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Age Calculation Using X-ray Microfocus Computed Tomographical Scanning of Teeth: A Pilot Study*

ABSTRACT: To correlate dental age with an individual's chronological age based on the calculated volume ratio of pulp versus tooth volume measured, an X-ray microfocus computed tomography unit (μCT) with 25μm spatial resolution was used to non-destructively scan 43 extracted single root teeth of 25 individuals with well-known chronological age. Custom-made analysis software was used by two examiners to obtain numerical values for pulpal and tooth volume. The ratio of both was calculated and statistically processed. No significant intra- or inter-examiner differences were found. In fact, a very strong concordance correlation coefficient was found. Linear regression analysis showed a coefficient of determination (r) of 0.31 which suggests that there is a rather weak correlation between the volume ratio of pulp versus tooth and biological age. Although rather time consuming, this technique shows promising results for dental age estimation in a non-destructive manner using X-ray microfocus computed tomography.

KEYWORDS: forensic science, forensic odontology, dental age calculation, tomography, X-ray computed, non-destructive

Forensic identification includes processing long bones and teeth in order to verify a person's age. Whereas bones and teeth can be both used postmortem, teeth can be inspected also to some degree during life.

The aging of teeth is a unique process. The coronal surface of a tooth consists of enamel, which is the hardest structure of the human body. Under physiological conditions enamel—after formation—does not show age-related changes except for a loss in permeability, an increase in brittleness and a small amount of wear. On the other hand, pathological conditions and behavioral habits may result in caries, erosion, attrition and abrasion; and thus an irreversible loss of enamel after time.

The remaining part of the tooth consists of a pulpodentinal complex (PDC) and a cementum layer on the external root surface. Especially this PDC shows more age-related changes. Morse (1) found that the principal changes of the PDC during life are an increased mineralization of the primary dentin, an accelerated formation of secondary dentin, and a decreased circulation and innervation of the dental pulp characterized by a marked reduction in cells, a relative increase in fibers, fat infiltration and calcification. Altogether the volume of the pulp canal system considerably reduces over time.

Based on these age-related changes a variety of methods for dental age estimation were proposed. Most of them require extraction (indirect measurement) with or without preparation of microscopic sections. These methods are time-consuming and expensive, and the destructive approach may not be acceptable for ethical, religious, cultural, or scientific reasons (2–4).

Since 1982 dental radiography, a non-destructive and simple technique used daily in dental practice, has been employed in methods of age estimation (5). Initial attempts were based on direct and indirect measurements of secondary dentin deposits at the pulp. In 1995 Kvaal et al. (6,7) refined this technique by measuring directly the pulpal size and root size from radiographs of several teeth from the same dentition, and by calculating the ratios of both. They concluded after principal component and multiple regression analysis that measurements on dental radiographs are a non-invasive technique for estimating the age of adults.

Although quite useful, data obtained by X-ray transmission are restricted to two-dimensional information at a low resolution. Improvement of the X-ray transmission technique might be obtained by using X-ray microfocus computed tomography (μCT), a high resolution imaging modality, which allows non-destructive testing. In μCT the same scanning principle as in medical computed tomography (CT) is utilized; however, the spatial resolution is orders of magnitude higher. During the past 20 years, μCT has shown its potential in various fields (8). In dental research, the technique has been used to analyze the three-dimensional structure of root canal systems of extracted teeth before and after endodontic instrumentation (9).

μCT could develop a potential in forensic research as well. The advantages of the technique together with the use of appropriate software may lead to more precise formulas for age estimation based on the quantitative measurements of volumes. The aim of the present pilot study was to correlate the volume ratio of pulp versus tooth with the chronological age of an individual using μCT.

Materials and Methods

Specimen Selection and Hardware

Forty-three teeth (30 incisors, 8 canines, 5 premolars) were collected from 25 different individuals, ranging in age from 24 to

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TABLE 1—*Age and gender distribution of the sample studied.*

Patient	Tooth	Gender	Age
1	31	F	66
2	43	M	65
2	34	M	65
3	42	M	65
4	42	M	63
5	13	F	60
5	23	F	60
6	43	F	55
6	34	F	55
7	32	M	55
7	31	M	55
7	32	M	55
8	21	M	54
8	32	M	54
8	42	M	54
9	31	M	52
9	41	M	52
10	21	M	51
10	31	M	51
11	11	F	51
11	31	F	51
12	41	F	50
12	31	F	50
13	23	M	50
14	11	M	48
15	41	F	48
15	31	F	48
16	43	F	47
17	24	F	47
18	21	F	45
18	22	F	45
19	11	F	44
20	22	M	42
21	41	M	39
21	31	M	39
21	32	M	39
22	43	F	39
22	42	F	39
23	31	M	31
23	32	M	31
24	24	F	28
24	14	F	28
25	23	M	24

66 years of age. The age and gender distribution of the material is shown in Table 1. The selection was restricted to upper and lower single rooted teeth that had been extracted for a number of reasons being mostly periodontal disease or orthodontic treatment. Only those teeth that revealed neither profound caries nor restorations were included. The teeth were stored in individual bottles with alcohol 94% before use.

Scanning was performed using a desktop X-ray micro-CT scanner (SkyScan bvba, Aartselaar, Belgium) providing datasets that were used later for qualitative and quantitative purposes. The device consisted of a microfocus sealed X-ray tube (Hamamatsu Photonics K.K., Hamamatsu-City, Japan), operated at 110, 6 kV and 300 μA, a precision object manipulator with two translations and one rotation, and an X-ray CCD-camera (1024 × 1024 pixels) consisting of a scintilator, lens optics. The geometrical magnification was chosen according to the principle of cone-beam geometry.

Scanning Technique

Each tooth was vertically positioned on a metal holder in the center of the scanner, whereby the apex was fixed with a cyanoacrylate glue (SuperGlue3®, Loctite®, Brussels, Belgium).

FIG. 1—*Proximal projection of a reconstructed tooth after scanning.*

During acquisition (110, 6 kV, 300 μA, 8, 5× magnification), which was the first stage of the μCT procedure, hundreds of two-dimensional projection images through 180° of rotation were sent in a digital form to the computer memory. The time taken for the scanning procedure depends on a number of factors, all related to the level of resolution required. In this study approximately 35 min were needed for each complete tooth scan (Fig. 1).

During reconstruction, which was the next and final stage of the μCT procedure, the data containing projection images were reconstructed into axial cross sections, also called slices, in order to gain the third dimension. As each slice had the same thickness, the stack of such consecutive slices was a true dimensional image volume, the image points of which are called "voxels" (12.5 μm × 12.5 μm × 25.0 μm). Time needed for reconstruction was nearly four hours per tooth. All data were stored for later use by software during post processing.

Post Processing

The three-dimensional set of contiguous sectional images is presented as a gray scale image were each gray scale value corresponds with the intensity value of the voxel. Custom made software, written on top of the public domain Visualization ToolKit (VTK) package (10) was developed for both segmentation and volume measurements by voxel counting.

Segmentation, a step to select structures of interest that are present in the volume data, was done by assessing a threshold. This basic segmentation method will extract voxels whose value falls within a user defined density range. The optimal threshold for the segmentation of the inner and outer contour was visually assessed for each tooth and resulted in an identification of the dental hard tissues and the pulp (Fig. 2) (11).

Volume measurements were calculated on the binary images using region growing technique starting from a seed point placed in a segmented area of the three-dimensional volume. All voxels falling into some kind of user-defined conditions (density threshold) are joined together iteratively from the user-specific seed point until no more growing is possible. The software allowed counting the voxels of the pulp (below the threshold) by using a seed point in the pulpal area. Next, the voxels of the remaining part of the tooth (above the threshold) were counted. The summation of both values gives the total voxel quantity, corresponding with the total volume of the tooth. Dividing pulpal volume by the tooth volume resulted in the volume ratio of pulp versus tooth, which might be correlated to the dental age. Post processing took nearly 25 min for a single tooth. Since the threshold was assessed visually, analysis of all teeth was done and repeated by two independent examiners in order to evaluate reproducibility and accuracy of the method.

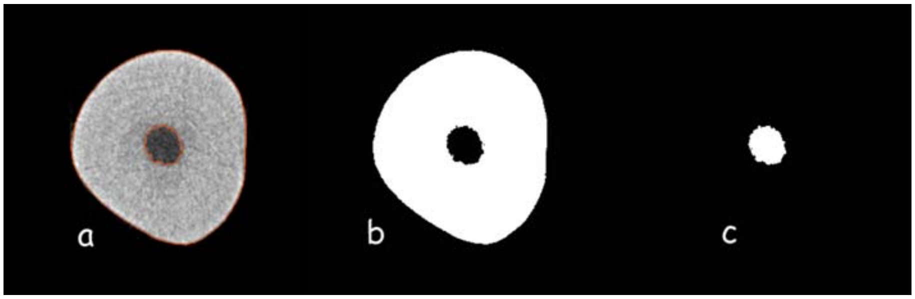

FIG. 2—*Reconstructed axial cross section (a) showing segmentation of the hard tissues (b) and the pulp (c).*

Statistical Analysis

All measurements and additional information (type of tooth, individual's age and gender, threshold value, and voxel count of pulp and tooth) were entered in a spreadsheet (Microsoft® Excel®, Microsoft, Seattle). Statistical analysis was carried out using SAS statistical software package (SAS Institute, Cary, NC). The ratio between pulpal volume and tooth volume (pulp/tooth volume ratio) was calculated and used in the analysis to reduce the effect of variation in tooth size.

Calculation of the concordance correlation coefficient was used to study the intra- and inter-examiner variations. The concordance correlation coefficient (Cc) evaluates the degree to which pairs of measurements fall on the line of agreement. Any departure from this line would produce Cc < 1 (12).

As more than one predictor is available from which to estimate age, a principal component regression analysis of the predictors was performed. Additionally, a regression analysis was performed, using chronological age as the dependent variable and the principal components as independent variables with inclusion level at $P < 0.05$.

Results

Threshold values varied from 75 to 150 (observer A) and from 76 to 141 (observer B). Pulp/tooth volume ratios varied from 0.000102 to 0.028346 (observer A) and from 0.000222 to 0.028575 (observer B).

The analysis of principal components showed that only the pulp/tooth volume ratio had a coefficient significantly different from zero, and therefore the remaining components, being gender and type of tooth were disregarded. Regression analysis with age as dependent variable and the pulp/tooth volume ratio as independent variable showed a weak coefficient of determination: $R^2 = 31\%$. The formula was given as:

$$\text{age} = 61.78 - (788.94 \times \text{calculated ratio}) \text{ (Fig. 3)}$$

Residue analysis revealed a normal distribution.

The concordance correlation coefficients were very high (0.97–0.99), indicating a very strong agreement between two independent measurements of one observer and also between the two observers.

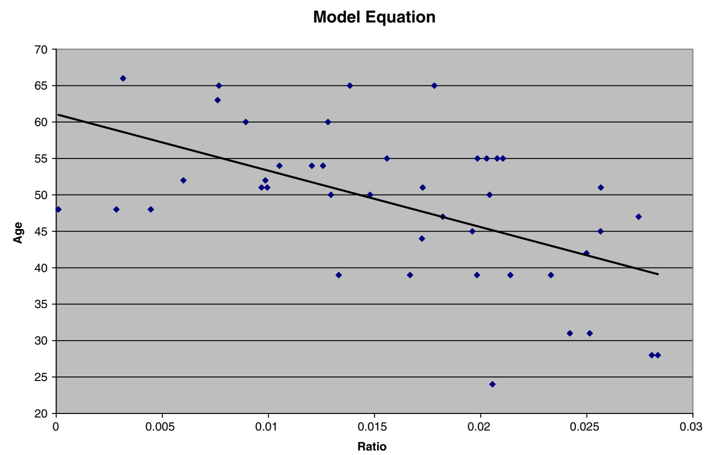

FIG. 3—*Linear regression model with corresponding regression equation.*

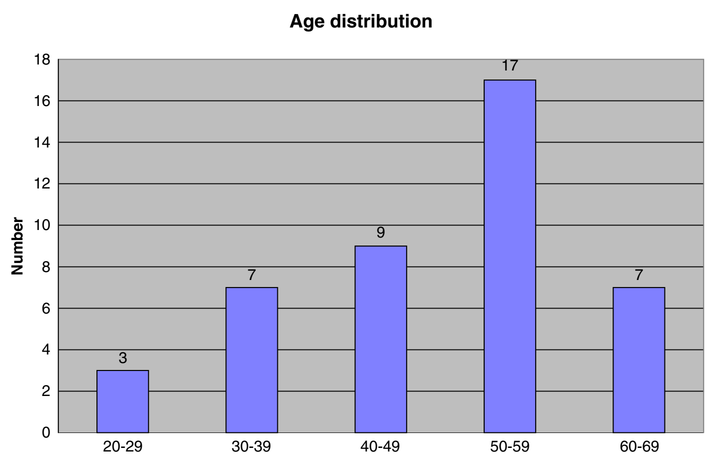

FIG. 4—*Age distribution of the selected samples.*

Discussion

Collection of teeth from individuals in different ages was difficult since they had to be free of decay. Due to dental hygiene, young persons preserve their teeth as long as possible, which resulted in a nonproportional distribution of age (Fig. 4).

A random sample of extracted single rooted teeth was collected ($n = 43$), being incisors, canines and premolars, both from upper and lower jaw. Often, several teeth from the same individual were used.

In previous studies of dental age estimation from two-dimensional dental radiographs, the ratio between the pulpal size and root size and the ratio between the pulpal size and tooth size have successfully been used (5,7). As the size of the pulp is reduced with age, the correlation coefficient between age and the ratio is negative. Based on these earlier studies the ratio between pulpal volume and tooth volume has been chosen as a possible age indicator in the present study, whereas use of the μCT technique added the third dimension.

The aim of the study was to evaluate the usefulness of μCT for age estimation. μCT allows only evaluation of small objects as e.g., single-rooted extracted teeth. In that way, it was possible to image volumes of those teeth non-destructively at a high resolution. Both the pulpal volume and the tooth volume could be processed and their ratio calculated.

Concerning the practical use of μCT, the total procedure including scanning, reconstructing, and post processing was rather time consuming, being 5 h per sample. This could be a first major drawback for implementing the technique in daily practice or on large scale. The use of a threshold might be a second drawback, because reproducibility can be questioned. However, there was strong agreement between two independent readers. Other drawbacks are costs and training periods inherent to μCT technology.

The coefficient of determination R^2, ranging between 0 and 1, is the fraction of the variability in Y (age) that can be explained by the variability in X (pulp/tooth volume ratio) through their linear relationship. So in this study 31% of the age variability can be explained by the variability of the pulp/tooth volume ratio.

The analysis of principal components showed that gender and type of tooth could be disregarded during regression analysis. Nevertheless, in previous studies they found a correlation for some teeth (5,7).

As already mentioned in literature, not every tooth was just as useful. Kvaal found lower correlations for maxillary canine and significant differences between the ratios from the left and right mandibular central incisors, even when using more teeth from each individual. The fact that we used a rather small sample size with a high proportion of mandibular incisors ($n = 12$) could be an explanation that our results are not as good as expected.

Since attrition, caries and periodontal recession may influence tooth volume measurements, further investigation should be done on the use of pulp/root volume ratios which might give a better result and could facilitate the collection of samples. To improve our results, the use of a greater sample of teeth has to be investigated. Adaptation of the software may result in automatic segmentation of pulp, root and tooth, and the calculation of different ratios.

Conclusion

X-ray microfocus computed tomography is a non-destructive tool for imaging internal structures of teeth. The presented methodology shows promising results for estimating biological age based on the pulp/tooth volume. Optimization of scanning and measuring technique as well as increased numbers of samples can therefore be the subject of further investigation.

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