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Invited article

Accuracy and reliability of enamel and dentin thickness measurements on micro-computed tomography and digital periapical radiographs[#]

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ABSTRACT

In the application of scientific human skeletal variation in medico-legal matters, virtual anthropology is the current technique performed to examine skeleton and its body parts. Digital imaging techniques are used in many areas of dentistry and forensic dentistry. Among all digital imaging modalities, digital periapical radiography (PR) are the most widely used, however, new contemporary imaging techniques micro-computed tomography (Micro-CT) can be also used. The aim of this study was to assess the accuracy and reliability of enamel and dentin thickness measurement through intra and inter-observer error analysis, and comparison was made between periapical radiographs and Micro-CT methods. In this study 15 maxillary first premolar were used which extracted for various reasons. Enamel and dentin thicknesses and maximum cervical crown widths (MCCW) of 15 premolar teeth were examined in both Micro-CT and periapical radiographs. The results obtained with the exact maximum cervical crown widths were compared Image J software version. A digital caliper was used to measure the actual MCCW of the teeth. Results exhibited no significant differences in the measurements by the intra or inter-observer error analyses. The intraclass correlation coefficients (ICC) were more than 0.95 by both intra and inter-observer error analyses. There was significant differences in the measurements by PR and Micro-CT methods. By parameters, Micro-CT showed the highest R value (0.962) with the least error in different methods and observers. In conclusion, dentin and enamel measurements by Micro-CT was highly accurate and reliable as in the conventional method (PR). Micro-CT evaluations should be recommended for implementation in the future anthropological studies especially in countries with limited source of dental data.

1. Introduction

Digital imaging techniques are used in many areas of dentistry. In the evaluated of dental anatomy, the measurement of enamel-dentin thicknesses, the planning of the implant, the determination of the distance of the maxillary sinus from the alveolar crest, the measurement of root canal length in endodontics and post-core applications, the radiological evaluation of dental age in forensic dentistry studies, digital radiology can be used [1–3]. Among digital imaging modalities, periapical radiography (PR), panoramic radiography, computed tomography (CT), cone beam computed tomography (CBCT) and micro-computed tomography (Micro-CT) can be displayed [4–7].

Periapical radiographs (PR) are radiographs which are used frequently in dentistry routine and provide detailed images from the related region. The ease of use and accessibility have advantages such as less radiation dose than the other three-dimensional radiographs. However, in addition to these advantages, it has increased the need for 3D imaging due to the limited anatomical structure of the teeth and the resulting complications [8].

Micro-CT images were developed at the beginning of the 1980s are composed of voxels in the range of 5–50 μm. The image in Micro-CTs has a much better spatial resolution as the image is created with voxels about 1000,000 times smaller than CTs [9]. Although at first used in industrial research laboratories, Micro-CTs are now used in dentistry, especially the internal anatomy of the teeth that we cannot see in the clinic with the naked eye [10,11]. Micro-CT can be used in many subjects such as examination of root canal morphology and C-shaped root canal in molar teeth, effectiveness of endodontic materials, finite element analysis and physical properties of materials, tissue engineering, mineralizations of dental structures, dental implant structures and peri-implantitis cases after implant surgery, as well as in vivo and in vitro [9,11–13]. The use of micro-CT within forensic practice

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remains an emerging technology, principally due to its current limited availability to forensic practitioners. Some applications of micro-CT in forensic practice; evaluate the enamel-dentine and pulp cavity, bone pathology, post-mortem interval [14,15].

The aim of this study was to assess the accuracy and reliability of enamel and dentin thickness on periapical radiographs and Micro-CT methods.

2. Material and methods

Based on the literature, a power analysis using GPOWER 1.0 (Faul, Erdfelder, Lang, & Buchner, 2007) was conducted to determine the sample size, which indicated that differences could be detected with at least 15 teeth at a power 80%, 0.8 effect size (d), 95% confidence. This study was approved by the local ethics committee of our faculty.

The teeth had been extracted in our faculty for various reasons unrelated to the present study (mainly due to periodontal disease or orthodontic treatment). For this study, 15 maxillary premolar teeth (7 from females, 8 from males) without major destructions, no caries or no restoration were selected among the teeth that were extracted and kept them in 5% sodium hypochlorite for 24 h to remove periodontal tissue and calculus.

2.1. Periapical radiography evaluation

Periapical radiography was taken using Gendex Expert (Gendex Expert DC, Des Plaines, IL) with a standard #2-size phosphor plaque (Visualix eHD; Gendex) and the following parameters: 70 kV, 7 mA, and exposure time 0.2 s. All radiographs were performed using the paralleling technique.

2.2. Micro-CT evaluation

A high resolution, desktop, Micro-CT system (Skyscan 1172, Bruker-MicroCT, Kontich, Belgium) was used to scan all the teeth. The teeth were scanned at 100 kV, 100 uA beam current, 0,5 mm Al filter, 13.68 µm pixel size, rotation at 0.5 steps. The beam hardening artifact correction of%65 was applied. The mean time of scanning was around 1 h NRecon software (version 1.6.10.6, SkyScan, Kontich, Belgium) was used for the reconstruction of raw images. Other settings included beam hardening correction, as described, and input of optimal contrast limits according to manufacturer's instructions and based on prior scanning and reconstruction of the specimens.

2.3. Image analyses

Micro-CT images were reconstructed with NRecon (ver. 1.6.10.4, SkyScan, Kontich, Belgium) and CTAn (ver. 1.16.1.0, SkyScan, Aartselaar, Belgium) software were used to reconstruct using modified Feldkamp to obtain two-dimensional (2D) axial images. For image reconstruction, ring artifact correction and smoothing were fixed to zero and the beam hardening artifact correction was set to 40% (Fig. 1).

All images obtained from two different devices were converted to TIFF format and then measured using Image J software version 1.52a and bundled with 64-bit Java for Windows (National Institutes of Health, Bethesda, MD) [16,17]. The program was downloaded from http://imagej.nih.gov/ij/download.html. First of all, this software has been calibrated by determining the unit of length in which the pixels are equivalent. Then, the calibrated images were measured in millimeters.

Examination of the crown morphology and all measurements performed in sagittal section. The enamel thickness measurement based on the shortest distance between the outer enamel crown surface and enamel-dentin interface. The dentinal thicknesses were measured as distances between the enamel-dentin interface and the for each pulp horn [6,13]. The other parameter, the distance between the enamel-cement

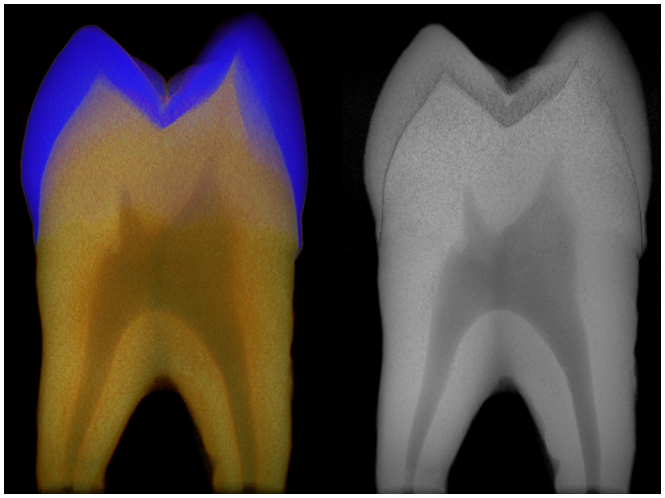

Fig. 1. 3D representation of the teeth using Micro-CT.

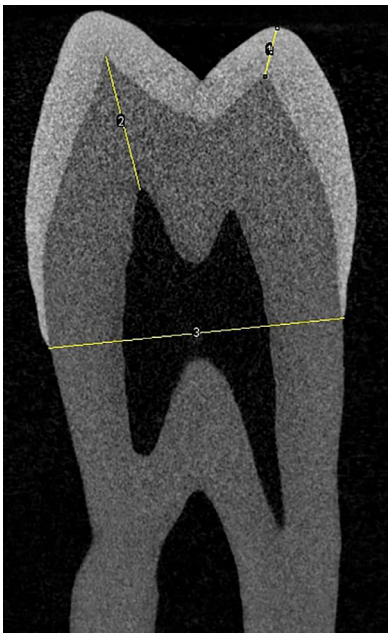

Fig. 2. Measurement in Micro-CT. 1: Enamel thickness, 2: Dentin thickness, 3: Maximum cervical crown width.

junction (CEJ) on both buccal and palatal surfaces was measured to find the maximum cervical crown width (MCCW) [18] (Fig. 2). A digital caliper was used to measure the actual MCCW of the teeth. The same measurements were repeated on two-dimensional images obtained from the periapical radiography images (Fig. 3). Measurements were made by two dentomaxillofacial radiologists and the results were recorded. All measurements were taken twice by the same observers, and the mean values of all measurements were included in the statistical analysis. The observers also performed the study twice with an interval of 2 weeks to detect intra-observer variability.

2.4. Statistical analysis

Kappa coefficients were calculated to assess both intra- and inter-observer agreements for each image set. Kappa values were interpreted according to the guidelines of Landis and Koch [19], adapted by Altman [20] $\kappa \leq 0.20$, poor; $\kappa = 0.21$–0.40, fair; $\kappa = 0.41$–0.60, moderate; $\kappa = 0.61$–0.80, good; and $\kappa = 0.81$–1.00, very good. Moreover, scores obtained from PR and Micro-CT were compared with the gold standard

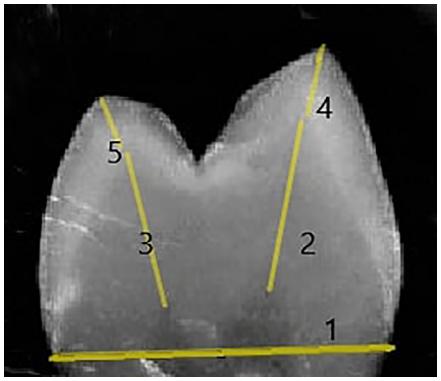

Fig. 3. Measurement in periapical radiography. 1: Maximum cervical crown width, 2: Buccal dentin thickness, 3: Palatinal dentin thickness, 4: Buccal enamel thickness, 5: Palatinal enamel thickness.

via receiver operating characteristic (ROC) analysis. The areas under the ROC curves (AZ values) were calculated using the NCSS 2007 statistical software (NCSS and GESS, NCSS, LLC. Kaysville, Utah). Mann-Whitney U test that the mean of two independent groups was used for statistical analysis. Within-group comparisons were performed using Wilcoxen signed rank significant difference test. P values < 0.05 were considered to indicate statistical significance. Statistical analyses were carried out using the SPSS 21.0 software (SPSS, Chicago, IL, USA).

3. Results

Table 1 shows the intra-observer kappa coefficients calculated for each observer by image type. Higher intra-observer agreement was obtained by comparing images from Micro-CT than PR images. Intra-observer kappa coefficients ranged identical values of 0.965–0.982 suggesting a very good for the Micro-CT images while the PR ranged from 0.721–0.773 suggesting notably good intra-observer agreement. Table 2 shows inter-observer kappa coefficients for both the first and second readings by image type. Higher inter-observer agreement was obtained from the Micro-CT images and compared with those PR images. Very good inter-observer agreement was found for the first and second readings from Micro-CT images. Moreover, moderate inter-observer agreement was found for PR images. The areas under the ROC curves (Az values) for the different observers, readings, and image types were calculated and are shown in Table 3. Higher Az values were obtained for the Micro-CT images than for PR images. The Az values of readings from both observers were higher for Micro-CT images (Table 3).

In particular, Micro-CT, the average enamel and dentin thickness in buccal tubercle was measured as 1.43 mm and 3.76 mm, respectively. The average enamel and dentin thickness in palatinal tubercle was measured as 1.55 mm and 3.72 mm, respectively. In PR, the average enamel and dentin thickness in buccal tubercle was measured as 1.80 mm and 4.03 mm, respectively. The average enamel and dentin thickness in palatinal tubercle was measured as 1.74 mm and 3.99 mm, respectively (Table 4). There was no statistically significant difference between PR and Micro-CT measurement ($p > 0.05$). Although it is not statistically significant in micro-CT measurements, palatinal dentin thickness is higher in males. Although the measurements performed on PR were not statistically significant, the values except the palatinal enamel thickness were higher in males. There was no statistically significant difference between genders in enamel-dentin thickness in both micro-CT and PR measurements ($p > 0.05$) (Table 5).

Table 1
Intra-observer agreement calculated for each observer by image type. PR: periapical radiology.

	Observer 1		Observer 2	
	Kappa	SE	Kappa	SE
PR	0.773	0.027	0.721	0.027
Micro-CT	0.982	0.071	0.965	0.038

Table 2
Inter-observer kappa coefficients among observers for first and second readings. PR: periapical radiology.

	First reading Obs1-Obs2		Second reading Obs1-Obs2	
	Kappa	SE	Kappa	SE
PR	0.652	0.027	0.613	0.027
Micro-CT	0.988	0.054	0.966	0.038

The MMCW was measured as 8.49 mm in Micro-CT and 8.06 mm in PR. The actual length of MCCW was measured as 9.73 mm. MCCW measurements in Micro-CT were significantly lower than the actual MCCW of the teeth ($p < 0.05$). Similarly, MCCW measurements performed on periapical radiographs were significantly lower than the actual MCCW measurements of the teeth. ($P < 0.05$) (Table 6). Since the values in the two groups were lower than the actual size, the difference between the measured values and the actual values was obtained to determine which of the measurements were closer to the actual values. As a result, it was seen that the measurements made with Micro-CT showed statistically significantly more realistic results. ($p < 0.05$).

4. Discussion

Forensic dentistry allows to investigate not only the pathological formations in the teeth, but also the differences between teeth and periods. There are literature studies that dental morphological variations can be used to estimate the dissociation times and migration patterns among local races. Morphological measurements of the teeth can also give an idea about the habits of people. For example, the presence of bruxism may occur in young patients with a reduction in enamel thickness [21]. Studies on tooth size in human society aim to explain the evolutionary mechanism of tooth size and to determine the differences between sexes within societies [22]. In our study, there was no statistically significant difference between enamel-dentin thickness and genders. On the other hand, values except palatinal enamel thickness are higher in males. We think that this is the result because of the small sample size.

In the literature, maximum crown length and bucco-lingual diameter was preferred for the measurement of dental morphology in forensic dentistry [23]. Therefore in accordance with the literature, the maximum crown width, buccal and palatinal enamel-dentin thickness were measured in our study.

The evaluated materials in forensic sciences without harm is important in order to repeat the measurements [3,14]. The advantages of Micro-CT are non-invasive, not causing deformity in the evaluated samples, a better understanding of dental structures due to different lighting and color are used during the creation of images, and allowing the internal anatomy of teeth to be evaluated from different angles [24,25]. In the literature have shown that Micro-CT's are very sensitive to the 3-dimensional surface analysis to understand the morphology of the teeth and to evaluate the anatomy of the teeth that cannot be seen directly with two-dimensional radiography [26–28].

In the forensic dentistry literature, Vandervoorte et al. [29] and Aboshi et al. [30] have reported the use of micro-CT to evaluated the enamel, dentine, and pulp cavity for age estimation purposes. Also, Asami et al. [31] estimated the volume change with age in the premolar crown using Micro-CT. In this study, Micro-CT was preferred to measure the enamel and dentin thickness.

In digital systems, the image consists of image particles called

Table 3
AZ values, standard errors and significance levels for all observers and their readings. PR: periapical radiology.

	Observer 1 First reading			Second reading			Observer 2 First reading			Second reading		
PR	0.6784	0.0452	0.0000	0.6665	0.0244	0.0000	0.6324	0.0339	0.0000	0.6595	0.0167	0.0000
Micro-CT	0.9730	0.0209	0.0000	0.9865	0.0156	0.0000	0.9865	0.0156	0.0000	0.9730	0.0119	0.0000

pixels. Calibration is performed by determining the unit of length in which various computer software and these pixels are equivalent. Linear measurements in millimeters can be performed on calibrated images. In this study, one of such programs, Image J, was used [16,17]. However, different methods have been used in the measurement of enamel-dentin thickness in the literature [5,32–34]. Munhoz et al. [32] evaluated that enamel-dentin thickness in maxillary first premolars using a digital caliper and measured that the mean of mesial and distal enamel thickness in left side was 1.22 mm and 1.39, respectively. In the right side, the average of mesial and distal enamel thickness were 1.22 and 1.28, respectively. They reported that no statistically significant differences between measurements obtained for right and left teeth. These results are thought to be smaller than our study due to the use of different techniques. Macha et al. [33] studied the stereoscopic microscope images of 40 maxillary first premolar (20 left, 20 right) teeth and similar to our work, measured that the mesial and distal enamel thickness were as 1.19 mm and 1.29 mm, respectively. Fernandes et al. [34] calculated the enamel thickness of the mandibular second premolar using a digital caliper and found that the range enamel thickness was 1.35 to 1.46 mm and the average length of the buccolingual was 8.67 mm in right, 8.65 mm in the left side. Although the methods used are different, the results are similar to our study.

Stroud et al. [5] measured the enamel and dentin thickness of the mandibular premolar teeth using bitewing radiography and found that the mean enamel thickness in female and male were measured as (1.99–2.36 mm) and (2.08–2.43 mm), respectively. The mean dentin thickness in female and male were measured as (4.92–4.83 mm) and (5.21–5.19 mm), respectively. The mean of enamel and dentin thickness is smaller than our study. In addition, in contrast to our study, they stated that there was a statistically significant difference between dentin thickness and gender. However, in accordance with our study, enamel thickness shows no significant sex differences. The reason for these differences can be considered as the difference between the technique used and the tooth groups.

There are few studies in the literature that evaluated enamel and dentin thickness using Micro-CT. Suwa and Kono measured buccal enamel thickness on Micro-CT scans in their study and stated that the thickness of the enamel increases in the "functional" cusps. In that study, it was reported that the buccal enamel thickness range between 0.94 mm and 1.55 mm [6]. Similar to our study Hara et al. [15] evaluated that enamel thickness in maxillary first premolars using micro-CT and calculated the mean of the buccal and palatal enamel thickness were 0.36 mm and 0.54 mm, respectively. Dudic et al. [35] evaluated that the root resorption after orthodontic treatment in the premolar was evaluated in both periapical radiography and Micro-CT. Root resorption was observed in Micro-CT scans at a rate of 86%, while root resorption was observed in only 55% in periapical radiography scans. They reported that periapical radiographs had borderline accuracy in the detection of apical root resorption. Similar to this study, in our study, it was seen that measurement on the micro-ct images were closer results in reality.

Bisecting technique and parallel technique are can be used to obtain images in periapical radiographs. However, standardization cannot be achieved in the image bisecting technique and magnifications are seen in the image. Therefore, the use of parallel techniques with film holders is recommended to obtain accurate measurements in the horizontal and vertical directions [4,36]. Therefore, in our study, periapical radiography was performed using a parallel technique.

Siddiqui et al. [37] in their study, measured the dentin thickness of mandibular second premolars using periapical radiography. As a result, the dentin thickness was measured as 4.45 mm minimum and 4.69 mm maximum. They stated that the thickness of the dentin increased with age. These calculated values are higher than those in our study. Differences in dentin thickness can be considered due to reference to different points.

There are some limitations to this study. The first is the small number of samples. Research can be expanded using more examples. Another limitation is that the actual thickness of the enamel and dentin is unknown. In our study, only the real distance of the maximum cervical crown width is known. More accurate comparisons can be made by knowing the thickness of enamel and dentin.

As a result, although morphometric studies performed on Micro-CT images show results that are closer to reality, it is known that periapical radiographic routinely used in the clinic. Morphometric studies can be performed in periapical radiographs due to the fact that it is easy to use, and the magnification rate is low in the radiographs taken in parallel technique. Therefore, both methods can be used to measure the thickness of enamel and dentin in forensic dentistry.

Declaration of Competing Interest

The authors have no relevant conflict of interest to declare.

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Table 4
Measurements of all parameters in Micro-CT and PR (mm). PR: periapical radiology.

		n	Mean	Median	Minimum	Maximum	Standard deviation
Dentin thickness (Buccal Cusp)	Micro-CT	15	3.76	3.71	3.01	5.10	0.62
	PR	15	4.03	4.01	3.20	4.81	0.48
Enamel thickness (Buccal Cusp)	Micro-CT	15	1.43	1.41	1.05	1.87	0.26
	PR	15	1.80	1.80	1.41	2.09	0.19
Dentin thickness (Palatinal Cusp)	Micro-CT	15	3.72	3.45	2.60	5.22	0.79
	PR	15	3.99	4.03	3.16	4.69	0.52
Enamel thickness (Palatinal Cusp)	Micro-CT	15	1.55	1.57	1.02	1.98	0.26
	PR	15	1.74	1.69	1.22	2.38	0.30

Table 5
Comparison of periapical radiography and micro-CT measurements by gender. MCCW: maximum cervical crown width. SD: standard deviation.

		Micro-CT					Periapical radiography				
		n	Mean	Median	Minimum	Maximum	SD	Mean	Median	Minimum	Maximum	SD
Dentin Thickness (Buccal Cusp)	Male	8	3.85	3.64	3.01	5.10	0.67	4.18	4.06	3.68	4.81	0.43
	Female	7	3.66	3.72	3.07	4.84	0.61	3.86	3.79	3.20	4.52	0.52
Enamel Thickness (Buccal Cusp)	Male	8	1.52	1.53	1.16	1.87	0.27	1.83	1.80	1.41	2.09	0.22
	Female	7	1.34	1.38	1.05	1.76	0.24	1.77	1.80	1.50	1.98	0.16
Dentin Thickness (Palatinal Cusp)	Male	8	4.04	3.74	3.05	5.22	0.81	4.05	4.18	3.16	4.65	0.54
	Female	7	3.36	3.20	2.60	4.62	0.64	3.93	3.92	3.40	4.69	0.54
Enamel Thickness (Palatinal Cusp)	Male	8	1.55	1.66	1.02	1.98	0.32	1.73	1.72	1.56	1.96	0.15
	Female	7	1.55	1.53	1.18	1.82	0.20	1.74	1.49	1.22	2.38	0.43
MCCW	Male	8	8.60	8.82	7.63	9.36	0.67	8.20	8.40	7.01	8.89	0.71
	Female	7	8.36	8.50	6.86	9.49	0.80	7.89	8.01	6.17	9.19	0.89

Table 6
The lengths of the MCCW of the teeth in exact in (PR) and micro-CT (mm). MCCW: maximum cervical crown width. PR: periapical radiography SD: standard deviation.

	n	Mean	Median	Minimum	Maximum	SD
MCCW (Micro- CT)	15	8.49	8.53	6.86	9.49	0.72
MCCW (PR)	15	8.06	8.02	6.17	9.19	0.79
MCCW (exact)	15	9.73	10.00	8.00	11.00	1.03

Helsinki declaration

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1964 and later versions. Informed consent was obtained from all patients for being included in the study.

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