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Assessment of Age and Gender Using Pulp Tooth Volume Ratio Of Permanent Maxillary Canine In A Subset Of Indian Population-A CBCT Study

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ABSTRACT
The aim of this study was to assess age and gender using pulp tooth volume ratio (PTVR) of permanent maxillary canine in a subset of Indian population using CBCT. CBCT scans showing permanent maxillary left and right canine of patients older than 16 years of age were selected. A total of 92 scans were selected for study by excluding the periapical pathology, decayed canine, poor resolution and artifacts. PTVR was calculated for each tooth. Linear regression analysis was performed in order to predict age estimation by using PTVR. ANOVA analysis indicated that the regression model predicted the age as a dependent variable significantly well. Correlation analysis of age showed a negative relationship of age with all the variables that indicated a linear relationship. Intra observer and inter observer agreement as suggested by kappa analysis was perfect. The highest correlation was found when age was correlated with average of left and right maxillary canine pulp tooth volume ratio. This study revealed that tooth volume(TV), average tooth volume(ATV) and left canine pulp volume(PV) was gender dependent. PTVR of permanent maxillary canine showed significant correlation with age and gender.
Keywords: *CBCT; Pulp Volume; Tooth Volume; Pulp tooth volume ratio*

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INTRODUCTION

In forensic science, positive identification requires a multidisciplinary team effort as well as speculative or absolute approaches. Age, sex, race, and ethnicity of an individual are some of the most important factors in determining a person's identification, whether living and deceased [1]. When no contextual evidence is available and visual recognition is impossible (for example: skeletal remains), age is one of the vital pieces of information that must be used to reconstruct the identity of the body. In anthropology and forensics odontology, determining age might be difficult at times, but it is crucial [2]. In forensic medicine/dentistry age prediction role is not limited to post-mortem reconstructive identification but, it has a major role in identification of unknown in disasters or events that involve crime, major accidents, employment, labor acts, child marriage, child adoption, illegal immigrants, unaccompanied minor asylum seekers [3]. There is a need to develop scientific evidence for identification based on relevance, reliability, and acceptance in every subject.[1]Teeth are regarded as one of the most reliable ways of personal identification in forensic sciences as they are least affected by genetics, nutritional, environmental and hormonal factors [4].

Therefore, estimating chronological age (CA) through morphological and radiological study of teeth is becoming increasingly essential [5]. Dental age estimation methods rely on developmental, morphological and biochemical changes in teeth. However biochemical methods used aspartic acid racemization which is time-consuming and pernicious [6]. Till 16 years of age, development and eruption of tooth is a good method for determining the age of an individual but after 16 years of age evaluating the dental age is difficult as after 16 years of age only growth of 3rd molar is evaluated which is missing most of times and also it has a lot of variability and debatable accuracy [7].

The teeth consists of enamel, dentin and pulp and there are 3 types of dentin: the primary dentin which is formed until the tooth is functional, the secondary dentin which is formed after root completion and the tertiary dentin which is formed as a reaction to injury [8]. The formation of secondary dentin is caused by attrition, abrasion, erosion, caries, changes in osmotic pressure throughout the pulp chamber and aging. Also, there is a decrease in volume of dental pulp chambers with increasing age. Hence, volume changes of

pulp chamber in intact teeth are considered as a dental age predictor.[9] The size of the pulp chamber has been studied using a variety of approaches, including tooth cross-sections and radiography. The pulp/tooth area ratio (PTAR) of maxillary canines has been assessed using both panoramic and periapical radiography. Radiographs have the fundamental disadvantage of being two-dimensional projections that are vulnerable to significant magnification and distortional errors [2].

The main disadvantages of 2D radiographs are superimposition of structures upon one another and, failure to assess the pulp changes and recognition of overall shape of the tooth.[7] Cone-beam computed tomography's (CBCT) clinical launch has opened up new avenues for obtaining three-dimensional tooth radiographs, with provision of volumetric analysis at optimal image quality and minimal radiation dosage [4]. Correlation of age and the pulp tooth volume ratio (PTVR) was first assessed by Vandervoort et al [10] using Computed tomography (CT), which demonstrated a linear association [10]. CBCT has also been employed to assess the correlation between PTVR and age using automatic or manual segmentation methods [11]. However, different studies have used different teeth like mandibular canines, premolars, incisors and have reported variable results [2, 4].

Maxillary canine has a considerably bigger pulp chamber and generally survives better than other teeth, thus making it ideal for assessing correlation between PTVR and age [12]. Multiple studies have been done that have used maxillary canine for assessing PTVR using CBCT [4-6]. However, all the studies have used automatic segmentation through variable software's. The automatic segmentation may not be representative of the entire volumetric detail.[13] It's likely that variances in primary dentine production exist between males and females, which could explain this disparity. The Y chromosome controls the thickness of dentine, while the X chromosome exclusively affects the thickness of enamel, according to evidence.[7] Also males have large tooth size as compared to females. Thus, the present study was undertaken to assess the correlation of pulp tooth volume ratio of maxillary canine with age using manual segmentation in CBCT. Also, the previous studies have been done in various subsets of population and none have been reported in the North India population. Thus, this would be the first study to assess the PTVR in maxillary canine using manual segmentation and correlating it with age and gender in the subset of North India population.

MATERIAL AND METHODS

A prospective study was done on the CBCT scans available at SGT Center of Maxillofacial Imaging. The study was conducted after the approval by the Institutional review board held on 25th october 2019. CBCT scans were obtained using Planmeca Romexis with 90 kvp and 10 mA. The scans that were exposed for the routine dental care needs of the patient were included in the study. No patient was exposed only for the study. The medium and large FOV(field of view) that showed right and left maxillary canines were included in the study.

Scans were screened to exclude any pathology, poor resolution and artifacts. A total of 1027 scans were screened of which 182 scans showed both maxillary canines which were fully developed with closed apex. However, out of those 32 scans were excluded due to decay in canine, 29 scans were excluded because of periapical pathology in canine, 11 scans were excluded because of poor quality of radiograph and 18 scans were excluded because of crown placement on canine. Thereby leaving only 92 scans for the study.

Methodology:

Multiplanar Reformatted images (axial, sagittal, coronal sections) of maxillary canines (bilateral) were evaluated. Tooth volume (TV) and pulp volume (PV) were recorded using the free region grow tool of Romexis software version 5. Manual segmentation of tooth was done with minimum slice thickness of 0.400 mm and at each slice thickness markings were recorded in axial section and volume was created using the create region of free region grow tool. Similarly PV was recorded on both maxillary canines in the axial section. (Figure 1, 2, 3, 4, 5, 6)

TV and PV of the left and right maxillary canine were entered in the excel sheet. PTVR was calculated using the above-mentioned measurements using the Planmeca Romexis software.

After completing all the samples by principal investigator every 5th sample was again re-evaluated and PTVR was measured and ratio was calculated for intra observer agreement.

The selected sample sizes were reevaluated by co-investigator for assessment of inter-observer agreement.

Statistical Analysis:

Data was analyzed using Statistical Package for Social Sciences (SPSS) version 21, [IBM SPSS statistics (IBM corp. Armonk, NY, USA released 2011)]. Descriptive data was reported for each variable. Kruskal Wallis test was used for comparison of two or more groups and Mann Whitney U test for two groups (gender). A value of $p < 0.05$ was considered statistically significant. ANOVA analysis was done with age as dependent variable and ratio as predictor. Correlation analysis was done for comparing age and volume.

RESULTS

The descriptive analysis regarding distribution of patients according to age and gender showed that subjects were not homogeneously distributed according to age and gender. (Graph 1) ANOVA analysis indicated that the regression model predicted the age as a dependent variable significantly well. (Table 1). The results of Linear regression analysis between each canine PV, PTVR is shown in Table 2. Correlation analysis of age showed a negative relationship of age with all the variables that indicated a linear relationship. (Table 3) (Graph 2) TV of left and right maxillary canine and their average showed statistically significant results with higher TV in males. The left PV showed significant results when compared between both genders with higher significant levels in males. Intra observer and inter observer agreement as suggested by kappa analysis was perfect. Hence, it was statistically significant (p = 0.001). Equations for linear regression analysis between PV, PTVR and average PV and average PTVR with age as dependent variable is predicted in (table 4). Mann Whitney U test regarding the comparison of left canine TV, right canine TV, AVT and left canine PV to gender showed statistically significant relation as depicted in (table 5)

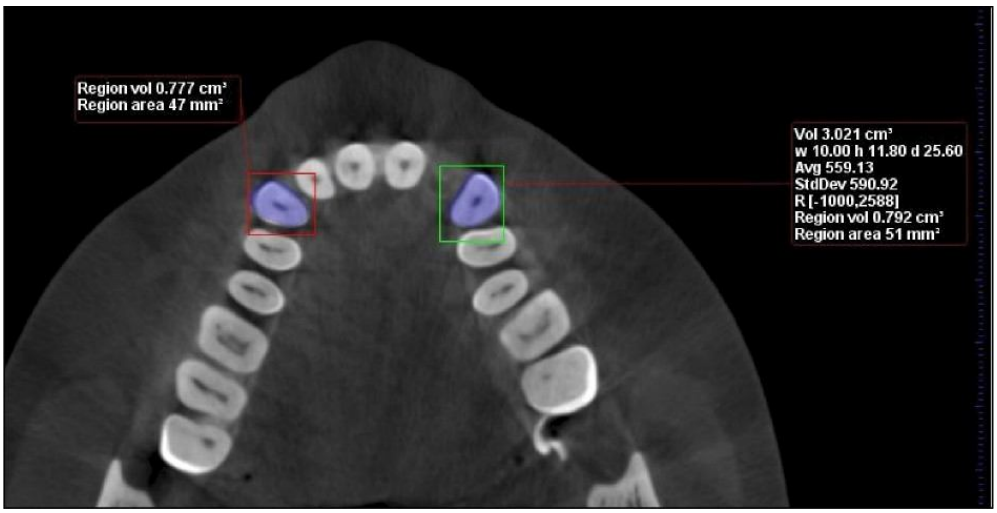


Figure1Maxillary Right canine and maxillary left canine tooth volume in axial section

Figure 2: Maxillary Canine tooth volume in sagittal section

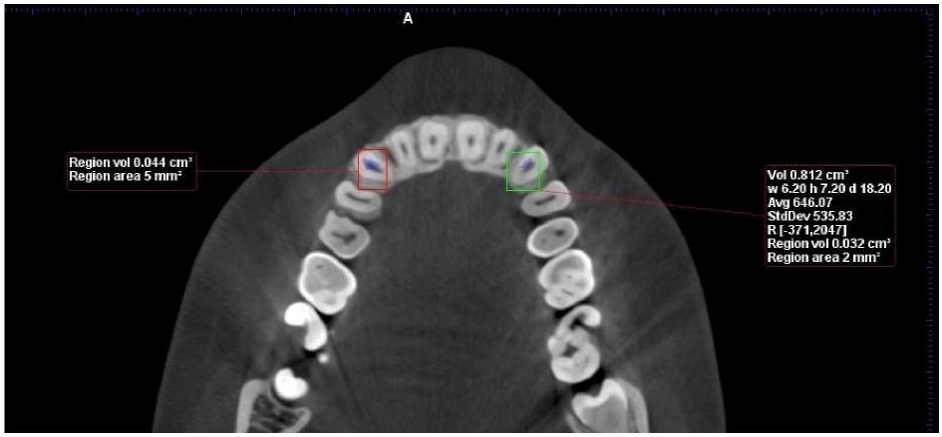


Figure 3: Maxillary left canine Pulp volume and maxillary right canine pulp volume in axial section

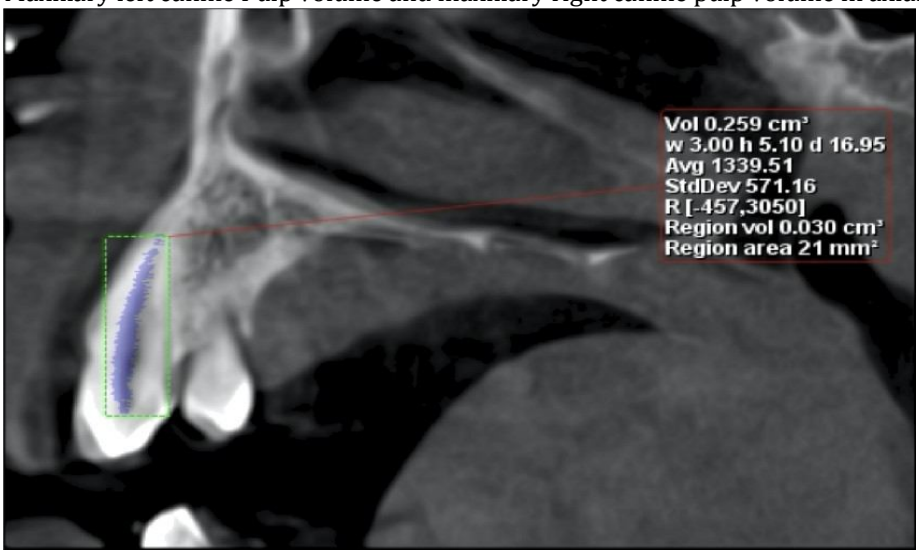


Figure 4 Maxillary canine Pulp volume in sagittal section

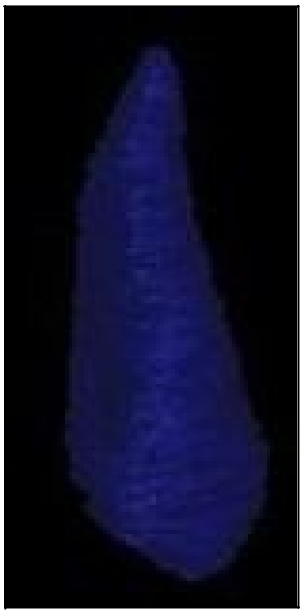


Figure 5 Maxillary canine tooth volumes in three dimensions

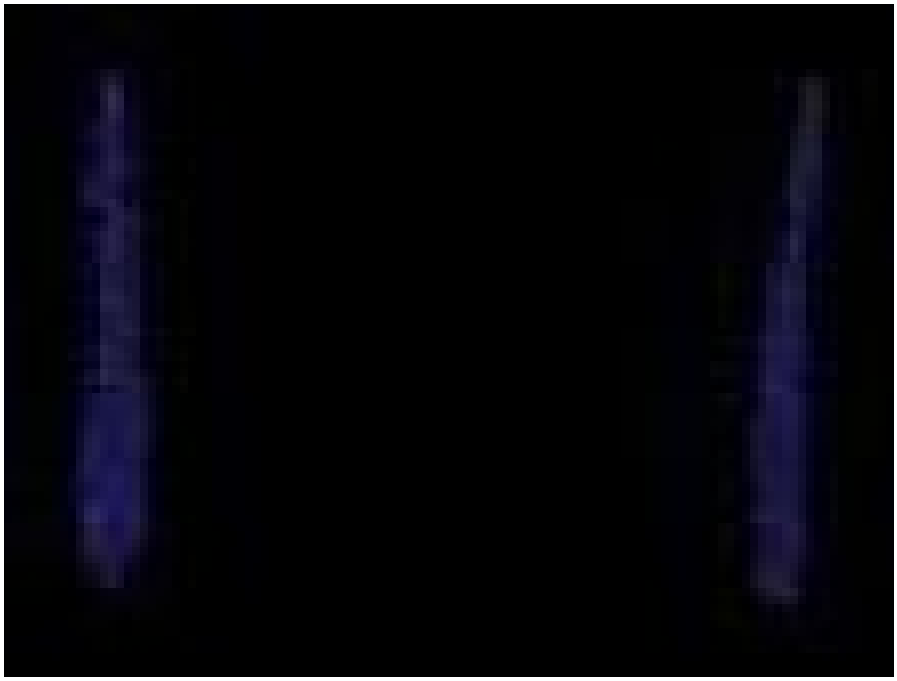
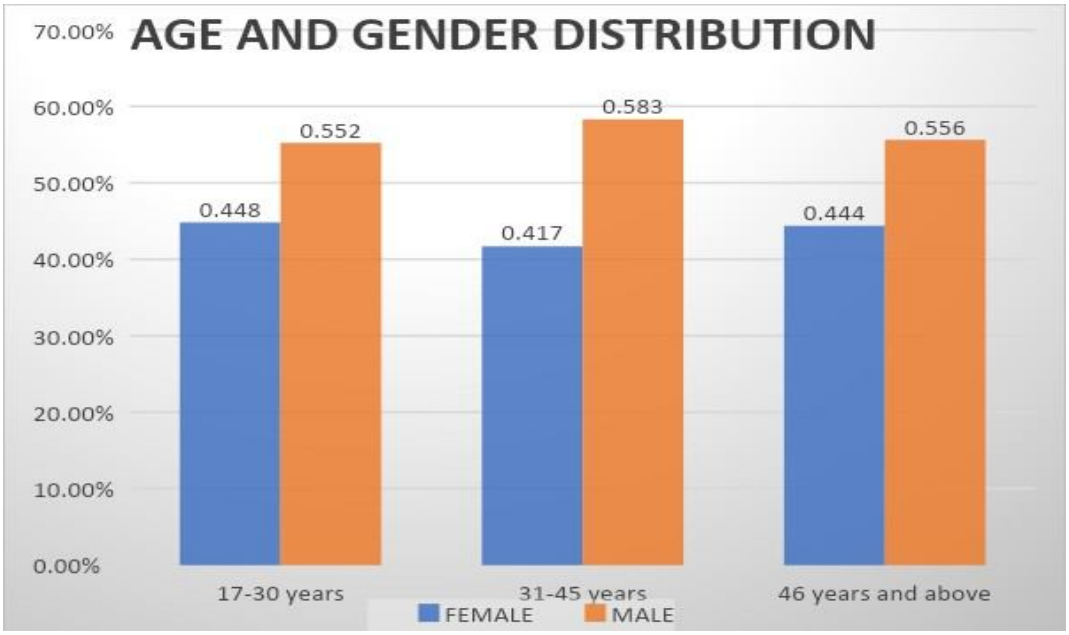


Figure 6: Maxillary canine pulp volume in three dimensions



(Graph 1 Distribution of age and groups and gender)

(Table 1 ANOVA analysis with age as dependent variable and ratio as predictors p=0.003*)

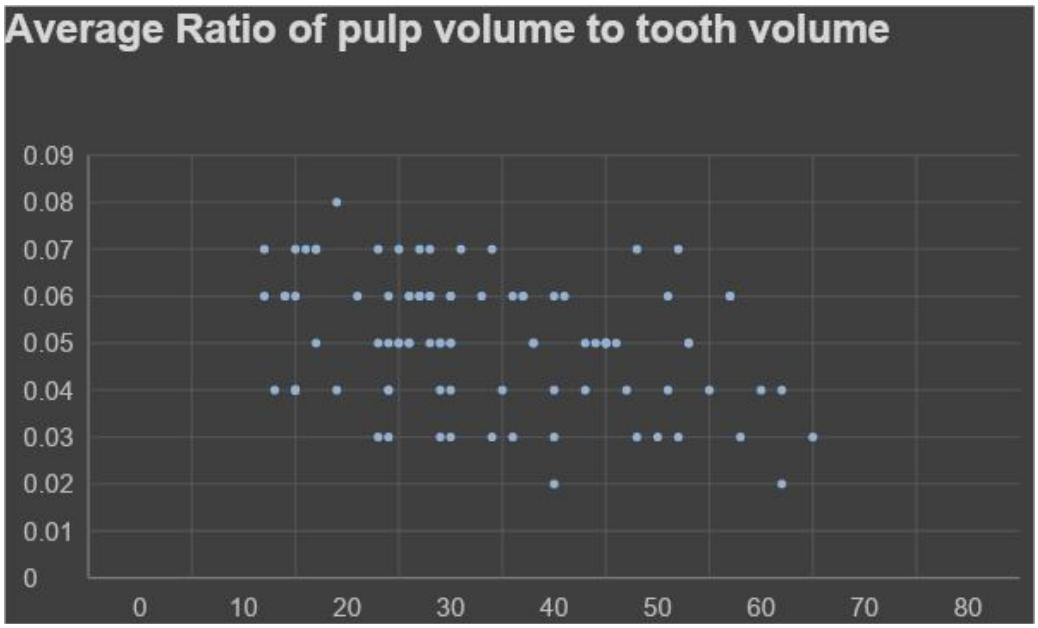
ANOVA[a]						
Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regressio n	3474.616	6	579.103	3.590	.003
	Residual	13713.242	85	161.332		
	Total	17187.859	91			
a. Dependent Variable: Age						
b. Predictors: (Constant), Ratio of left canine pulp volume to tooth volume, Right canine tooth volume, Ratio of right pulp volume to tooth volume, Left Canine tooth volume, Right canine pulp volume, Left canine pulp volume						

Table 2 Linear regression analysis between each canine PV, PTVR

=	Unstandardized Coefficients		Standardized Coefficients	t	p value
	B	Std. Error	Beta		
(Constant)	48.592	3.994		12.167	.000
Left Maxillary canine PV	-307.461	112.085	-.278	-2.743	.007*
(constant)	51.408	4.702		10.933	.000
Left maxillary canine PTVR	-281.433	96.642	-.293	-2.912	.005*
(constant)	47.690	4.319		11.041	.000
Right maxillary canine PV	-264.491	115.279	-.235	-2.294	.024*
(constant)	53.742	4.816		11.160	.000
Right maxillary canine PTVR	-311.934	93.414	-.332	-3.339	.001*
(constant)	48.650	4.236		11.485	.000
Average PV	-300.116	116.196	-.263	-2.583	.011*
(constant)	53.720	4.913		10.935	.000
Average PTVR	-321.004	98.354	-.325	-3.264	.002*

Table 3 Correlation Coefficient p<0.05*

	Correlation Coefficient	Sig. (2-tailed)
Left Canine tooth volume	-.122	.247
Left canine pulp volume	-.275*	.008*
Ratio of left canine pulp volume to tooth volume	-.261*	.012*
Right canine tooth volume	-.078	.460
Right canine pulp volume	-.217*	.038*
Ratio of right canine pulp volume to tooth volume	-.303*	.003*
Average pulp volume	-.100	.344
Average tooth volume	-.261*	.012*
Average of pulp volume to tooth volume (ratio)	-0.325	0.002*



Graph 2: Correlation between age and average pulp tooth volume ratio showed statistically significant result

Table 4: Equation for linear regression analysis with age as dependent variable

Linear regression analysis between left canine PV and age	Age= -307.46 (left canine PV)+48.59
Linear regression analysis between left canine PTVR and age	Age= -281.433 (left canine PTVR)+51.40
Linear regression analysis between right canine PV and age	Age= -264.491 (right canine PV)+47.69
Linear regression analysis between right canine PTVR and age	Age= -311.934 (right canine PTVR)+53.74
Linear regression analysis between average PV and age	Age= -300.116 (average PV)+48.65
Linear regression analysis between average PTVR and age	Age= -321.004 (average PTVR)+53.72

Table 5 Comparison of tooth volume, pulp volume and ratio of pulp volume to tooth volume according to gender

	GENDER	N	Mean	Std. Deviation	Mean difference	z value	p value
Left Canine tooth volume	Female	40	0.583	0.098	-0.116	-4.927	0.001*
	Male	52	0.699	0.107			
Right canine tooth volume	Female	40	0.597	0.100	-0.100	-4.403	0.001*
	Male	52	0.697	0.112			
Average tooth volume	Female	40	0.590	0.098	-0.108	-4.761	0.001*
	Male	52	0.698	0.107			
Left canine pulp volume	Female	40	0.031	0.011	-0.005	-2.037	0.042*
	Male	52	0.035	0.013			
Right canine pulp volume	Female	40	0.033	0.012	-0.004	-1.423	0.155
	Male	52	0.037	0.012			
Average pulp volume	Female	40	0.032	0.011	-0.004	-1.891	0.059
	Male	52	0.036	0.012			
Ratio of left pulp volume to tooth volume	Female	40	0.051	0.016	0.003	-0.149	0.881
	Male	52	0.048	0.014			
Ratio of right pulp volume to tooth volume	Female	40	0.047	0.013	0.001	-0.843	0.399
	Male	52	0.046	0.015			
average Ratio of pulp volume to tooth volume	Female	40	.0491	.01386	0.00201	0.684	0.496
	Male	52	.0471	.01405			

Mann Whitney U test, level of significance set at $p < 0.05$, * statistically significant)

DISCUSSION

Age estimation is an important parameter in the field of forensic science for living as well as dead individuals for proper management of records and identification of subjects. The accurate age estimation method is extremely important in cases of criminal conduct or in case of child labor. The skeletal and dental maturation is complete in adults, hence, the number of age assessment methods in living adults are considerably decreased [14]. Skeletal method for estimating age has disadvantage because of variation in bone development which is influenced by nutritional and environmental factors [15]. Radiographic tooth growth has been demonstrated to be a more reliable way of age assessment since it is mostly genetically influenced and thus less susceptible to nutritional and environmental variables [16]. Also, teeth being the strongest structures of body are resistant to external factors, such as the decomposition process and extreme temperatures (up to 1100 °C) [17]. Therefore, teeth are used as predictor in estimating age in forensic medicine for identification of living individuals. Pulp space evaluation is a new and golden opportunity for age estimation by dental radiologists. Deposition of secondary dentine with age is considered as an effective method as deposition of secondary dentin increases with age causing reduction of pulp cavity space [2]. Secondary dentine deposition can be assessed using 2D dental radiography methods or by extracting and sectioning a tooth. Previous studies have shown a link between secondary dentine deposition along the pulp chamber walls and age as well as the ability to detect and measure these changes using nondestructive periapical, RVG or panoramic radiographs, which are commonly employed in dentistry [18]. These two dimensional radiographs provide limited information with magnification and

distortion errors that can hamper the results of any research. Hence, simultaneous assessment of mesio-distal and bucco-lingual dimensions of teeth is always recommended [2]. CBCT is superior in estimating tooth dimensions and volume and does not require tooth extraction for sectioning.[6]Van De Voort et al (2004) was the first to look into the possibility of utilizing Micro CT to estimate age.[10] CBCT has the advantage over micro CT in that it has arelatively large scanning area, whereas micro CT has a constrained scan region and high radiation dose [19]. Until now, the pulp/tooth area ratio(PTAR) has been employed to adjust for 2D radiograph magnification or angulation problems.[3] An earlier study examined the use of the PTAR (rather than PTVR) to predict age and revealed that the formula developed for an Italian population could also be used to estimate age in Indians [20]. However our study was done by evaluating PV, TV and PTVR in living individuals using CBCT and uniqueness of our study was that in Indian population age estimation using PTVR has not been done before on sound teeth. Previous studies have included extracted teeth for evaluating PV and TV or PTVR for estimating age.[21]Astudy by Ranjan Vet al [22] has been done on Indian population using PTVR but the demerit of the study was the less sample size than our study. They used vector-based segmentation in On Demand software and did volume rendering after getting minimum and maximum threshold value of pulp of each individual on maxillary canine. The method we used in our study was manual segmentation of teeth which was not done previously in the Indian population for measuring PV and TV. Also manual segmentation of tooth gives more clear picture of tooth and pulp and it was easy to mark the outline of tooth and pulp using this method as automatic segmentation of tooth and pulp in apical region is unreliable [7]. Using this method it was easy to see any hidden groove or cusp of tooth. The accuracy of our method was justified by the fact that there was perfect agreement between intra observers and inter-observer agreement. The study utilizing vector based segmentation by Gulsahi A et al[6]showed moderate intraobserver and interobserver agreement whereas study by Kazmi Set al[7] in which manual segmentation method was used showed excellent agreement.

Biuki N et al [23] concluded that teeth in both jaws are reliable for estimating age. However, a stronger relationship was observed for maxillary teeth.[23] The maxillary canines have large pulp chambers and are less resistant to wear and are usually the ones that stay in the mouth the longest. Hence, both the maxillary canines were used in our study. In our study, we preferred axial section images for calculating PV/TV whereas study done by Gulsahi A *et al* [6] used the sagittal section for calculating PV/TV. It was easy to mark the outline of tooth and pulp using a free region grow tool in each slice on the axial section.

Based on the results of this present study, a negative correlation was observed between age and PTVR. A linear regression with biological age has been demonstrated in our study. PTVR of left canine ranges from 0.01 to 0.08 and PTVR of right canine ranges from 0.02 to 0.08. These results are similar to study done by Gulsahi A *et al* [6]. However, the contrasting result from the Gulsahi A et al [6] study was that the highest coefficient of determination was seen in maxillary central incisor. This difference is because of different tooth morphology of canine and incisors. The results of our study are similar to a study by Kazmi Set al [7] in which correlation of left maxillary canine PV with age had the highest coefficient of determination. According to study by Biuki N *et al* [23] a strong correlation was seen in maxillary central incisors and canines. The coefficient of determination in a study by Adisen MZ et al [11] on maxillary canine showed statistically significant results for women. In a study done by Abdinian M *et al* [24], coefficient of determination was almost similar in mandibular central incisor and maxillary canine and strongest correlation between age and PTVR was reported for mandibular central incisor followed by maxillary canine. In a study by Salemi F et al [25] on Iranian population using the PTAR of right maxillary canine a significant inverse correlation was noted between age and all measured variables which was similar to our study. However, the correlation coefficient of their study (R = - 0.88) was higher than our study (R = -0.325). This could be because of the difference in the population studied. Another study was done by Jagannathan N et al [2] on Indian population using extracted mandibular canine that showed a moderate negative correlation between PTVR and age with value of correlation coefficient higher (R = -0.63) than our study value (R = -0.325) This difference in correlation coefficient value with same significant inverse relationship of PTVR with age is because of different method and different software used in thesestudies [2, 22].

A study was done by Rai A et al [26] in which they investigated age based on PTAR on maxillary canine in three different planes of CBCT and they concluded that linear regression analysis in axial plane showed statistically significant result with correlation coefficient of 0.32 which is similar to our results. Hence, it supports our study in which the measurements are done on axial plane and concluded that axial plane gives better result than study done by Kazmi S et al [7] Another study by Haghanifar S et al [27] supported the fact that there was a more significant relationship between the age and PTAR in axial section compared to sagittal section.

The earlier study by Erbudak H et al [28] showed that teeth on either side i.e left or right had no effect on age estimation. However, in our study, the ratio of right PTVR (R = -0.303) gives a more significant result

than left ratio of PTVR (R = -0.261) for calculating the age. While comparing PV with age, left canine PV (R = -0.275) gives more significant results than right canine PV (R =-0.217)
Based on the results of our study average TV also showed a statistically significant relation with age (p = 0.01). On correlating average TV with age there was a negative correlation observed which concluded that with increase in age there is decrease in average TV of maxillary canine. This is supported by the fact that as the individual's age increases there are chances of attrition, abrasion and abfraction of teeth that causes decrease in size of tooth thereby decreasing TV.
On comparing TV, PV and PTVR with gender there was a significant correlation of left, right and average TV of canine with gender with higher relation being reported in males in our study. On comparing left PV with gender using Mann Whitney U test there was a significant relation with males showing high PV. The result of our study is similar to the study done by Yayun Wu et al [29] in which there was a significant difference between males and females in a Chinese population and reported that in males, the canal/root diameter ratio had a greater correlation with estimated age compared to females. A study by Kazmi S et al [7] showed a significant difference in PV of males and females similar to our study. It's likely that variances in primary dentine production exist between males and females, which could explain this disparity. The Y chromosome controls the thickness of dentine, while the X chromosome exclusively affects the thickness of enamel, according to evidence [7].
The result of left canine PV, left canine PTVR, right canine PV, right canine PTVR, average PV and average PTVR with age as dependent variable showed significant relation in our study. Hence, these equations can be used for estimating age but as correlation coefficient was highest for average PTVR, equation using average pulp tooth volume ratio will give more accurate results. (Table 4)
The mean absolute error of our study was 13.10 +/- 1.39 which is acceptable in the field of forensic science for estimating age. The regression equation given by Jagannathan N et al [2] for age estimation on maxillary canine was age = 57.18 + (- 413.41 x pulp/tooth volume ratio) and this is the commonly used equation for age estimation in the Indian population. This equation of Jagannathan N et al [2] is a close approximation of our equation for average pulp tooth volume ratio.
The intra examiner and inter examiner agreement of our study analyzed by kappa agreement showed perfect agreement (ranges from 0.8 to 1). This concluded that there was high agreement between observers. However in a study by Gulsahi A et al [6] inter observer agreement varied between moderate to good which was in contrast to our study.

LIMITATION OF OUR STUDY

Age estimation research is highly affected by the number of individuals in each age group and selected age range in sample size.[7] Our study lack the uniform distribution sample size in each group, therefore a large data sample size characterized by homogeneous age distribution (that is approximately equal number of individuals in each age group) should be used to estimate age and correlation between PTVR and age. Also our study was done on a subset of North Indian population which was not representative of the whole Indian population. In our study only maxillary canine was used however using multiple types of teeth may improve the accuracy of age estimation using pulp tooth volume ratio.

CONCLUSION

We concluded that the measurement of PTVR of permanent maxillary canine can be used as an adjuvant parameter for estimating age using CBCT scans. This study revealed that TV, average TV and left canine PV was gender dependent. There was mild correlation found between age and PTVR of left and right maxillary canine. The highest correlation was found when age was correlated with average of left and right maxillary canine PTVR. Hence it was concluded that while estimating age using maxillary canine both left and right maxillary canine should be considered for better results using CBCT.

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