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Age Estimation from Pulp/Tooth Area Ratio of Canines using Cone-Beam Computed Tomography Image Analysis: Study of an Egyptian Sample

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Abstract

Accurate, non-invasive and conservative methods for dental age estimation of unknown Individuals are an important aspect in different legal, forensic and anthropological situations. The pulp/tooth area ratio (PTR) used to estimate age by measuring the amount of decrease in the volume of the dental pulp cavity due to secondary dentin deposition. The study aims to age estimation based on PTR of canines measured in sagittal and axial planes obtained from Cone-Beam Computed Tomography (CBCT) image data. One hundred fifty CBCT images of Egyptian individuals (age range: 14-68 years) were analysed. PTR were measured by AutoCAD 2013. Linear regression analysis was used to assess the correlation between age and PTR, and to establish a mathematical model for the human age estimation. Study results revealed that the maxillary canines and sagittal sections measurements produced a higher correlation between chronological age and PTR. Therefore, maxillary canine sagittal section was the most accurate for age estimation (r=-0.919). The Standard errors of estimates (SEE) of the regression analyses for the individual canine sections and section combinations were found to be (range: ± 4.76 - 8.1 years) which an acceptable range in for forensic application (SEE ± 10 years). In conclusion, this study showed that PTR calculation of canines is a reliable parameter for human age estimation and that CBCT is an easy and conservative approach with reasonable precision and accuracy.

Keywords: Age estimation; Forensic odontology; Pulp/tooth ratio; Cone-beam CT; Canines; Egyptian

Introduction

Accurate age estimation in human adult is an important issue in forensic sciences, anthropological situations, in addition to criminal and civil proceedings. To this end, forensic practitioner is requested to perform an age assessment on a living individual for legal requirements for age estimation as in case of refugee and asylum seekers, criminal responsibility, and child pornography, falsification of age, competency and quantification of uncertainty [1-3]. Therefore, age estimation important for both living and deceased persons, children, adolescents, and adult age groups [4]. Whereas an accurate sex determination can be done by DNA analysis recently, age determination is not as straightforward issue.

Traditional morphological routines utilized by anthropologists for age estimation, especially in adult are often less accurate, whereas the chemical analysis of the dentin layer, such as racemization of aspartic acid in dentin and the radiocarbon analysis of the enamel layer, has shown more precise results for age estimation [5].

Teeth are known one of the strongest structures of the human body and usually minimally affected by the taphonomic process. Therefore, teeth have long been recognized as one of the most reliable methods of age estimation of a person in forensic sciences [6]. Different methods are used for dental age prediction in adults, depending on the determination of degenerative and morphologically age related changes of teeth, including wear and tear [7], tooth cementum emulations [8], root dentin transparency [9] and racemization of aspartic acid [10]. However, these methods require sophisticated laboratory preparations and they are invasive and require tooth extraction and often tooth destruction, which is infeasible in living individuals [11].

Apposition of secondary dentin in the tooth pulp cavity occurs throughout life on all the popular walls, reducing the size of the pulp chamber [12,13]. It is least affected by environmental factors [14]. Therefore, non-invasive radiographic techniques have been developed to estimate this reduction in the dental pulp size associated with increasing age due to the formation of secondary dentin deposition and used for a significant age estimation especially in living adults [15,16]. This reduction in pulp cavity was correlated with chronological age, and linear regression equations were formulated for age estimation by using periapical radiographs [17,18] and panoramic radiographs [19].

Radiographic evaluation of secondary dentine was used as a non-invasive method for age estimation by Cameriere et al. [18,20-23] since 2004, using the conventional two-dimensional (2D) radiographic images (periapical and panoramic radiographs) where they measured pulp/tooth ratio on mesiodistal view, firstly for maxillary canine but subsequently, included different teeth.

Despite of the significant results of this method, some potential errors may be rise due to radiographs reproduce three-dimensional (3D) tooth structure being projected into 2D image, which can lead to including superimposition and no equal magnification [24,25].

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In addition, buccolingual views show the largest area of the tooth while the mesiodistal view does not [26]. Since 2000, Cone Beam Computed Tomography (CBCT) has provided a recent method to acquire the 3D images of teeth in living individuals. It is compact, faster and is a safer version of the regular CT due to small radiation dosage [27,28].

In recent years, with the wide use of three dimensional images in radiography practice, several studies used to estimate age by using three-dimensional CBCT in order to overcome conventional two-dimensional radiographs limitations [29-32].

Thus, the aim of the present study was to investigate the age estimation based on the pulp-tooth area ratio of the maxillary and the mandibular canines measured in two planes (sagittal and axial) obtained from CBCT image, and to identify the reliability and applicability of this method for age estimation in human cases.

Materials and Methods

Samples

The study included a total of one hundred fifty Egyptian patients (85 males and 65 females) with age ranged from 14 - 68 years. The patients were recruited from those attend to a private dental centre in Cairo between May 2017 and February 2018. The CBCT radiographs data using (Planmeca ProMax 3D Classic CBCT machine) were enrolled in this study. The CBCT radiographic images utilized within this study were made for the purpose of diagnosis, clinical evaluation and routine treatments.

Patients were excluded who demonstrated any tooth anomalies, maxillofacial deformities or variation in the tooth size or shape as well as any patient has root resorption (internal or external) caused by trauma or orthodontic treatment, furthermore, patients have any disorders affecting the pulp-tooth area, such as cysts or tumors, teeth with restorations, cavities or attrition were excluded from the study.

According to the agreement with some previous investigations [33], a clearly visible canine which has the biggest root and the highest remaining rate in the dental arch was selected for the measurement in each subject.

Measurements

CBCT images were taken for both mandibular and maxillary canine (available left or right) using the following settings: The tube current: 10 mA, 86 kVp, exposure time: 11.2 s, field of view: 8 × 8 cm, 534 × 534 × 534 dimension, voxel size 150 µm. In order to obtain the maxillary and mandibular canine image, Romexis 1 version 4.4.1.R (Planmeca, Helsinki, Finland) was used with the following image settings: window width 4000, window level 1500.

Sagittal and axial cuts were taken for the canine. The following adjustment was performed in order to fix the cuts during obtaining the measurement (Figure 1), adjust the long axis of the canine, from the cusp tip to the root apex. Then, adjust the buccolingual plane from the axial view in the most convex point of the buccal crown then the sagittal cuts images were taken.

While the horizontal sectional images were obtained by the following process: Following the long axis of the canine set the axial plane to the cementoenamel junction (CEJ) then the axial cuts were taken. These images were saved as high-resolution JPEG files on a desktop computer.

According to Cameriere et al. [20-23], Measurements of the pulp and tooth areas were done. Briefly, the already saved section images were imported to AutoCAD 2013 computer-aided drafting software program (Autodesk Development Sarl, Neuchâtel, Switzerland). Wherein the tooth's image file was opened and zoomed in, enlarge the working area, Brightness/contrast and sharpness, if needed, were adjusted.

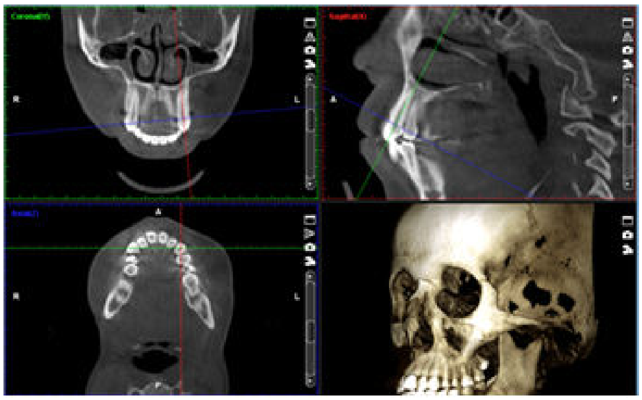

Figure 1: CBCT images of maxillary canine obtained for the study subjects showing how to adjust the long axis within both the sagittal and axial cuts.

For each image, at least twenty points were marked on the outline of the tooth, a minimum of ten points was also marked on the pulp outline and connected with the line tool using the point and line tools on AutoCAD's Draw Toolbox (Figure 2); then the area was measured for both tooth and pulp of that image using the AutoCAD's Draw Toolbox (Figure 2). The data were saved in Microsoft Office 2010 Excel spreadsheet and the pulp/tooth area ratio (PTR) calculated for all the two CBCT sections for each tooth.

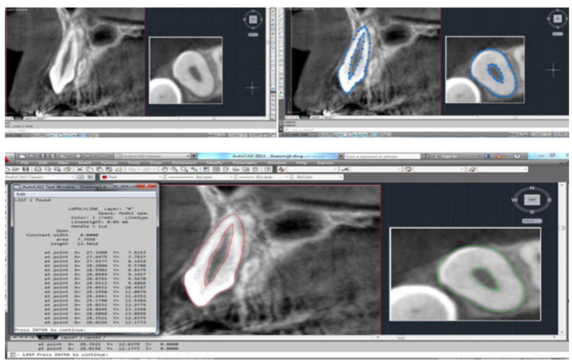

Figure 2: Shows the sagittal and axial section of maxillary canine and how to delineate the tooth as well as their pulp. This was accomplished first by defining the pulp and tooth outlines using the point and line tools on the AutoCAD 2013 software program's Draw Toolbox; the areas of the outlined pulp and tooth were then calculated for both sections by the same software program.

All measurements were carried out by the same observer. Repeat measurements on 20 randomly selected radiographs were undertaken after an interval of one month to assess the intra-observer reproducibility and the values subjected to a paired t-test to detect potential intra- and inter-observer error.

In the present study teeth were chosen either from the left or the right side, whichever were best suited for measurement. As according to Kvaal et al., [16], there are no significant differences between permanent teeth from the left and right side of the jaw.

Statistical analysis

Data were coded and entered using the statistical package SPSS version 25 (IBM Company, Chicago, IL). Data was summarized using mean and standard deviation for quantitative variables and frequencies (number of cases) and relative frequencies (percentages) for categorical variables. Comparisons between primary and repeated measurements were done using paired Student t-test [34].

Correlations between quantitative variables were done using Pearson correlation coefficient [35]. The S.E.E. was calculated to predict the deviation of the estimated age from the actual age, which in turn, reflects the accuracy of age prediction. Linear regression analysis was done to predict age using different measurements [36], and linear regression models were developed. p-values less than 0.05 were considered as statistically significant.

Ethical approval

Informed consent was taken from each patient. The protocol of this study was reviewed and approved by the Ethical Committee of Faculty of Medicine, Beni-Suef University.

Results

Age and sex distribution of patients included in this study are listed in Table 1. The descriptive statistics of pulp/tooth ratios in both sagittal and axial sections of the tested canines were shown in Table 2. The paired t-test revealed no statistically significant intra- and inter-observer differences (p>0.05) for all the examined sections Table 3.

While, it was observed that the accuracy of the observer was better for sagittal section measurements of the teeth. Our measurements in both sections did not reveal any significant differences between male and female, and consequently sex was not included in the statistical models.

The linear regression analysis, linear regression formulae, regression, correlation coefficients, standard errors of estimates (SEE), and regression equations derived for each tooth section as well as different sections combinations are presented in Table 4.

The regression analysis and Pearson correlation coefficient test (r) revealed a stronger correlation between the pulp/tooth ratios and age for the sagittal section measurements than for the axial section measurements. In addition, the maxillary canine measurements produced a higher correlation than the mandibular canine measurements.

The maxillary canine sagittal section was the most closely correlated with age (r=-0.919) followed by mandibular canine sagittal section (r=-0.827). The mandibular canine axial section revealed the lowest correlation (r=-0.687) (Figure 3).

Value		Sex					
		Male		Female		Total	
		Count	%	Count	%	Count	%
Age groups (years)	14 - 20	14	16.50%	4	6.20%	18	12.00%
	21- 30	21	24.70%	7	10.80%	28	18.70%
	31 - 40	16	18.80%	17	26.20%	33	22.00%
	41 - 50	17	20.00%	19	29.20%	36	24.00%
	51 - 60	12	14.10%	9	13.80%	21	14.00%
	> 60	5	5.90%	9	13.80%	14	9.30%
	Total	85	100.00%	65	100.00%	150	100.00%

Table 1: Age and sex distribution of patient's CBCT tomographs included in the study.

Sections	Minimum	Maximum	Mean	± SD	No.
Sagittal PTR of maxillary canine	0.03	0.3	0.15	0.07	150
Axial PTR of maxillary canine	0.01	0.22	0.08	0.05	150
Sagittal PTR of mandibular canine	0.02	0.34	0.14	0.06	150
Axial PTR of mandibular canine	0.01	0.21	0.07	0.04	150

Table 2: Descriptive statistics of pulp/tooth area ratios in both sagittal and axial sections of the maxillary and mandibular canines of the studied samples.

Parameter/Variable	Primary Measurement		Repeat Measurement		T-values	P-values
	Mean	± SD	Mean	± SD		
Sagittal PTR of maxillary canine	0.17	0.07	0.16	0.06	0.48	0.642
Axial PTR of maxillary canine	0.08	0.05	0.08	0.04	0	1
Sagittal PTR of mandibular canine	0.15	0.06	0.15	0.06	-0.361	0.726
Axial PTR of mandibular canine	0.07	0.04	0.07	0.04	-1.964	0.181

Table 3: Comparison between the primary measurements and corresponding repeat evaluations of pulp/tooth area ratio on the two sections (sagittal and Axial) for 20 of the 150 CBCT Tomography of the studied samples.

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Parameter/variable	Regression equation	r	R2	SEE (Years)	P value
Maxillary canine sagittal section	Age=73.106+(−222.323 × Sagittal PTR of Maxillary Canine)	-0.959	0.919	4.76	<0.01
Maxillary canine axial section	Age=59.286+(−285.016 × Axial PTR of Maxillary Canine)	-0.885	0.783	7.81	<0.01
Mandibular canine sagittal section	Age=62.615+(−382.821 × Sagittal PTR of Mandibular Canine)	-0.909	0.827	6.98	<0.01
Mandibular canine axial section	Age=60.687+(−255.382×Axial PTR of Mandibular Canine)	-0.829	0.687	8.09	<0.01
Maxillary canine sagittal +Axial sections	Age=68.782+(−151.272× Sagittal PTR of Maxillary Canine)+(−84.159× Axial PTR of Mandibular Canine)	-0.933	0.87	5.24	<0.01
Mandibular canine sagittal +Axial sections	Age=67.389+(−116.127 × Sagittal PTR of Mandibular Canine)+(−142.126× Axial PTR of Mandibular Canine)	-0.897	0.805	6.41	<0.01
Canine sagittal sections Maxillary+mandibular	Age=70.074+ (−153.840 × Sagittal PTR of Maxillary Canine)+(−56.990 × Sagittal PTR of Mandibular Canine)	-0.928	0.861	5.42	<0.01
Canine axial sections Maxillary+mandibular	Age=60.651+(−169.623 × Axial PTR of Maxillary Canine) +(−85.652 × Axial PTR of Mandibular Canine)	-0.83	0.689	8.1	<0.01
All four teeth sections	Age=69.906+(−122.809 × Sagittal PTR of Maxillary Canine)+(−51.793 × Axial PTR of Maxillary Canine)+(−45.176 × Sagittal PTR of Mandibular Canine)+(−124.362 × Axial PTR of Mandibular Canine)	-0.938	0.881	5.05	<0.01

Table 4: Linear regression analysis, linear regression formulae, Pearson correlation coefficient (r), Coefficient of determination (R2), standard error of the estimate values (SEE) and regression equations for the different ratios in both sagittal and axial sections of both maxillary and mandibular canines.

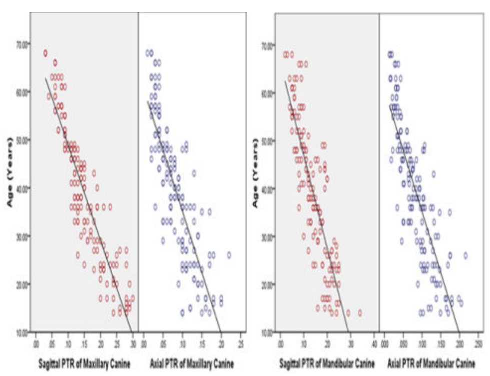

Figure 3: A Scatter plot and estimated line represent a linear regression relation between the age and Pulp/Tooth area ratio in the sagittal and axial section of canines.

Among section measurement combinations, All four teeth sections taken together had a good correlation with age (r=-0.881). Overall, all individual sections/section combinations had highly statistically significant correlation with age (p<0.001), linear regression equations were determined separately for the individual canine sections and section combinations.

The standard errors of estimates (SEE) of the regression analyses for the individual canine sections and section combinations were found to be ranged from ± 4.76 to 8.1 years Table 4. It was noted that all the estimates (SEE) were more than the threshold generally accepted in forensic practice (SEE ± 10 years) [34].

Discussion

In forensic science, the age estimation of the human either living or the dead is considered an important research issue. Teeth are considered as the most reliable human body structures for forensic age estimation since it is usually minimally affected by the environmental and taphonomic process [6]. There is a various proposed dental age estimation approach in forensic Odontology practice. In particular, if the method is non-invasive, easy, simple, and fast method that can be applied to living individuals [15,16,29]. One of the most important physiological age-related changes is secondary dentin deposition. Therefore, measurement of the size of the pulp chamber and pulp/tooth area ratio by dental radiographs (panoramic and periapical radiography) is a well-known non-invasive age estimation technique for adults [13,16]. However, application of the two-dimensional imaging methods (panoramic and intraoral periapical radiographs) is difficult in the case of malpositioned (e.g., rotated or crowded) tooth. So, 3D Tomographic techniques are a valuable method as they eliminate any complications of tooth malposition and any image superimposition [31,32,35].

CBCT is a newly effective and noninvasive three-dimensional diagnostic imaging modality in dental practice, resulting in a highly accurate image with a low radiation dose and affordable costs [24]. In addition, CBCT has a several advantages over conventional radiographic methods, including easy accessibility, ease of handling, magnification, lack of superimposition, absence of geometric distortion, and ability to offer a dataset of multiplanes cross-sectional and 3D reconstructions (in a single scan), therefore leading to improved structure visualization and diagnostic efficacy [36,37]. In this study, canines were selected as they are less expected to be damaged by trauma in comparison to the other single rooted anteriors, less likely to suffer erosion, abrasion, attrition or carious in comparison with molars, and they are the single root teeth with the longest root and widest pulp area; thus the easiest tooth to analyze. Moreover, it is more possible to find the canine in a dry skull in comparison to the molars [38-41]. Previous studies did not find a significant difference among the teeth from the right and the left side of the jaw; teeth from either the right or from the left side were processed depending on whichever were best appropriate for measurement.

The method of PTR which was planned by Camiere et al. is one of the simplest and widely applicable methods. It is performed by using a radiologic image so that there is no need for any difficult maneuvers or sophisticated laboratory work [20-23]. However, since these PTR dental methods use 2D (2 dimension) radiographs (intraoral periapical and panoramic), the PTR can be affected by malpositioned teeth. So, in our study we use 3D images produced by CBCT. In addition, we used a different process to calculate PTR in comparison to the preceding researches. The previous researches measured the PTR from the mesiodistal projected canine view. But, in the current study, the sagittal (bucco-lingual section) views and the axial section (horizontal) views of the CEJ of the canine was used for analysis. These areas are where the maximum level of calcified precipitation occurs. Furthermore, measurements of PTR in the sagittal section, on the long axis and the widest part of the tooth, could solve the problems caused by conventional 2D radiographs [24,31,42]. In the current study, we used 2 different methods to get the PTR in comparison to the previous researches. The sagittal cuts provide the bucco-lingual projection view of the cuspid which can reflect the complete quantity of pulp calcification related to a 2D image. The calcification of the pulp does not occur regularly from the pulp chamber to the end of the root canal. Therefore, it is better to measure the calcification of the pulp throughout the crown and the root in the sagittal views. The pulp calcification due to aging occurs mainly in the CEJ. In the current study, axial cuts images of the CEJ were analysed. Practically, the accuracy of the easing of these dental procedures for age estimation should be verified in different populations. Until now, In Egypt, there have only been a few studies proposing a method for dental age estimation by using PTR in living adults. Therefore, in the present study, we use the PTR of the both canines measured in two planes (sagittal and axial) obtained from the CBCT image for estimating the age and to establish an age estimation model in the Egyptian population.

In this study, no statistically significant differences in intra-observer repeated measurement of PTR for re-examined tomography on the two sections ($p>0.05$) which indicate a high reproducibility of the measurements, and this can be clarified by the simplicity of the used method. In addition, measurements of PTR show no statistically significant sex difference, as reported by previous studies which measured PTR in single-root teeth and the results shown that sex has no significant influence on the age estimation [15,39,40,43].

The results of the current study show a statistically significant correlation of PTR to age in both sagittal and axial planes of the studied canine's images ($p<0.05$). In addition, the results also show that age and PTR (S) on sagittal (bucco-lingual) views had the maximum Pearson correlation coefficient ($r=-0.959$) in comparison to the axial sectional views (Table 4). These results are in agreement with Lee et al., [44], who used CBCT to view the buccolingual and horizontal dimensions of the maxillary cuspid of adult Korean patients and found that the PTR gained from sagittal sectional views was more powerful for age estimation than horizontal sectional views. The high accuracy of measurements at sagittal sections more than axial ones may be explained due to the advantage that the ratio was not influenced by any image alteration caused by tooth malposition. Furthermore, PTR frequently has a fault in measurement caused by small tooth area in axial cuts, but the pulp and the tooth area of a sagittal cuts image is larger than axial cut images. This advantage effect increase when we examine the patient with lower resolution in order to decrease the radiation dose (large area decrease measurement errors). This reason also can explain the best result obtained from the maxillary canine compared with the mandibular canine; the maxillary canine size is bigger than the mandibular canine.

The high correlation coefficient and coefficient of determination on the results of the present study as shown in maxillary canine sagittal section ($r=-0.959$ and R2=91%), considered as the highest values of correlation coefficients amongst the studies using the CBCT for the age estimation [45-49], and consistent with the studies using a conventional radiography to determine the PTR. Therefore, our finding showed that the physiological pulp/tooth area ratio could be used to estimate the chronological age in Egyptian population.

A linear regression analysis was designed with age (as a dependent variable) and the physiological pulp/tooth area ratio (as independent variable), for both maxillary and mandibular canine, in both sagittal and axial sections, and a multiple linear regression analysis was carried out using all variables. Linear regression equations were resulted separately for the individual section and sections combinations, to estimate the chronological age by introducing PTR (Table 4). The Standard Errors of Estimates (SEE) in the linear regression analysis for one section and multiple sections combinations in both canines ranged from (± 4.76 - 8.1 years) which is indicates the high accuracy of those methods based on pulp/tooth ratio calculation, and this was in agreement with the best results described in previous articles using 3D to the analysis of PTR [50]. Finally, it is necessary to mention that our findings were promising for dental age estimation by a non-invasive technique using CBCT (SEE ± 4.76), which will be suitable for forensic application as SEE < ± 10 years is considered acceptable in forensic age prediction.

Conclusion

In conclusion, our study investigated the relationship between chronological age and pulp/tooth area ratio by a non-invasive technique using CBCT image analysis for dental age estimation. The results revealed a strong relationship between chronological age and PTR obtained from buccolingual (sagittal) sectional images for maxillary canines (r=-0.959). The standard errors of estimates (SEEs) of the linear regression analysis were found to be (range: ± 4.76 - 8.1 years) which an acceptable range in for forensic application (SEE ± 10 years). The derived regression equations for the different ratios can be useful for adult dental age estimation. Therefore, this method of dental age estimation is a reliable predictor of chronological age. Moreover, this method of dental age estimation is gender independent of the Egyptian population.

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Conflict of Interest Statement

Neither the author nor any of the co-authors have any potential conflict of interests related to the publication of this paper. The authors state that there are no any financial and personal relationships with other people or organizations that could inappropriately influence their work.

References

1. Franklin D, Flavel A, Noble J, Swift L, Karkhanis S (2015) Forensic age estimation in living individuals: Methodological considerations in the context of medico-legal practice. Res Rep Forensic Med J 5: 53-66.
2. Lynnerup N (2013) Forensic anthropology and human identification. Scand J Forensic Sci 19: 16-38.
3. Black S, Aggrawal A, Payne-James J (2010) Age estimation in the living: The practitioners guide. John Wiley & Sons, West Sussex.
4. Solheim T, Vonen A (2006) Dental age estimation, quality assurance and age estimation of asylum seekers in Norway. Forensic Sci Int 159: S56-60.
5. Alkass K, Buchholz BA, Ohtani S, Yamamoto T, Druid H, et al. (2010) Age estimation in forensic sciences: Application of combined aspartic acid racemization and radiocarbon analysis. Mol Cell Proteomics 9: 1022-1030.
6. Gustafson G (1950) Age determination on teeth. J Am Dent Assoc 41: 45-54.
7. Prince DA, Kimmerle EH, Konigsberg LW (2008) A Bayesian approach to estimate skeletal age-at-death utilizing dental wear. J Forensic Sci 53: 588-593.
8. Czermak A, Czermak A, Ernst H, Grupe G (2006) A new method for the automated age-at-death evaluation by tooth-cementum annulation (TCA). Anthropol Anz 64: 25–40.
9. Prince DA, Ubelaker DH (2002) Application of Lamendin's adult dental aging technique to a diverse skeletal sample. J Forensic Sci 47: 107–116.
10. Yekkala R, Meers C, Van Schepdael A, Hoogmartens J, Lambrichts I, et al. (2006) Racemization of aspartic acid from human dentine in the estimation of chronological age. Forensic Sci Int 159: S89–S94.
11. Meinl A, Tangl S, Pernicka E, Fenes C, Watzek G (2007) On the applicability of secondary dentin formation to radiological age estimation in young adults. J Forensic Sci 52: 438-441.
12. Gupta P, Kaur H, Shankari GSM, Jawanda MK, Sahi N (2014) Human age estimation from tooth cementum and dentin. J Clin Diagn Res 8: ZC07-10.
13. Drusini AG, Toso O, Ranzato C (1997) The coronal pulp cavity index: a biomarker for age determination in human adults. Am J Phys Anthropol 103: 353-363.
14. Arora J, Talwar I, Sahni D, Rattan V (2016) Secondary dentin as a sole parameter for age estimation: Comparison and reliability of qualitative and quantitative methods among North Western adult Indians. Egyptian J Forensic Sci 6: 170-178.
15. Star H, Thevissen P, Jacobs R, Fieuws S, Solheim T, et al. (2011) Human dental age estimation by calculation of pulp-tooth volume ratios yielded on clinically acquired cone beam computed tomography images of monoradicular teeth. J Forensic Sci 56: S77-82.
16. Kvaal SI, Kolltveit KM, Thomsen IO, Solheim T (1995) Age estimation of adults from dental radiographs. Forensic Sci Int 74: 175-185.
17. Babshet M, Acharya AB, Naikmasur VG (2010) Age estimation in Indians from pulp/tooth area ratio of mandibular canines. Forensic Sci Int 197: 125.
18. Cameriere R, Ferrante L, Belcastro GM, Bonfiglioli B, Rastelli E, et al. (2007) Age estimation by pulp/tooth ratio in canines by peri-apical X-rays. J Forensic Sci 52: 166-170.
19. Bosmans N, Ann P, Aly M, Willems G (2005) The application of Kvaal's dental age calculation technique on panoramic dental radiographs. Forensic Sci Int 153: 208-212.
20. Cameriere R, Ferrante L, Cingolani M (2004) Variations in pulp/tooth area ratio as an indicator of age: A preliminary study. J Forensic Sci 49: 317-319.
21. Cameriere R, Ferrante L, Cingolani M (2004) Precision and reliability of pulp/tooth area ratio (RA) of second molar as indicator of adult age. J Forensic Sci 49: 1319-1323.
22. Cameriere R, Brogi G, Ferrante L, Mirtella D, Vultaggio C, et al. (2006) Reliability in age determination by pulp/tooth ratio in upper canines in skeletal remains. J Forensic Sci 51: 861-864.
23. Cameriere R, Ferrante L, Belcastro MG, Bonfiglioli B, Rastelli E, et al. (2007) Age estimation by pulp/tooth ratio in canines by mesial and vestibular periapical X-rays. J Forensic Sci 52: 1151–1155.
24. Yang F, Jacobs R, Willems G (2006) Dental age estimation through volume matching of teeth imaged by cone-beam CT. Forensic Sci Int 159: S78-83.
25. Someda H, Saka H, Matsunaga S, Ide Y, Nakahara K, et al. (2009) Age estimation based on three-dimensional measurement of mandibular central incisors in Japanese. Forensic Sci Int 185: 110-114.
26. Kambe T, Yonemitsu K, Kibayashi K, Tsunenari S (1991) Application of a computer assisted image analyzer to the assessment of area and number of sites of dental attrition and its use for age estimation. Forensic Sci Int 50: 97–109.
27. Arai Y, Iwai K, Hashimoto K, Shinoda K (2000) Fundamental efficiency of limited cone-beam x-ray CT (3DX multi image micro CT) for practical use. Dent Radiol 40: 145-154.
28. Scarfe WC, Farman AG (2008) Oral Radiology (6th edn): Principles and interpretation: Cone beam computed tomography (Ch 14), White S, Pharoah M. St Louis: Elsevier pp: 225-243.
29. Jagannathan N, Neelakantan P, Thiruvengadam C, Ramani P, Premkumar P, et al. (2011) Age estimation in an Indian population using pulp/tooth volume ratio of mandibular canines obtained from cone beam computed tomography. J Forensic Odontostomatol 29: 1–6.

30. Ge ZP, Ma RH, Li G, Zhang JZ, Ma XC (2015) Age estimation based on pulp chamber volume of first molars from cone-beam computed tomography images. Forensic Sci Int 253: 133-137.
31. Pinchi V, Pradella F, Buti J, Baldinotti C, Focardi M, et al. (2015) A new age estimation procedure based on the 3D CBCT study of the pulp cavity and hard tissues of the teeth for forensic purposes: A pilot study. J Forensic Leg Med 36: 150–157.
32. Porto LV, Celestino da Silva Neto J, Anjos-Pontual AD, Catunda RQ (2015) Evaluation of volumetric changes of teeth in a Brazilian population by using cone beam computed tomography. J Forensic Leg Med 36: 4–9.
33. Du C, Zhu Y, Hong L (2011) Age-related changes in pulp cavity of incisors as a determinant for forensic age identification. J Forensic Sci 56: S72-76.
34. Solheim T, Sundnes PK (1980) Dental age estimation of Norwegian adults: A comparison of different methods. Forensic Sci Int 16: 7-17.
35. Tardivo D, Sastre J, Catherine JH, Leonetti G, Adalian P, et al. (2014) Age determination of adult individuals by three-dimensional modelling of canines. Int J Legal Med 128: 161–169.
36. Kamburoglu K, Kiliç C, Ozen T, Horasan S (2010) Accuracy of chemically created periapical lesion measurements using limited cone beam computed tomography. Dentomaxillofac Radiol 39: 95-99.
37. De-Vos W, Casselman J, Swennen GR (2009) Cone-beam computerized tomography (CBCT) imaging of the oral and maxillofacial region: A systematic review of the literature. Int J Oral Maxillofac Surg 38: 609–625.
38. Malikaew P, Watt RG, Sheiham A (2006) Prevalence and factors associated with traumatic dental injuries (TDI) to anterior teeth of 11-13-year-old Thai children. Community Dent Health 23: 222-227.
39. Cameriere R, Cunha E, Sassaroli E, Nuzzolese E, Ferrante L (2009) Age estimation by pulp/tooth area ratio in canines: Study of a Portuguese sample to test Cameriere's method. Forensic Sci Int 193: 128.
40. Cameriere R (2008) Age Estimation project: Cameriere's methods for age estimation. Macerata (Italia): Eum, Edizioni Università di Macerata.
41. Aboshi H, Takahashi T, Komuro T (2010) Age estimation using microfocus X-ray computed tomography of lower premolars. Forensic Sci Int 200: 35–40.
42. Juneja M, Devi YB, Rakesh N, Juneja S (2014) Age estimation using pulp/tooth area ratio in maxillary canines: A digital image analysis. J Forensic Dent Sci 6: 160-165.
43. Nemsi H, Haj-Salem N, Bouanene I, Ben-Jomaa S, Belhadj M, et al. (2017) Age assessment in canine and premolar by cervical axial sections of cone beam computed tomography. Leg Med 28: 31–36.
44. Lee SM, Oh S, Kim J, Kim YM (2017) Age estimation using the maxillary canine pulp/tooth ratio in Korean adults: A CBCT buccolingual and horizontal section image analysis. J Forensic Radiol and Imag 9: 1–5.
45. Sakuma A, Saitoh H, Suzuki Y, Makino Y, Inokuchi G, et al. (2013) Age estimation based on pulp cavity to tooth volume ratio using postmortem computed tomography images. J Forensic Sci. 58: 1531–1535.
46. Ge ZP, Ma RH, Li G, Zhang JZ, Ma XC (2015) Age estimation based on pulp chamber volume of first molars from cone-beam computed tomography images. Forensic Sci Int 253: 133.
47. De Angelis D, Gaudio D, Guercini N, Cipriani F, Gibelli D, et al. (2015) Age estimation from canine volumes. Radiol Med 120: 731–736.
48. Afify MM, Zayet MK, Mahmoud NF, Ragab AR (2014) Age estimation from pulp/tooth area ratio in three mandibular teeth by panoramic radiographs: Study of an Egyptian sample. J Forensic Res 5: 231.
49. Agematsu H, Someda H, Hashimoto M, Matsunaga S, Abe S, et al. (2010) Three-dimensional observation of decrease in pulp cavity volume using micro-CT: Age-related change. Bull Tokyo Dent Coll 51: 1-6.
50. Gulsahi A, Kulah CK, Bakirarar B, Gulen O, Kamburoglu K (2018) Age estimation based on pulp/tooth volume ratio measured on cone-beam CT images. Dentomaxillofac Radiol 47: 20170239.