained from all patients. Sixty permanent molar and premolar teeth with suspected occlusal caries lesions were included in the study. Our samples were selected from teeth freshly extracted either for orthodontic, periodontal, or surgical reasons. Teeth were cleaned with a pumice-water slurry using a rotary brush; remnants and remaining stains were removed with hand instruments. Teeth were stored in 10% neutral-buffered formalin solution throughout the study.

Images of occlusal surfaces were acquired, and the suspected spot (test site) on every single tooth was recorded. The same reference points on each tooth, i.e., marked on photographs, were used for comparing the efficacy of the various visualization methods. First, the examiners together explored how to conduct each method before evaluating the caries lesions. All measurements were performed twice by the same two experienced and calibrated examiners independently. And, measurements were then repeated 1 week later.

All of the obtained scores, described for each evaluation method separately, were combined according to “sound/enamel caries/dentin caries” classification to compare the methods via statistical analysis. For the D1 threshold, a score of 0 was considered as “sound”, and scores of 1 and 2 were considered as “caries”. For the D3 threshold, scores of 0 and 1 were considered as “sound”, and a score of 2 was considered as “caries”.

Examination methods

Visual inspection

The test sites, marked on photographs, were examined with a light reflector and without magnification by two trained examiners. First, teeth were examined without drying the surface and then again after drying for 5 s with compressed air. The examiners did not use instruments such as a mouth mirror or explorer, which could have destroyed remineralizable tissues at the area of interest. Visual examination was scored according to the following criteria of the International Caries Detection and Assessment System-II (ICDAS-II).15

0. Sound tooth;
1. First visual change in enamel;
2. Distinct visual change in enamel;
3. Localized enamel breakdown;
4. Underlying dark shadow originating from dentin;
5. Distinct cavity with visible dentin;
6. Extensive distinct cavity with visible dentin.

Scores 1 and 3 were the cut-off points for the ICDAS-II visual examination criteria for the two thresholds D1 (caries) and D3 (dentin caries), respectively.
Radiographic method

Three teeth were selected randomly and mounted in a block of silicone to facilitate the examination. A wax block, 1 cm on a side, was placed in front of the buccal side of the silicone block to represent soft tissues. Focus-to-film distance was set to 21 cm. The x-ray device (Belmont Phot-X II, Osaka, Japan) was set to 70 kV, 7 mA, and 0.13 s exposure time. E-speed radiographic films (Eastman Kodak, NY, USA) with a bitewing film holder were used according to standard conditions. After exposure, all films were developed automatically (Velopex, London, UK) on the same day and were viewed independently using a light box without magnification (Figure 1). Criteria modified from the criteria described by Ekstrand et al. were used to score caries lesions as follows.

0. No radiolucency visible;
1. Radiolucency visible in enamel;
2. Radiolucency visible in the outer half of dentine;
3. Radiolucency visible in the inner half of dentine.

Scores of 1 and 2 were the cut-off points for the radiographic method for thresholds D1 and D3, respectively.

Micro-CT examination

Tooth crowns were separated from their roots to reduce ring artifacts. Images of tooth crowns were obtained with a SkyScan 1774 unit (SkyScan, Kontich, Belgium). The device was set to 50 kV, 800 µA, a pixel size of 15 µm (at 1024 × 1024 resolution), and 1st rotation step. Mean time of the exposure was 73 min. SkyScan NRecon software was used to reconstruct the images; in this way, 700 to 1000 slices were obtained for each tooth (Figure 2). Images were assessed by the examiners using SkyScan Data Viewer software. The criteria used for the radiographic method was also applied for the micro-CT examination.

Scores of 1 (D1) and 2 (D3) were the cut-off points for the defined criteria.

Histological examination

Histological examination was used as the gold standard for validating lesion depth. Tooth crowns were embedded in methyl methacrylate, and two to four sections from the area of suspected caries of each tooth were obtained in the bucco-lingual direction with a diamond saw placed on a microtome (Isomet 1000, Buehler Ltd., Lake Bluff, USA). After grinding and polishing, crown slices of ~100 µm thickness were examined under a light microscope (Leica Dental Corp., Wetzlar, Germany), as shown in Figure 3. In case of a disagreement among the examiners, consensus was reached by repeating the examination. Downer’s criteria were used for lesion depth used for this study.

0. No enamel demineralization;
1. Enamel demineralization limited to the outer 50% of the enamel layer;
2. Demineralization involving the inner 50% of the enamel, extending to the enamel-dentine junction;
3. Demineralization involving the outer 50% of the dentine;
4. Demineralization involving the inner 50% of the dentine.

Scores of 1 and 3 were the cut-off points for the Downer’s criteria for thresholds D1 and D3, respectively.

Statistical analysis

Statistical analysis was performed using SPSS 15.0 (SPSS Inc., Chicago, USA) and NCSS 2007 (NCSS, Kaysville, USA) software. The intra- and inter-examiner agreements were evaluated using the Kappa coefficient. Data were analyzed separately for each observer. Sensitivity, specificity, accuracy, and AUC values were calculated at thresholds D1 and D3 for all methods. Each diagnostic method and gold standard were compared using the McNemar test. The McNemar test and Nam’s test were used to compare sensitivity, specificity, and accuracy of the methods for paired samples of intra-examiner evaluation at thresholds D1 and D3. ROC analysis was also performed for each method at both thresholds. Differences between

Figure 1. Bitewing radiography images.
Validation of micro-computed tomography for occlusal caries detection: an in vitro study

AUCs were determined with the Z-test, and $p < 0.05$ was considered statistically significant.

Results

In total, 60 occlusal test sites were analyzed. According to histological criteria, occlusal surfaces of 11 samples were sound ($D_0$), 9 had initial enamel caries ($D_1$), 13 had advanced enamel caries ($D_2$), 19 had initial dentin caries ($D_3$), and 8 had advanced dentin caries ($D_4$). The reliability of caries detection — as assessed with the Kappa coefficient — is presented in Table 1.

The Viera and Garrett\textsuperscript{18} criteria were adapted when comparing the reliability of the caries detection methods. Micro-CT, histology, and radiography methods indicated almost perfect inter- and intra-examiner agreement, and visual examination indicated substantial agreement. The greatest Kappa coefficient was recorded for examiner 1 for the micro-CT analysis, for examiner 2 for bitewing radiography, and for inter-examiner agreement for the histology analysis.

Specificity, sensitivity, accuracy, and AUC values for both examiners and thresholds are presented in Tables 2 and 3, and these values for ICDAS-II application to compare the micro-CT and histological examinations are presented in Table 4.

At threshold $D_1$, radiographic method had the lowest statistically significant sensitivity (0.694–0.714) whereas visual examination had the lowest statistically significant specificity (0.545–0.364) for both examiners. Micro-CT had the highest values for accuracy and AUC (0.917–0.949).

At threshold $D_3$, micro-CT had the highest values for almost all thresholds, and these values were quite similar to those for the histological gold standard as seen at threshold $D_1$ for micro-CT values.

At both thresholds, ICDAS-II values were similar for both micro-CT and histological examination.

Discussion

In dental practice, radiographic methods and visual and tactile examinations are the most-preferred techniques for caries detection. Compared with these conventional techniques, new methods such as laser fluorescence and transillumination are not as routinely used.\textsuperscript{1,19,20,21} Although radiographic examination often yields a poor outcome at threshold $D_1$,\textsuperscript{1,3,19,20} in this regard we observed a relatively improved correlation between radiographic results and outcome. This can be explained by the inclusion of a greater number of samples with advanced caries in our study compared...
with other studies, although the teeth in our study were selected based on minimal occurrence of fissure caries. ICDAS-II, a relatively new visual examination method, exhibited acceptable performance except for specificity values at threshold D1. Although the teeth were carefully cleaned, these false-positive results were reported by both examiners, probably owing to color changes and deep fissures at the selected occlusal sites. In the case of dentin caries (D3), the ICDAS-II classification system also exhibited good performance similar to the findings of previous studies.5,6,22,23

Bitewing radiography exhibited greater specificity but lesser sensitivity than visual examination. Therefore, radiography and ICDAS-II should be combined for caries detection. However, Neuhaus et al.19 reported that radiography and ICDAS-II lacked sufficient performance with respect to occlusal surfaces of primary teeth, and they recommended the use of bitewing radiographs for approximal caries lesions rather than occlusal caries. Some studies have reported that primary teeth exhibit thinner enamel and a relatively different enamel structure compared with permanent teeth,19,20,21 which may explain such dissimilar results.

Removing caries lesions and moving teeth apart with orthodontic separators are the gold-standard

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**Table 2.** Sensitivities, specificities, accuracies, and AUC values for each detection method at threshold D1.

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Accuracy</th>
<th>AUC</th>
</tr>
</thead>
<tbody>
<tr>
<td>E1</td>
<td>E2</td>
<td>E1</td>
<td>E2</td>
<td>E1</td>
</tr>
<tr>
<td>BW</td>
<td>0.694</td>
<td>0.714</td>
<td>1.00</td>
<td>0.909</td>
</tr>
<tr>
<td>ICDAS-II</td>
<td>0.939</td>
<td>0.939</td>
<td>0.545</td>
<td>0.364</td>
</tr>
<tr>
<td>Micro-CT</td>
<td>0.898</td>
<td>0.898</td>
<td>1.00</td>
<td>1.00</td>
</tr>
</tbody>
</table>

E1: Examiner 1; E2: Examiner 2; D1: D0 = sound, D1-D4 = decayed.
Within columns, significant differences are represented by different superscript letters (McNemar and Nam’s test, α = 0.05).

**Table 3.** Sensitivities, specificities, accuracies, and AUC values for each detection method at threshold D3.

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Accuracy</th>
<th>AUC</th>
</tr>
</thead>
<tbody>
<tr>
<td>E1</td>
<td>E2</td>
<td>E1</td>
<td>E2</td>
<td>E1</td>
</tr>
<tr>
<td>BW</td>
<td>0.778</td>
<td>0.667</td>
<td>1.00</td>
<td>1.00</td>
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<tr>
<td>ICDAS-II</td>
<td>0.741</td>
<td>0.741</td>
<td>0.970</td>
<td>1.00</td>
</tr>
<tr>
<td>Micro-CT</td>
<td>0.889</td>
<td>0.926</td>
<td>1.00</td>
<td>1.00</td>
</tr>
</tbody>
</table>

E1: Examiner 1; E2: Examiner 2; D3: D0-D2 = sound, D3, D4 = decayed.
Within columns, significant differences are represented by different superscript letters (McNemar and Nam’s test, α = 0.05).

**Table 4.** Sensitivity, specificity, accuracy, and AUC values with respect to 95% confidence intervals for ICDAS-II associated with micro-CT and histological examination at both thresholds.

<table>
<thead>
<tr>
<th></th>
<th>Micro-CT</th>
<th>Histology</th>
</tr>
</thead>
<tbody>
<tr>
<td>D1</td>
<td>E1</td>
<td>E2</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>0.977 (0.882-0.996)</td>
<td>0.932 (0.818-0.976)</td>
</tr>
<tr>
<td>Specificity</td>
<td>0.500 (0.280-0.720)</td>
<td>0.250 (0.102-0.495)</td>
</tr>
<tr>
<td>Accuracy</td>
<td>0.850 (0.760-0.940)</td>
<td>0.750 (0.640-0.860)</td>
</tr>
<tr>
<td>AUC</td>
<td>0.858 (0.758-0.958)</td>
<td>0.761 (0.641-0.882)</td>
</tr>
<tr>
<td>D3</td>
<td>E1</td>
<td>E2</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>0.750 (0.551-0.880)</td>
<td>0.800 (0.609-0.911)</td>
</tr>
<tr>
<td>Specificity</td>
<td>0.917 (0.782-0.971)</td>
<td>1.000 (0.901-1.000)</td>
</tr>
<tr>
<td>Accuracy</td>
<td>0.850 (0.760-0.940)</td>
<td>0.920 (0.847-0.987)</td>
</tr>
<tr>
<td>AUC</td>
<td>0.865 (0.768-0.962)</td>
<td>0.897 (0.799-0.995)</td>
</tr>
</tbody>
</table>

E1: Examiner 1; E2: Examiner 2; D0 = sound, D1-D4 = decayed; D3: D0-D2 = sound, D3, D4 = decayed.
methods for in vivo studies, whereas histological examination is commonly used for in vitro studies to validate caries detection methods. Rodrigues et al. reported different results in terms of standardization when comparing four different validation techniques, and they showed that stereomicroscopy may yield false-positive results. Based on their findings, here we used light microscopy, which also provides detailed information regarding lesion boundaries.

A micro-CT device does not damage samples and can evaluate approximately 1000 sections of a tooth at once. A few studies have tested the validity of micro-CT for caries detection. In our present study, the validity of micro-CT was assessed as an alternative to histological examination. Both histological (0.895, 0.947, 0.973) and micro-CT (0.873, 0.898, 0.975) methods indicated almost perfect agreement with respect to intra- and inter-examiner reproducibility.

Micro-CT performed best for both thresholds D1 and D3 with only a few exceptions. Interpolation of the data presented by both examiners yielded a value of 0.939 for visual examination at D1 in terms of sensitivity. In addition to this value, the AUC value for radiographic examination (0.976) for examiner 1 at D3 yielded the best performance. However, this radiographic examination value for examiner 1 did not differ significantly from the value of micro-CT (p > 0.05). Willekens et al. emphasized that increasing the resolution of micro-CT to detect caries lesions requires much more scanning time, and Taylor et al. reported that image quality can be improved by scanning at higher resolution. Mitropoulos et al. claimed that micro-CT examination cannot be used as the gold-standard technique for caries detection because they recorded 0.83–0.84 for sensitivity, 0.87–0.88 for specificity, and 0.85 for accuracy without AUC values. Contrary to their findings, our micro-CT results at threshold D1 were 0.898 for sensitivity, 1.00 for specificity, 0.917 for accuracy, and 0.949 for AUC obtained with ROC analysis. Similar results were obtained at D3. Thus, micro-CT showed favorable results that are in accordance with the findings of Kamburoğlu et al. and Soviero et al. who recommended micro-CT as an alternative to histological examination for in vitro studies.

Our ICDAS-II sensitivity, specificity, accuracy, and AUC values correlate well with other reports. Specificity values for ICDAS II were low, but the other values were high, and this pattern was similar for both micro-CT and histology.

Our results corroborate the utility of micro-CT as an alternative to histological examination for in vitro studies. Obviously, it is not possible to attain 100% accordance with gold-standard techniques as demonstrated by Rodrigues et al. It seems it is more important to establish a more practical and valid gold-standard method.

Carrying out micro-CT evaluations during routine clinical examinations is not feasible because it entails a high radiation dose and requires too much time for scanning, reconstruction, and taking measurements from images. As an in vitro diagnostic technique, however, it is not destructive, and scan time can be increased to reach higher image resolution for enamel caries detection. Additional studies must be undertaken to assess the validity of micro-CT as the new gold standard and to compare it with other established techniques. However, within the limitations of the present study, we observed a high correlation between micro-CT and histological methods.

**Conclusion**

Micro-CT can be preferred as the new gold-standard technique to investigate the depth of caries under laboratory conditions.
References