

## ORIGINAL ARTICLE

# Pulp Volume - An In-depth Tool in Age Estimation- A Comparative Retrospective Forensic Based Cone Beam CT Study

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## Abstract:

The field of forensic utilizes various cranio-facial structures and skull in identifying an unknown deceased. This identification deals with assessing the gender and age of skeletonized remains based on eliciting the ethnicity of the population at archaeological sites and comparison of post-mortem records with the presumed antemortem records. Interestingly, even a single tooth can be used to assess the age of an individual and this is widely used in forensic for investigating legal matters as well as in scientific research purpose. Teeth are resistant to environmental insults and post-mortem decomposition and hence can be retained without distortion. The objective of this study is to analyse the volumetric data of canine and first molars in cone beam CT for estimating age among samples of various age groups and to compare between those values to evaluate which tooth indexed volumetric data gives more specificity in age estimation. It is a retrospective Institutional based Forensic study conducted using 180 samples from 90 full skull CBCT images whose age ranged between 20-65 years, acquired from the dental archives of department of Oral Medicine and Radiology were equally divided among both genders. Further the samples were categorized into three groups as (20-35), (36-50) and (51-65) years in both the genders. All data samples were assessed using the ITK –SNAP 3.8.0 software. Using semi-automatic active contour segmentation method, the volumes of pulps of upper canine and upper first molar were analysed and calculated data were statistically analysed using discriminant functional analysis and multivariate regression analysis to evaluate the correlation of pulp volumes with respect to chronological age.

The multivariate regression analysis done for estimating age among the given groups of samples using upper canine gave statistically highly significant p value of 0.001, 0.004 and 0.039 for group I [20-35 years], group II [36-50 years] and group III [51-65 years] respectively. The regression analysis done using upper first molar for age estimation gave statistically highly significant p value of 0.001 for group I, whereas group II and group III gave out insignificant results. The comparative analysis between multivariate regression analysis for age estimation of upper canine and upper first molar to determine which tooth index has more specificity, gave statistically significant results for canine samples in all age groups. The samples were also analysed for Pearson correlation in estimating age, which resulted in high correlation in group I samples for both canine and first molar, moderate correlation for group II and group III samples for canine pulp volumes and low correlation for group II and group III for molar pulp volumes. Over the years, CBCT has evolved a lot and today they are being used in various sectors of dentistry. In the field of forensic, various oral and maxillofacial structures are being used as an adjuvant in personal identification with more precision using cone beam CT. The results of the present study show statistically highly significant correlation between age and upper canine samples in all the age groups analysed. Hence, based on the results of present study, the upper canine can be used as a promising adjuvant tool in forensic odontology for age estimation.

**Keywords:** Pulp volume; CBCT; Oral and maxillofacial radiology; Forensic odontology; Age estimation.

## Introduction:

The science of dentistry as related to the law is known as forensic dentistry or forensic odontology.<sup>1</sup> The theory behind forensic dentistry is that “no two mouths are alike.” Kieser-Nielsen<sup>2</sup> defined Forensic odontology as the branch of dentistry that deals with proper handling and estimation of dental evidence and proper evaluation and presentations of dental findings in the interest of dental science. Age estimation plays an important role

in Forensic Dentistry for dead individual identification as well as for alive persons to clarify criminal and civil liability issues.<sup>3</sup> Teeth, skeleton or both structures are used in age estimation as maturity indicators. However, the teeth maturation provides a valuable index of dental age and serves as a better index of the maturation than any other index as they are seldom affected by systemic, nutritional and endocrine status<sup>4</sup> and most of the individuals would have been to dentist in their mean course of life and thus, their ante-mortem data would be available which help in victim identification more precisely. The oral and maxillofacial radiographic field had discovered tracks which are advantageous to mankind in which the radiographs play a crucial role in personal identification. Studies have demonstrated that CBCT is a highly accurate method in personal identification in forensic due to their resolution and the quality of producing images of high

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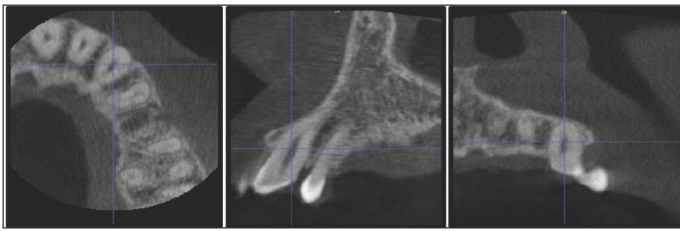


Figure 1. Standardisation.

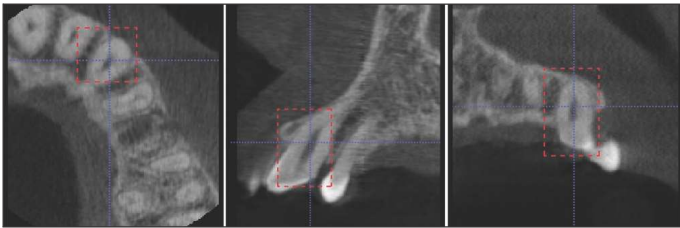


Figure 2. Active contouring of region of interest.

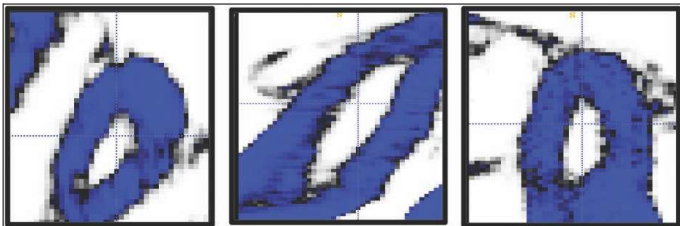


Figure 3. Conversion of grey scale images into blue white window.

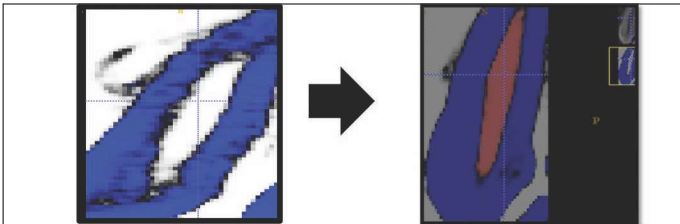


Figure 3. Pre-processing, initialization and evolution.

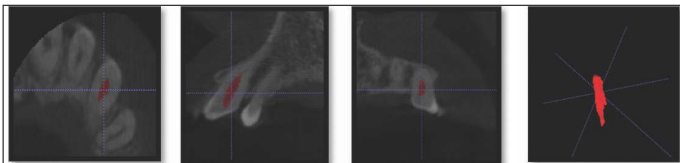


Figure 4. Extraction of pulp volumes from canine.

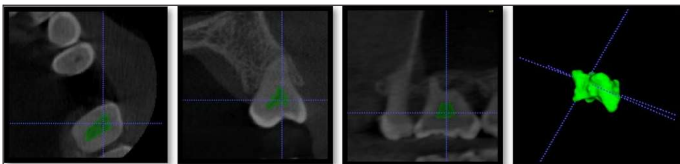
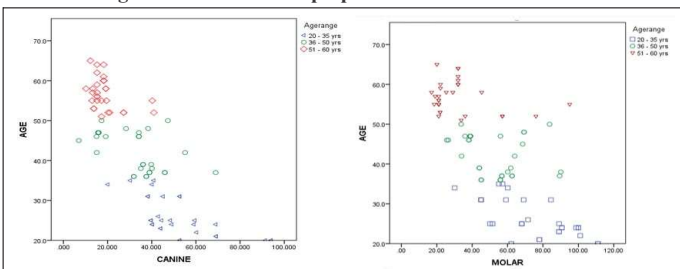


Figure 5. Extraction of pulp volumes from first molar.



Graph 1 and 2. Pearson correlation.

contrast.<sup>5</sup> This present study evaluated pulp volumes of upper canine and upper first molar irrespective of sides to estimate age and to analyse which tooth index gives high specificity with respect to age.

**Materials and Methods:**

Full skull CBCT images of the 90 patients reported to the institution for various dental problems taken in the time period between 2020–2022 were acquired from the dental archives. The scans were generated using the Planmeca Promax 3D Mid Proface CBCT machine obtained at 90Kvp, 10mA and with large FOV 13x15cm. The study population included 45 males and 45 females, CBCT images of good quality and the study group was divided into three subdivisions; group I: age 20-35, group II: age 36-50, group III: age 51-65. Each group contained 60 samples, so total of 180 samples were analysed for estimating age using pulp volumes. All the images were assessed using ITK-SNAP software version 3.8.0. The images with artifacts, developmental disturbances, pathologies in the jaw region were excluded.

**Methodology:**

Step 1: Standardisation [figure 1]. All the CBCT images were standardised prior to measurements by placing the intersection of horizontal and vertical toggle in the pulpal region of tooth of interest in all the 3 sections [Axial, Coronal and Sagittal].

Step 2: The region of interest [figure 2]. From the field of view, the region of interest is marked and highlighted by an active contour segmentation mode in such a way that it only involves the tooth of interest from which the volume of pulp is going to be extracted. This process is done in all the 3 sections coinciding each other and the complete tooth structure should be included to avoid the discrepancies

Step 3: Volume generation using auto segmentation in ITK SNAP software [figure 3]. The cropped out selected region of interest is updated into 3D segment option in ITK SNAP software, which

Table 1 & 2: Multivariate regression analysis for age estimation using canine constant as indicator.

Table 1. Model Summary.<sup>a</sup>

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1 [20 - 35 years]	.663 <sup>b</sup>	.639	.419	3.7519
2 [36-50 years]	.510 <sup>b</sup>	.560	.234	4.3755
3 [51-65 years]	.379 <sup>b</sup>	.414	.113	3.7183

a. Age range. b. Predictors: (Constant), CANINE

Table 2. Coefficients.<sup>a,b</sup>

Model		Unstandardized Coefficients		Standardized Coefficients	t	P-value	95.0% Confidence Interval for B	
		B	Std. Error	Beta			Lower Bound	Upper Bound
1	(Constant)	35.350	2.151		16.431	.000	30.943	39.756
	Canine	-.183	.039	-.663	-4.682	.0001	-.264	-.103
2	(Constant)	48.188	2.068		23.304	.000	43.953	52.424
	Canine	-.185	.059	-.510	-3.140	.004	-.305	-.064
3	(Constant)	60.757	1.932		31.449	.000	56.799	64.714
	Canine	-.211	.097	-.379	-2.169	.039	-.411	-.012

1(A)] Age range = 20 - 35 years, 2] Age range = 36 - 50 years, 3] Age range = 51-65 years. B. Dependent Variable: Age

**Table 3. Descriptive analysis of mean value and standard deviation for individual age groups using one way anova for canine samples.**

Anova <sup>a, b</sup>						
	Model	Sum of Squares	df	Mean Square	F	Sig.
1	Regression	308.644	1	308.644	21.925	.000 <sup>c</sup>
	Residual	394.156	28	14.077		
	Total	702.800	29			
2	Regression	188.731	1	188.731	9.858	.004 <sup>c</sup>
	Residual	536.069	28	19.145		
	Total	724.800	29			
3	Regression	188.731	1	188.731	9.858	.004 <sup>c</sup>
	Residual	536.069	28	19.145		
	Total	724.800	29			

a. Age range [1(20 - 35 years), (36 - 50 years) and (51 - 60 years)]  
 b. Dependent Variable: Age, c. Predictors: (Constant), Canine.

**Table 4 & 5. Multivariate regression analysis for age estimation using first molar constant as indicator.**

**Table 4. Model summary.**

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1[20 - 35 years]	.648b	.420	.399	3.8150
2[36-50 years]	.273b	.075	.042	4.8942
3[51-65 years]	.259b	.067	.034	3.8814

a. Age range, b. Predictors: (Constant), Molar.

**Table 5. Coefficients.<sup>a, b</sup>**

Model		Unstandardized Coefficients		Standardized Coefficients	t	p-value	95.0% Confidence Interval for B	
		B	Std. Error	Beta			Lower Bound	Upper Bound
1	(Constant)	36.736	2.526	-.648	14.544	.000	31.562	41.910
	MOLAR	-.144	.032		-4.504	.0001	-.210	-.079
2	(Constant)	46.380	2.922	-.273	15.875	.000	40.396	52.365
	MOLAR	-.079	.052		-1.503	.144	-.186	.029
3	(Constant)	58.649	1.463	-.259	40.096	.000	55.653	61.645
	MOLAR	-.057	.040		-1.419	.167	-.139	.025

1(A). Age range = 20 - 35 years, 2] Age range = 36 - 50 years, 3] Age range = 51-65 years. B. Dependent Variable: AGE

changes the grayscale images of tooth in interest into a blue and white window, Then, the volume generation using auto segmentation will be done using 3 steps: Pre-processing, initialization and evolution.

- 1] Pre-processing: In this step, upper and lower thresholds are selected to delineate the pulpal area from rest of the tooth structure.
- 2] Initialization: The delineated pulpal area is filled with colour bubble using bubble at cursor option in all 3 sections in multiple areas which serves as a source to semiautomatic segmentation of the entire pulp in following processes.
- 3] Evolution step: In this step, the added bubble marks will start to spread in all directions completely covering the entire pulp without crossing the delineated areas, once the complete area of interest is segmented, it is cross checked in all three planes and 3- dimensional model of extracted pulp can be obtained [figure 4 & 5].

Step 4: Volumetric assessment: Once the area of interest is 3 dimensionally extracted, the volumetric assessment of it could be done by using the same software, using the segmentation mode which gives out precise volumetric data of pulp of tooth in

interest and the data were statistically evaluated.

**Results :**

The collected data were analysed with IBM SPSS Statistics for Windows, Version 23.0. (Armonk, NY: IBM Corp). To describe about the data descriptive statistics frequency analysis, percentage analysis was used for categorical variables and the mean & S. D were used for continuous variables. To find influence of canine & molar with age, the regression analysis model was used to fit the equation. This study evaluated and compared pulp volumes of maxillary canines and first molars in full skull CBCT images of male and female patients for age estimation. The descriptive analysis [One-way Anova] of the pulp volumes and age using canine as indicator, there is a statistically highly significant P value of 0.0001 and 0.004 for group I and group II samples and a statistically significant P value of 0.039 for group III samples [table 3] and for molar as an indicator, there is a statistically highly significant P value of 0.0001 for group I and non-significant P value of 0.144 and 0.167 for group II & III samples [table 6]. Multivariate regression analysis for age estimation using upper canine for both females and males showed statistically highly significant P value of 0.001, 0.004 and 0.039 with correlation [r value] of 0.663 , 0.510 and 0.379 with the error rate of 3-4% for age group I, II and III respectively [table 1 & 2] and a Multivariate regression analysis for age estimation using upper molars for both females and males showed statistically highly significant P value of 0.001 for group I sample with correlation [ r value] of 0.649 and an insignificant P value of 0.144 and 0.167 with correlation of 0.273and 0.259 for group II and III samples with the error rate of 3-4% [table 4 & 5]. Graph 1 and 2 depicts Pearson correlation of canine and molar pulp volumes as an age indicator in which canine showed high correlation between group I sample and moderate correlation between group II and III samples whereas in molar, the correlation was high in group I samples but low in group II and III samples. Comparative regression analysis of canine and first molar for age estimation in group I sample gave R value of 0.007 and shows statistically significant P value of 0.048 for canine and statistically non-significant P value of 0.082 for first molar and for group II, the estimated R value is 0.513 and shows statistically significant P value of 0.014 for canine and statistically non-significant P value of 0.760 for first molar and for group III , the estimated R value is 0.451 and shows statistically significant P

**Table 6. Descriptive analysis of mean value and standard deviation for individual age groups using one way anova for molar samples.**

ANOVA <sup>a, b</sup>						
	Model	Sum of Squares	df	Mean Square	F	Sig.
1	Regression	295.285	1	295.285	20.289	.000c
	Residual	407.515	28	14.554		
	Total	702.800	29			
2	Regression	54.099	1	54.099	2.258	.144c
	Residual	670.701	28	23.954		
	Total	724.800	29			
3	Regression	30.336	1	30.336	2.014	.167c
	Residual	421.831	28	15.065		
	Total	452.167	29			

a. Age range = [ 1(20 - 35 years), (36 - 50 years) and (51 - 60 years) ]  
 b. Dependent Variable: AGE, c. Predictors: (Constant), MOLAR

**Table 7 & 8. Multivariate regression analysis for age estimation using canine and first molar constant as indicator among group i sample [20-35 years of age].**

**Table 7. Model Summary.<sup>a</sup>**

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1[a]	.707 <sup>b</sup>	.500	.463	3.6091
2[b]	.513 <sup>b</sup>	.263	.208	4.4480
3[c]	.451 <sup>b</sup>	.204	.145	3.6515

1[a]. Age range = 20 - 35 years, 2[b] Age range = 36 - 50 years, 3[c] Age range = 51 - 65 years. b. Predictors: (Constant), MOLAR, CANINE.

**Table 8. Coefficients.<sup>a,b</sup>**

Model		Unstandardized Coefficients		Standardized Coefficients	t	p-value	95.0% Confidence Interval for B	
		B	Std. Error	Beta			Lower Bound	Upper Bound
1	(Constant)	37.649	2.430	-.407	15.494	.000	32.663	42.635
	Canine	-.113	.054		-2.070	.048	-.224	-.001
	Molar	-.079	.044		-1.806	.082	-.169	.011
2	(Constant)	47.665	2.700	-.550	17.654	.000	42.125	53.205
	Canine	-.199	.076		-2.627	.014	-.354	-.044
	Molar	.019	.060		0.309	.760	-.105	.143
3	(Constant)	62.427	2.230	-.981	27.999	.000	57.852	67.001
	Canine	-.547	.254		-2.153	.040	1.067	-.026
	Molar	-.143	.100		1.426	.165	-.063	.348

a. Age range = 20 - 35 years b. Dependent Variable: AGE

value of 0.040 for canine and statistically non-significant P value of 0.165 for first molar.

**Discussion:**

Forensic odontology is one of the integral parts of dentistry which is intended for the handling, examination and evaluation of evidences generated by dental structures, which are to be then evaluated, tabulated and presented to jurisprudence.<sup>6</sup> Forensic specialists initially focus on morphology of the skeletal remains followed by morphometrics in the process of personal identification. Human dentition is often considered as a hard tissue analogue like fingerprints.<sup>1</sup> In several situations, teeth and bones are frequently the only sources available for identification of degraded or fragmented human remains. For age estimation using teeth, increase in secondary dentin deposition and decrease in pulp volume remains one of the reliable and historical indicators.<sup>7</sup> The studies done by Kvaal et al. (1995),<sup>8</sup> Cameriere et al. (2007)<sup>9</sup> and Palak H. Shah et al. (2016),<sup>10</sup> Nima Biuki et al. (2017),<sup>11</sup> stated that there is a negative correlation between age and pulp volume stating that the volume of pulp decreases as the person ages which is also observed in the present study. So far in literature, several 2D imaging modalities have been applied to evaluate the decrease of pulp chamber volume, but it seems that the use of 3D images which demonstrate the real morphological change is the most suitable one in this kind of dental age estimation methods. The advent of Cone beam CT radiography for evaluation of oral and maxillofacial structures had been widely used in dentistry for clinical purposes. The application of CBCT for hard and soft tissues in the body, especially facial bones and teeth, that allows a clinician to obtain a detailed 3-dimensional images.<sup>12</sup> Another advantage of the CBCT machine is that they are easy to operate, ease of handling and ability to offer (from a single scan) a dataset of multiplanar cross-sectional

and 3D reconstructions.<sup>13</sup> In contrast to the present study, several studies were conducted for age estimation in literature using morphometric analysis such as tooth pulp ratio or tooth coronal index. The information obtained in those studies remains inadequate in assessing the fine details. Thus, with the introduction of specialised software technologies like ITK SNAP'S semiautomatic segmentation for volume analysis provides greatest advantage due to their ability to classify the structures precisely. Previous studies in various literature used linear mathematical models to estimate age, but a logarithmic model was developed in the present study. To compare with logarithmic models with linear models, a linear multivariate regression analysis was also conducted with age as dependent variable, pulp chamber volume as independent variable for maxillary first molars and maxillary canine, respectively, in the present study.

The present study focuses on estimating the age using semiautomatic segmentation of pulp volumes of upper canine and upper first molar using CBCT imaging. The results from the present study showed significant (p < 0.001) value for younger age samples and an insignificant p value for age group above 50 using first molar as age indicator which came in accordance with the study by Zhi et al. in 2015,<sup>14</sup> whereas Shiva Kumar et al. in 2016<sup>15</sup> analysed age using same method in Indian Population which also resulted in significant results between younger age group and maxillary molar pulp volumes with a correlation of 27.0% which is in accordance with the present study with the difference in correlation value of 64.0% between estimated age and actual age of samples of younger age groups. In CBCT analysis of age estimation using multi-rooted teeth by Faezeh Yousefi et al. in 2020<sup>16</sup> showed significance between maxillary, molar and age which also coincides with the current study.

Tardivo et al. in 2014,<sup>17</sup> Nima buki et al. in 2017,<sup>11</sup> Kasmi et al. in 2019<sup>18</sup> and Mehrdad Abdinian et al. in 2021<sup>19</sup> reported that use of maxillary canine is better and stronger in estimating age in their respective analysis with a significant correlation (p < 0.001) between maxillary canines and age. Whereas the results of the current study also stated that canine shows statistically high correlation (p < 0.001) between age and canine pulp volume of all the considered samples of different age groups, these results are also in accordance with above mentioned studies. The fundamental result of this study is that on comparing the volumes of maxillary first molar and maxillary canine among various age groups which gave out a statistically significant result (p < 0.001) for canine samples in all the subdivided age groups for both males and females. On the contrary, the results of the current study are in disharmony with the study done by Hazha star et al. in 2011<sup>20</sup> which might be due to reduced sample size and manual processing method of segmenting the pulp from rest all tooth structure unlike this particular study. This commenced study utilised advanced imaging system such as CBCT combined with the use of advanced software methods to obtain absolute pulp volume by using semiautomatic segmentation without using the morphometrics and formulae, which remains superior and can provide up to three times more practical volumetric estimations than manual methods and also to our best knowledge, this current

study is the first among literature to compare the accuracy of pulp volumes of monoradicular and multiradicular teeth of a same individual in estimating age among Indian population. One limitation of the present study is that only maxillary first molars and canines were included as age indicators, this makes difficult to study or estimate age of an unknown deceased person when these teeth are missing or lost or endodontically treated, hence, in near future if teeth volume analysis is estimated for anterior and posterior teeth of both arches using the methodology of current study, the data of such study could provide a better view on selection of tooth for age analysis in forensic context.

### Conclusion:

In the field of forensic, personal identification is an important component and fundamental aspect in the identification of deceased individuals. Circumstances where the comparative DNA samples and fingerprints are not found, the use of the other structures such as teeth or skeleton with radiological identification comes into play. There are many numbers of studies and researches that had been done focusing on the radiological identification of a person in forensic aspect which proved to an effective adjuvant method. As dental professionals, we play a key role in forensic by maintaining quality records of patients and thus could be a part of the investigating team in the field of forensics.

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