Pulp volume estimation using CBCT- an in vitro pilot study on extracted monoradicular teeth

Akshay Rathore[1*], Suma GN[2], Sharad Sahai[3], Manisha Lakhanpal Sharma[4], Siddharth Srivastava[5]

[1,5]Senior Lecturer, [4]Professor, I.T.S Centre for Dental Studies & Research, Muradnagar, Ghaziabad, Uttar Pradesh, India, [2]Professor & Head, Dept. of Oral Medicine & Radiology, SGT Dental College, Vill. Budhera, Dist. Gurgaon, Haryana, India, [3]Director & Consultant, Dental & Maxillofacial Diagnostics, Rajnagar, Ghaziabad, Uttar Pradesh, India

***Corresponding Author:**
Email: akshaythedoc@gmail.com

Abstract

Aim: Age estimation is an important aspect of forensic odontology. Calculation of pulp volume using CBCT and its correlation with age could be an effective, non destructive method. The aim of this pilot study was to verify and calibrate the proposed method of calculating pulp and tooth volume using CBCT.

Materials and Method: The study was conducted on six extracted monoradicular teeth which were scanned by Cone Beam CT (CS9300-C 3D.Carestream Health, Inc. Rochester, NY) after endodontic preparation. The tooth (TV2) and pulp volumes (PV 2) were then calculated using imaging software (Xelis Dental software (*INFINITT Inc., Seoul, South Korea).* Then actual tooth (TV1) and pulp volumes (PV1) were calculated based on the Archimedes principle. The pulp and tooth volume ratios R1 and R2 were also calculated. The estimated and actual values were compared and percentage error was calculated.

Results: The percentage error was also calculated to evaluate the accuracy which was found to be 8.84%, 2.6% and 12.7% for TV1 and TV2, PV1 and PV2 and R1 and R2 respectively.

Conclusion: These were in accordance with the results by Yang et al.[7] who obtained a percentage error of ±7.8% while the estimated error was quite less than that reported by Star et al[8] who reported the maximum percentage error to be 21% and 16% for pulp and tooth volumes respectively.

Keywords: Age Estimation, Forensic Odontology, Cone Beam CT, Pulp Volume

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Introduction

Age estimation is important in establishing identity of a person and is a sub-discipline of forensic sciences. Various medico-legal issues warrant accurate age estimation thus making it important in the identification process. Multiple age estimation methods have been reported in the forensic literature using teeth over the years.

Most methods described involve sectioning and extraction of teeth and thus are not possible on living individuals. Radiological examination of teeth, on the other hand, is a simple, non-destructive method used to obtain information and does not require extraction.[1,2] Thus techniques which are radiology dependent allow for age estimation in living individuals. Radiological studies that have been conducted mostly depend on developmental stages of teeth and their correlation with age and thus have a limited applicability only up to the age of twenty five years.[3,4,5,6]

The dental pulp can also be used as an indicator of age because it undergoes regression in size with increasing age due to secondary dentine deposition. This is a continuous process and thus it can be used as a parameter of age estimation even beyond 25 years of age**.** In 1995, Kvaal et al developed a new method for estimating the chronological age of adults based on the relationship between age and the pulp size on peri-apical dental radiographs.[1]

Two dimensional imaging is associated with its inherent errors of distortion and magnification and thus limit the accuracy. Cone-beam computed tomography (CBCT) is an imaging modality which generates three-dimensional (3D) data that is accurate and at lower cost and lower absorbed doses when compared to conventional computed tomography (CT).

Yang et al.[7] in 2006 estimated age through volume matching of teeth imaged by cone beam CT. The aim of this study was to attempt establishing a correlation between the chronological age of a certain individual and the pulp/tooth volume ratio (PTV) of one of the teeth. The images were analyzed using the custom-made software and linear regression analysis was performed. The results of the analysis showed a moderate correlation between the pulp/tooth volume ratio and biological age.[7]

3D images generated by a CBCT unit allow for the calculation of volume of each tooth and corresponding pulp chamber. Thus a study was planned to estimate age using calculation of pulp and tooth volume ratios on mono-radicular teeth and their correlation with age. To validate the research protocol a pilot study was

conducted on extracted teeth which verified the efficacy of the method when compared to the gold standard.

Materials and Method

The study was conducted in the Department of Oral Medicine and Radiology, I.T.S Centre for Dental Studies and Research, Muradnagar, Ghaziabad, Uttar Pradesh, India in collaboration with Dental and Maxillofacial Diagnostics, Ghaziabad, Uttar Pradesh, India between April 2013 and May 2013. Intact and fully developed six mandibular extracted monoradicular teeth i.e. mandibular lateral incisor, mandibular canine and mandibular first premolar were included which were free from any morphological abnormalities. Teeth with restorations, prosthetic rehabilitation, caries, attrition, abrasion, erosion, periapical pathology, root resorption, developmental anomalies were not selected.

The pulp canals of the six selected teeth were prepared endodontically. The cone-beam CT images were taken (CBCT Unit – CS9300-C 3D.Carestream Health, Inc. Rochester, NY) using the high resolution dental mode at 84 kV, 5mA. Pulp and tooth volume was calculated using the voxel counting software (Xelis Dental software (*INFINITT Inc., Seoul, South Korea*). The total scan time was 20seconds. Study images were reconstructed from the volumetric dataset, in planes perpendicular to the selected tooth axes i.e. (True and oblique axial, coronal and sagittal) with a thickness of 0.09 mm and an interval of 0.09mm. Cross-sectional images with a thickness of 0.09 mm and an interval of 1mm were also prepared. Image assessment was performed by specially trained Oral & Maxillofacial radiologist for volume estimation using the CBCT software.

The lines were dragged to reorient the tooth to be analyzed in all three axes and the file was renamed and saved. After reorienting, the data were re-sliced and the file was saved in a separate folder from the source file. Next the selected tooth was segmented using the segmentation tool of the software. A mask was created followed by selection of optimal grayscale threshold which showed the tooth within the bone. The mask was then cropped in all three axes to the closest dimension of the tooth. Then the regions not belonging to the tooth were selected and removed manually slice by slice thus removing parts of the cortical bone and adjacent teeth. This segmentation cannot be established by selecting optimal grayscale threshold because there is a minimal or no gray value difference between these closely apposed tooth anatomical structures. This segmentation process separates the tooth structure from within the bone and a final image is generated. A 3D volume calculation of this image using the "*Merge*" tool gives the tooth volume (TV2).

After this using the image segmentation, different regions of interest were identified within the pulp cavity on selected sequential slices. The "Pick and *Grow*" tool in object tools was then used to segment the pulp cavity from the rest of tooth structure, and the segmented pulp was saved as a new object and rendered in a different color (Green). Finally, the "*Merge*" tool was selected to display the volume of the pulp (PV2).

The object analysis tool in Xelis Dental software enabled calculation of ROI Histogram for volume assessment of the pulp cavity and the tooth. Then the PTV ratios were calculated for each study subject.

Next the teeth were filled with impression material (hydrophilic polyvinyl siloxane impression material), Aquasil Ultra XLV (Ultra Light Body), DENTSPLY using a Dispensing gun (*3M USA*). The volume of the whole tooth was measured by the method based on volume displacement by *Archimedes' principle*, which was generally used in determination of density of various materials. The buoyant force on a submerged object is equal to the weight of the liquid displaced by the object. The volume was calculated by, $V = (m_1g - m_2g) / \rho_1 g = (m_1 - m_2)/\rho_1$, where ρ_1 is the density of the liquid, V the submerged volume of the object, g the constant 9.8 N/kg, m_1 the mass of the object and m_2 is the apparent mass when submerged.[7]

Both m_1 and m_2 of all the teeth were measured in the Department of Biotechnology, I.T.S-CDSR, Muradnagar, and Ghaziabad. Electro-balance (Shimadzu Analytical India Pvt. Ltd) with a glass beaker (50ml, Borosil, India) of absolute ethyl alcohol with the density of 0.78 g/cm3 was used for volume measurements.

Each tooth was measured two times. First the actual mass, M_1 was determined**,** next when it was submerged in the alcohol, M_2 was determined. Then the tooth volume was calculated (TV1). Next the dental substrate was dissolved by immersing samples in successively 30% Hydrochloric Acid (HCl) for 36 h and 2.5% Sodium Hypochlorite (NaOCl) for 10 min. The same methodology was used to determine the volume of remaining silicon core which calculated the pulp volume (PV1).[7]

Finally the pulp/tooth volume ratios were calculated. This allowed comparing the outcome of the software with the gold standard measures of the pulp. The statistical analysis was done using SPSS (Statistical Package for Social Sciences) Version 16.0 Statistical Analysis Software.

Results

Six extracted mandibular monoradicular teeth were analyzed and their actual tooth (TV1) and pulp volume (PV1) was calculated by the method based on volume displacement by Archimedes' principle. This allowed comparing the gold standard measures of the tooth (TV2) and pulp volume (PV2) with the outcome of the software.

Mean of actual tooth and pulp volume was found to be 541.72±169.67 mm^3 and 27.63±3.13 mm^3 respectively (Table 1, Table 2) and mean of the estimated tooth and pulp volume by CBCT was found

to be 493.80±169.68 mm^3 and 28.35±4.49 mm^3 (Table 1, 2) while the mean for PTV Ratios R1 and R2 was found to be 0.0550± 0.0168 and 0.0620±0.017 respectively (Table 3).

The difference in the actual and estimated tooth volume was statistically significant [p=0.002] while the difference in actual and estimated pulp volume was not statistically significant [p=0.486].The difference in PTV Ratios R1 and R2 was statistically significant [p=0.002].

The percentage error was also calculated to evaluate the accuracy which was found to be 8.84%, 2.6% and 12.7% for TV1 and TV2, PV1 and PV2 and R1 and R2 respectively. (Table 4)

Table 1: Table showing mean actual and estimated tooth volumes for all 6 teeth in mm^3

Tooth Volume	Mean	S.D.	P-value
TV1	541.72	169.67	0.002
TV2	493.80	169.68	

Table 2: Table showing mean actual and estimated pulp volumes for all 6 teeth in mm^3

Pulp Volume	Mean	S.D.	P-value
PV1	27.63	3.13	0.486
PV2	28.35	4.49	

Table 3: Mean PTV ratios for all 6 teeth

Ratio	Mean	S.D.	P-value
R1 (PV1/TV1)	0.0550	0.0168	0.002
R2 (PV2/TV2)	0.0620	0.017	

Table 4: Percentage error for all 6 teeth

	Mean	S.D.	% Error
TV1-TV2	47.92	18.68	8.84%
PV1-PV2	0.72	2.35	2.6%
R1-R2	0.007	0.0028	12.7%

Fig. 1a: Endodontically prepared extracted tooth

Fig. 1b: Radiograph of endodontically prepared extracted tooth

Fig. 2: Dispensing gun (3M) with polyvinyl siloxane Cartridge

Fig. 3: Electric balance, Shimadzu, Japan

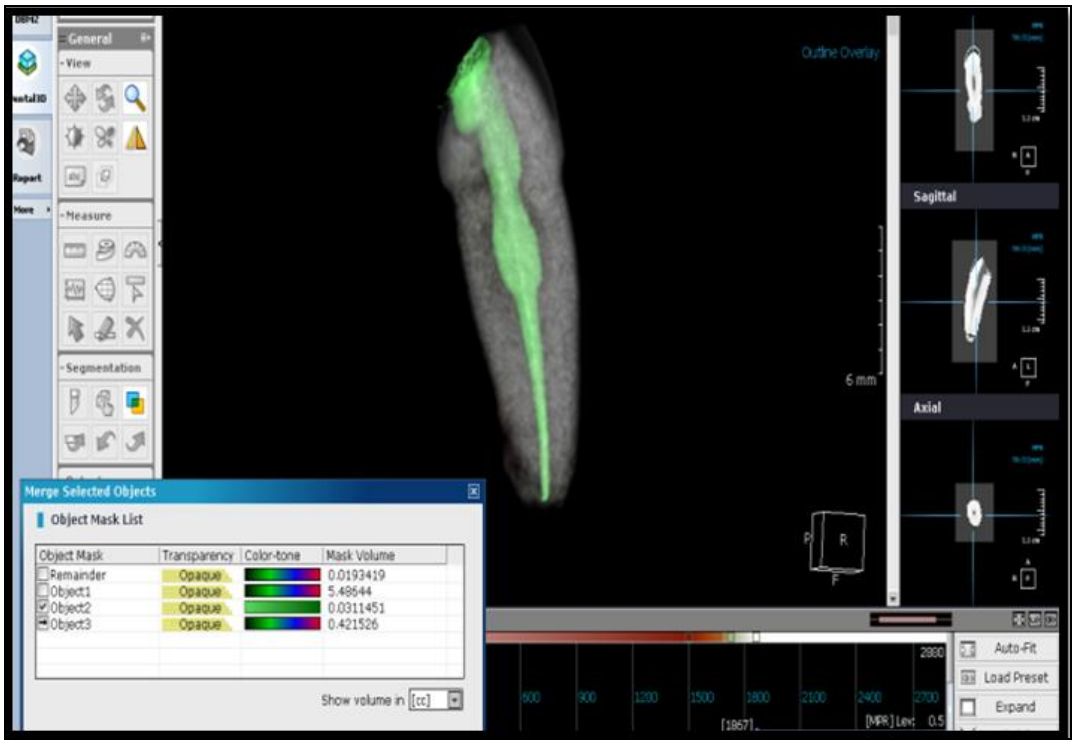

Fig. 4: Segmentation process showing the pulp volume marked with different color

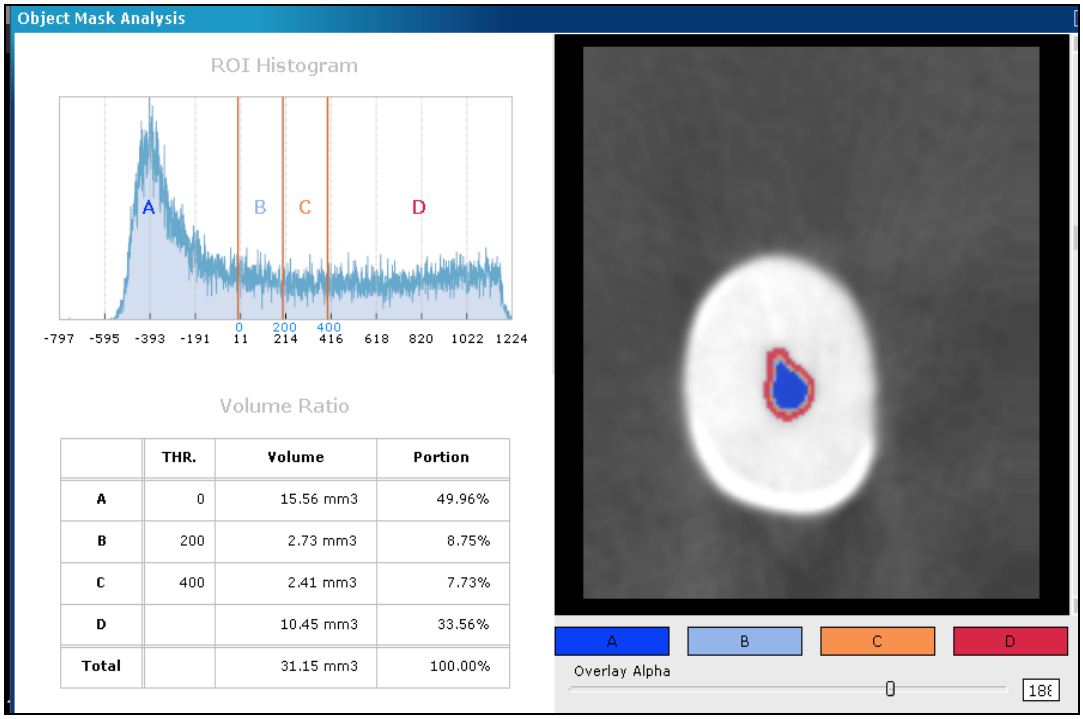

Fig. 5: The object analysis tool in Xelis Dental s/w enabled calculation of ROI Histogram for volume assessment of the pulp cavity

Fig. 6a: Tooth decalcification in 30% HCl

Fig. 6b: Post-decalcification remnant Silicone core

Discussion

The estimated age prediction outcomes allow authorities in their judgment on the chronological age of individuals with a questioned age and provide more accurate post-mortem profiling of unidentified body remains. Age estimation using teeth is viable, because teeth are highly resistant to mechanical, chemical, physical insults and also time. Secondary dentine apposition is a significant morphological dental age predictor.

The formation of secondary dentine is attributed to abrasion, erosion, attrition, caries, changes in pulpal osmotic pressure and aging thus leading to decrease in the pulp volume. Therefore, the volume changes of the pulp chamber in intact teeth are considered as a dental age predictor.

CBCT images of the extracted teeth enable the calculation of tooth and pulp volume non-destructively. Analysis in this study showed an acceptable reproducibility and accuracy with a percentage error of 8.84%, 2.6% and 12.7% for TV1 and TV2, PV1 and PV2 and R1 and R2 respectively.

These were in accordance with the results by Yang et al[7] who obtained a percentage error of ±7.8% while the estimated error was quite less than that reported by Star et al[8] who reported the maximum percentage error to be 21% and 16% for pulp and tooth volumes respectively.

The total procedure including scanning, image reconstruction, pre-processing and measurements took about 1 hour which is much faster than the 5 h per tooth processing time reported in a previous study on Micro CT.[9]

The segmentation process could be challenging because of its dependence on grey scale values and its dependence on inherent resolution of Cone Beam CT. Recent generations of cone-beam CT are available with better contrast resolution and detail which thus will enable improved visualization of the tooth segmentations. The technique needs to be evaluated on living individuals and could be a very objective and non- invasive method of estimating age.

Conclusion

Cone-beam CT scanning provides us a new method to acquire the 3D images of teeth in living individuals by which pulp/tooth volume can be calculated and correlated to age. The presented method shows promising results for age estimation in forensic odontology.

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