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Does the crypt: tooth ratio in the developing tooth correlate with chronological age in children?—a novel, radiographic, cross-sectional pilot study using ImageJ tools

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Abstract

Background: The bony cavity known as the crypt encloses the developing tooth, the size of which is influenced by the tooth movements during various phases of tooth eruption. The radiographic observations have revealed that the size of the crypt decreases as the root formation continues. The present study was conducted to assess the relationship between the crypt to tooth ratio (CTR) and the chronological age in children in the age range of 4 to 16 years using their digital panoramic radiographs (OPGs). The ImageJ tools were used to calculate the area of the bony crypt and the area of the developing mandibular second molar from 145 OPGs. The ratio between the area of the crypt and the area of the calcified portion of the tooth was obtained and compared with the calendric age (CA) to develop a regression model for age estimation.

Results: There was a significant negative correlation between the chronological age and the CTR values ($r = -0.898$, $p<0.001$) in all the age groups. The derived regression formula was age = 17.192–3.855 (CTR) with an r^2 value of 0.807 and a standard error of estimate (SEE) being 1.385. The derived formula was tested using 52 OPGs from a different set of patients in the same age group. The mean chronological age of the test sample was 9.57 years (± 3.42), their mean estimated age (EA) was 8.50 years (± 3.25), and the difference was statistically significant ($p<0.001$). A difference of more than 1 year was observed in 69% of boys and 54% of girls.

Conclusions: This method is an attempt to correlate the crypt to tooth ratio of developing the second molar tooth with the chronological age. A large sample study is required to validate this novel technique of age estimation in children.

Keywords: Bony crypt, Crypt to tooth ratio, Mandibular second molar, Age estimation, Orthopantomogram

Background

The tooth begins its development from its initiation through the invagination of the dental lamina and later expanding its growth within a designated space in the jaws. This bony space or cavity which encloses the developing tooth is known as crypt (Fig. 1) (Boughner and Dean 2004). The mineralization of the tooth germ is often graded or staged into developmental events involving the crown, the root, and the root apex. The crown starts with the first evidence of mineralization at the cusp tips and then coalescence of the cusps, followed by a complete cusp outline (Moorrees et al., 1969a). After the formation of the crown, the root formation initiates through the proliferation of Hertwig's epithelial root sheath (HERS). The root lengthens by the occlusal movement of the tooth germs, allowing Hertwig's epithelial

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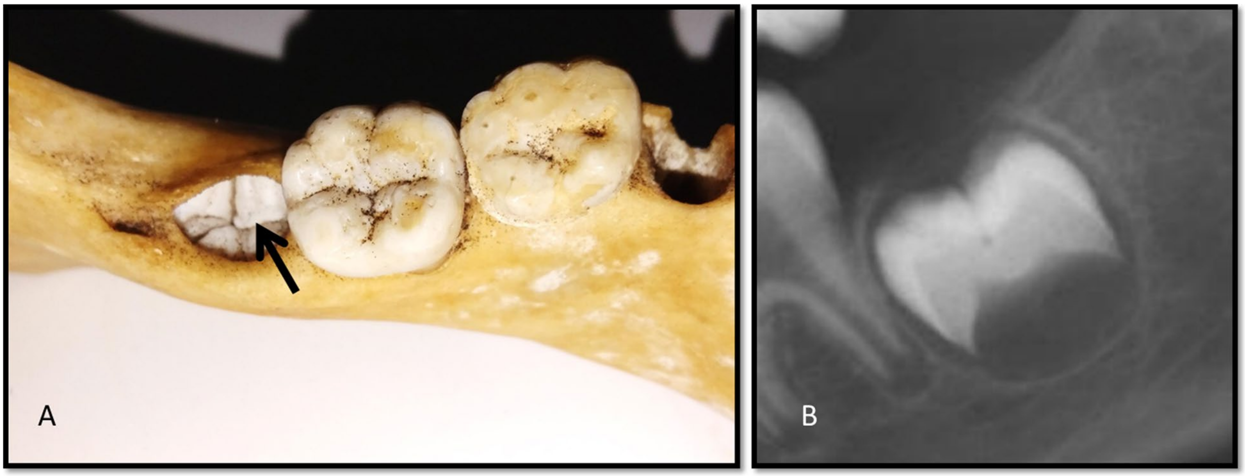

Fig. 1 **A** The image showing the developing mandibular second molar in the bony crypt. **B** The radiographic image showing the crown completed stage of the mandibular second molar in the bony crypt

root sheath to proliferate vertically (Sivapathasundaram and Logeswari 2019). The tooth's developmental journey ends as its crown reaches out into the oral cavity and establishes the full occlusion with the opposing tooth, which is termed as "eruption." During the pre-eruptive phase, a special relationship exists between the developing tooth and the surrounding alveolar bone. Usually, teeth begin their eruptive movement in the crypt with a half-formed root and emerge into the oral cavity when 3/4th of the root is complete (Sivapathasundaram and Logeswari 2019). From a general dentistry perspective, the most commonly used method to assess dental development is through clinically recording the emergence of teeth from the gingival surface (Eskeli 1999; Eveleth and Tanner 1990). However, from a forensic odontology perspective, there are several invasive and non-invasive methods and standards established for estimating the age using the dental parameters (Demirjian et al. 1973; Moorrees et al. 1963; Cameriere et al. 2006; Nolla 1963; Prince and Konigsberg 2008; Bang and Ramm 1970; Kvaal et al. 1994; Cameriere et al. 2004; Stott et al. 1982; Willems et al. 2001; Mincer et al. 1993). These scientific studies have related the stages of tooth development and the regressive changes in the tooth to the chronological age of the individual. From the anthropological aspect, the appearance of the cusp or cusps at the level of the alveolar crest is called "alveolar emergence" and the "clinical emergence" occurs at a later stage. For example, the alveolar emergence of the mandibular second molar takes place at 10.8 years (± 1.02) and the clinical eruption usually takes place after 2 years (Liversidge et al. 1998). The space available in the alveolar bone for the initiation of a tooth plays a significant role in the timing of its development. But there is hardly any study comparing the metric changes in developing tooth and the surrounding bony crypt. It is also a well-known fact that as the tooth mineralizes and increases in size, and the crypt space surrounding the tooth is being replaced by the bony component (Sivapathasundaram and Logeswari 2019). Thus, the objective of the present study was to evaluate the crypt to tooth ratio (CTR) using the radiographic area of the developing mandibular second molar and the area of its bony crypt and to the ratio value with the chronological age of the individual. We hypothesize that as the area of the developing tooth increases, the area of the bony crypt decreases.

Methods

This is a cross-sectional study undertaken with a randomly selected retrospective sample of 145 digital panoramic radiographs (OPGs) of children (72 boys and 73 girls) in the age range of 4 to 16 years. The mean chronological age of the study subjects was 9.75 years (± 3.14). The OPGs belonged to the patients visiting the departments of Pedodontia and Orthodontia for their respective treatments. The radiographs taken for treatment purposes were utilized for this study. The institutional ethical committee's approval was obtained for this study. The inclusion criteria included only those OPGs demonstrating clear radiographic details of the development stage of mandibular 2nd molar in both the quadrants. The date of birth and the date of x-ray were recorded, and the actual chronological age (CA) in years was calculated using the Microsoft Office Excel® 2007 (Microsoft®, Redmond, Washington, USA) tool. The OPGs were coded in such a way that the demographic details of the children were unknown to the investigators. The study subjects were categorized into four age groups according to the chronology of the events occurring during the development of the mandibular second molar (Table 1). The digital images of the OPGs were opened in the ImageJ software and using the threshold tool the visualization of the mineralized portion of the developing 2nd molar was enhanced. The crypt outline was drawn using the draw tool and the area in pixels was measured using the Analyze - Measure toolbar. The calcified area of the developing 2nd molar was also drawn, and the area was calculated as earlier (Figs. 2 and 3). All the measurements in the ImageJ software were taken to the nearest pixel value. Both the values were noted in the Excel sheet, and the crypt-to-tooth area ratio was calculated. To determine the reliability and the repeatability of the measurements, 20 OPGs were subjected to re-measurements and the values were compared with the earlier measurement and tested statistically. The crypt to tooth ratio (CTR) variable was compared to the actual age of the children, and statistical analysis was computed. The regression equations derived using the linear relationship between the CTR values and actual calendric age (CA) were tested on a test sample of 52 OPGs from a different set of children (26 boys and 26 girls) in the same age group with a mean age of 9.57 years (± 3.42) with a null hypothesis stating that there is no significant difference between the actual age (CA) and the estimated age (EA) using the newly derived age estimation formula.

Table 1 The frequency distribution of the study sample, according to age range (N=145)

Group	Age range (years)	*n*	%
I	4.01–7.00	38	26.2
II	7.01–10.00	38	26.2
III	10.01–13.00	37	25.5
IV	13.01–16.00	32	22.1

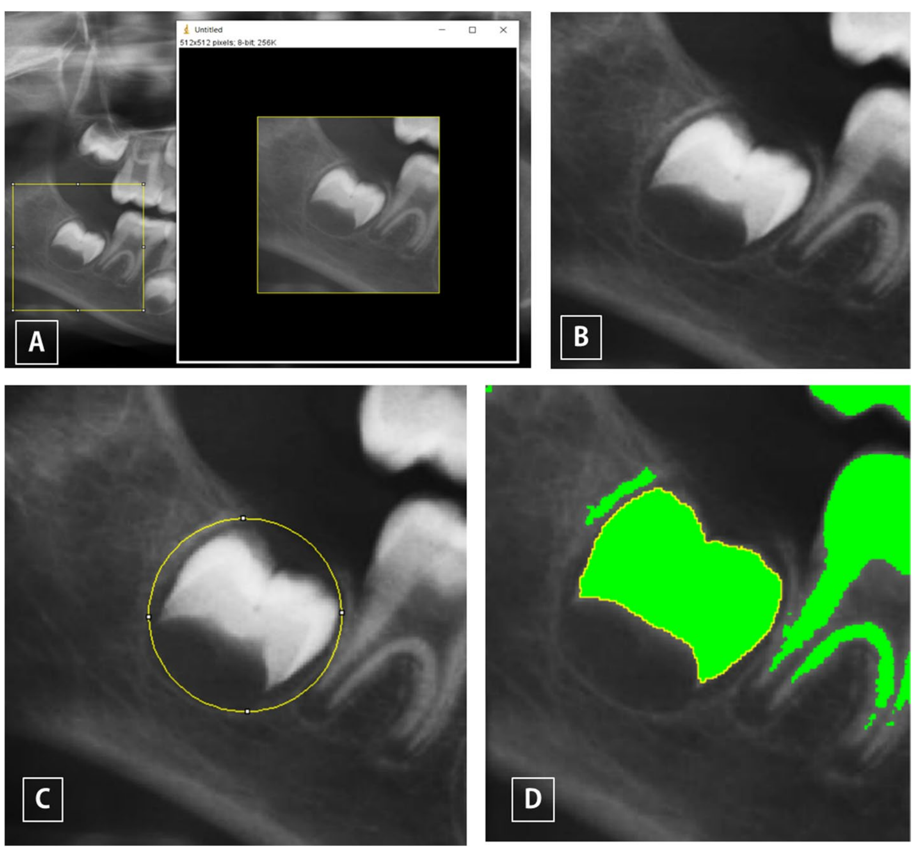

Fig. 2 The image showing the use of ImageJ tools for calculating the crypt area and the tooth area of the mandibular second molar. **A** Selecting the tooth understudy from the digital OPG; **B** using the image enhancement tools to visualize the mineralized component of the tooth; **C** using the elliptical tool and drawing the outline of the crypt; and **D** adjusting the threshold tool to select the mineralized part of the crown of the second molar

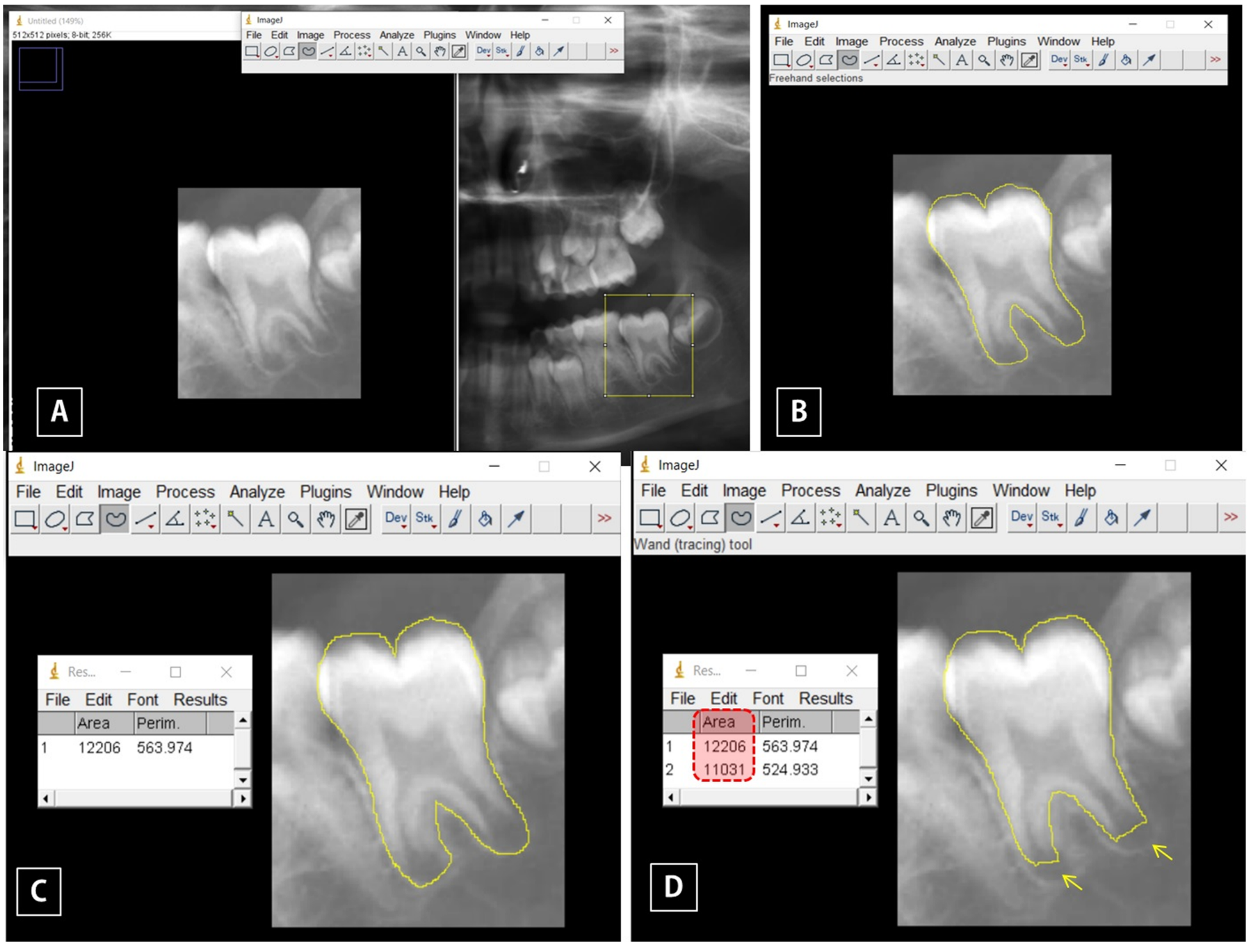

Fig. 3 The image showing the use of ImageJ tools for calculating the crypt area and the tooth area of the mandibular second molar. **A** Selecting the developing second molar in the digital OPG; **B** using the draw tool, the outline of the mineralized plus the follicular area is selected; **C** using the Analyze-Measure tool the area of the selection is calculated; and **D** using the draw tool, the outline of only the developing tooth alone is drawn and the area of the selection is calculated as before

Statistical analysis

The data was analyzed using the Statistical Package for Social Sciences (SPSS) software (SPSS for Windows, V.16.0. SPSS Inc., Chicago, IL, USA; now IBM Corp., Armonk, USA). A *p*-value of <0.05 was set as statistically significant. The normality of the quantitative data distribution was checked using the Shapiro-Wilks test and by the visual analysis of the normal Q–Q plots for the measured variables, the CTR value. The consistency and the reliability of the measurements were tested using the intraclass correlation coefficient (ICC), paired *t* test, and by applying Dahlberg's formula. Dahlberg's formula is defined as $D = \sqrt{\sum_{i=1}^{N} \frac{d_i^2}{2N}}$ where d_i is the difference between the two measured values and N is the sample size (N=20 in this case) (Galvão et al. 2012). The Mann-Whitney test was applied to test the difference in the CTR values between male and female samples. The analysis of variance (ANOVA) test was used to test the CTR values between the four age range categories of the sample. Pearson's correlation coefficient statistics was applied to identify the degree or strength of the linear relationship between the actual age and the crypt to tooth ratio values in all the groups. The linear regression equation was derived using age as the dependent variable and the CTR value as the independent variable.

Results

Sample characteristics

The Shapiro-Wilks test (p>0.05) and a visual inspection of their histograms and the normal Q-Q plots showed that CTR values were not normally distributed for both boys and girls, with skewness of 0.896 (SE=0.283) and kurtosis of 1.318 (SE= 0.599) for the boys and skewness of 1.388 (SE=0.281) and kurtosis of 1.722 (SE= 0.555) for the girls (Razali and Wah 2011). Excellent reliability was observed between the CTR values in the test and retest samples

Table 2 The results of the tests for the reliability of the variable, CTR (N=20)

Variable	*n*	Mean value	SD	Mean diff.	SD	Dahlberg's formula value	Paired *t* test (*p* value)*	ICC
CTR (1st attempt)	20	2.178	0.922	0.045	0.312	0.21	0.523	0.935
CTR (2nd attempt)		2.133	0.803					

*Significant at p<0.05; *CTR* crypt to tooth ratio, *SD* standard deviation, *ICC* intraclass correlation coefficient

Table 3 The results of the group-wise correlation between the mean age and the mean CTR values

Group	*n*	Mean age (S.D)	Mean CTR (S.D)	Correlation coefficient (*r*)	Sig.*
I	38	5.69 (0.79)	2.923 (0.61)	−0.713	0.000
II	38	8.62 (0.84)	1.940 (0.19)	−0.655	0.000
III	37	11.5 (0.81)	1.535 (0.16)	−0.588	0.000
IV	32	13.92 (0.61)	1.19 (0.18)	−0.718	0.000
Total	145	9.75 (3.14)	1.930 (0.73)	−0.898	0.000

*Significant at p<0.05

(Koo and Li 2016). Table 2 shows the results of the reliability tests. The single measures ICC was 0.935 with a 95% confidence interval from 0.844 to 0.974 ($F(df$=19) = 29.769, p<0.001). The mean CTR value for the overall sample was 1.930 (±0.73). There was a significant difference in the CTR values between the four age groups (df=3; F=168.070; p<0.001) and an overall significant negative correlation between the actual age and the CTR values in all the four groups (r= -0.898, p<0.001). The mean CTR value decreases as the age advances (Table 3). The mean CTR value in boys was higher than that of girls; however, the difference was not significant (p>0.05). The CTR value in boys showed a better correlation with age when compared to girls (Table 4). However, in the age groups 3 and 4, the girls showed a better correlation between CTR and age than boys. The regression model utilizing the CTR value yielded the regression equations for boys and girls separately and the overall sample (Table 5 & Fig. 4). The CTR values in the test sample were approximately normally distributed for both boys and girls, with skewness of −0.037 (SE=0.456) and kurtosis of −1.377 (SE= 0.887) for the boys and a skewness of −0.032 (SE =0.460) and kurtosis of −1.414 (SE= 0.890) for the girls. Table 6 shows the descriptive statistics of the test sample. The mean CTR value for the test sample was 2.26±0.84. The new regression formula underestimated the age of the test sample (Table 7). There was an excellent correlation between the estimated age (EA) and the calendric age (CA); however, the mean difference was statistically significant in both the genders and in the overall sample (mean = −1.08±0.484, df = 51, p<0.001). The tests of between-subjects effects between the age groups revealed a significant difference in the residual value (F(3,4.717), p<0.05,η^2= 0.243). The residual value between boys and girls in all four age groups was not significantly different. The estimated age was neared to the calendrical age in group 1 in both genders followed

Table 5 The regression equations derived using the age as the dependent variable and the CTR as the independent variable

Subjects	*n*	Regression equations	r^2	SEE	Sig.
Boys	72	1. Age = 17.894–4.166 (CTR)	0.839	1.285	0.000
Girls	73	2. Age = 16.646–3.611 (CTR)	0.785	1.463	0.000
Overall	145	3. Age = 17.192–3.855 (CTR)	0.807	1.385	0.000

*Significant at p<0.05. *SEE* standard error of the estimate; *CTR* crypt to tooth ratio

Table 4 The gender-wise frequency distribution and the descriptive statistics of variables in the study sample (N=145)

Group	Boys (n=72)								Girls (n=73)						Correlation coefficient	
	n	%	Age (years)		CTR		Correlation coefficient		*n*	%	Age (years)		CTR			
			Mean	SD	Mean	SD	*r*	Sig*.			Mean	SD	Mean	SD	*r*	Sig*.
I	20	27.8	5.74	0.84	2.881	0.51	−0.747	0.000	18	24.7	5.63	0.75	2.969	0.70	−0.710	0.001
II	18	25	8.52	0.73	2.00	0.16	−0.682	0.002	20	27.4	8.71	0.94	1.882	0.20	−0.644	0.002
III	19	26.4	11.53	0.85	1.550	0.20	−0.533	0.019	18	24.7	11.37	0.79	1.519	0.11	−0.798	0.000
IV	15	20.8	13.99	0.59	1.244	0.20	−0.747	0.001	17	23.3	13.87	0.63	1.154	0.14	−0.851	0.000
Total	72	100	9.68	3.17	1.97	0.69	−0.916	0.000	73	100	9.81	3.13	1.892	0.77	−0.886	0.000

*Significant at P<0.05. *CTR* crypt to tooth area ratio, *SD* standard deviation

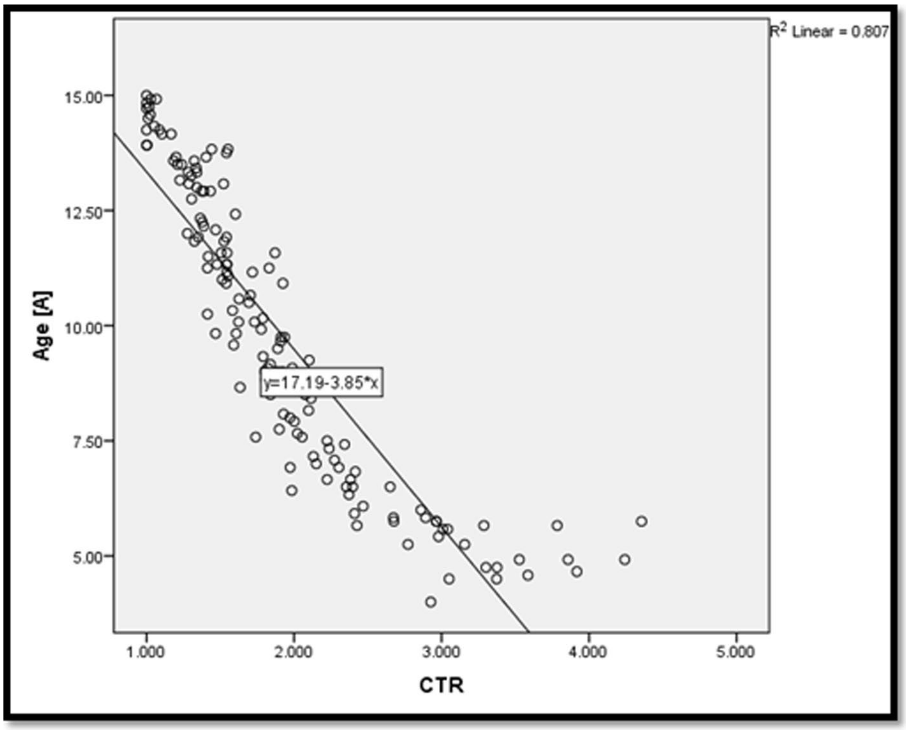

Fig. 4 The scatterplot diagram showing the linear relationship between the crypt to tooth ratio (CTR) and the chronological age of the children (*N*=145)

Table 6 The table showing the gender-wise descriptive statistics of the parameters in the test sample (*N*=52)

Parameters*	Boys (*n*=26)		Girls (*n*=26)		Total (*N*=52)		Sig**.
	Mean	SD	Mean	SD	Mean	SD	
CA	9.57	3.46	9.57	3.44	9.57	3.41	0.990
CTR	2.27	0.84	2.25	0.86	2.26	0.84	0.930
EA (gender-based formula)	8.45	3.51	8.53	3.10	8.50	3.28	0.929
EA-CA (gender-based formula)	−1.12	0.42	−1.04	0.59	−1.08	0.51	0.561
EA (general formula)	8.46	3.25	8.53	3.31	8.50	3.25	0.932
EA-CA (general formula)	−1.12	0.46	−1.04	0.52	−1.08	0.48	0.564

**CA* chronological age, *CTR* crypt to tooth ratio, *EA* estimated age, *SD* standard deviation

**Significant at $p<0.05$

by group 2 in boys and group 3 in girls (Table 8). A difference of more than 1 year was observed in 69% of boys and 54% of girls (Fig. 5).

Discussion

There are several age estimation methods based on the developmental and post-developmental changes in teeth. The present study is based on the spatial relationship of the developing tooth with its bony crypt. As the size of the mineralized component of the tooth increases during development, the size of the bony crypt decreases. This pilot study was limited to the developing mandibular second molar to find out the linear relationship between the area of the mineralized portion of the tooth and the area of the bony crypt. To the best of the author's knowledge, there was hardly any study till the submission of the present one, highlighting the relationship between the crypt to tooth ratio with the chronological age. Earlier, Cameriere et al. (2004) established a linear relationship

Table 7 The table showing the descriptive statistics of the test sample, according to the age groups

Group	*N*	Calendrical age (CA) (years)		CTR		Estimated age (EA) (years)		EA-CA (years)				
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	*F*	*df*	Sig*
I	16	5.54	0.84	3.23	0.25	4.74	0.95	−0.80	0.46	4.625	3	0.006
II	12	8.50	0.80	2.53	0.27	7.45	1.05	−1.05	0.44			
III	13	11.64	1.03	1.74	0.33	10.50	1.28	−1.14	0.48			
IV	11	14.17	0.54	1.16	0.18	12.73	0.68	−1.44	0.35			
Total	52	9.57	3.41	2.26	0.84	8.50	3.25	−1.08	0.48			

SD standard deviation

*Significant at $p<0.05$

Table 8 The mean difference between the estimated age (EA) and the calendric age (CA) in all the four groups between genders

Group	Difference between estimated age and calendric age (EA-CA)				Sig.*
	Boys		Girls		
	Mean	SD	Mean	SD	
I	−0.82	0.39	−0.79	0.55	0.886
II	−0.91	0.48	−1.19	0.38	0.297
III	−1.39	0.27	−0.93	0.54	0.800
IV	−1.45	0.33	−1.42	0.4	0.892
Total	−1.12	0.46	−1.04	0.515	0.564

*Significant at $p<0.05$, *SD* standard deviation

between the pulp area and the tooth area (Cameriere et al. 2004). The association of the tooth length and the alveolar eruption of all deciduous teeth, permanent central incisors, and first molars was reported by Liversidge and Molleson (Liversidge and Molleson 1999). Their findings suggest that the active phase of eruption is probably a rapid process and occurs during the first half of root growth. In the present study, it was observed that the minimum difference in the EA and CA was in the age range of 4 to 7 years when the crown formation is in the active phase. The root formation of the mandibular second molar usually starts after 8 years. The correlation between CTR value and chronological age was comparatively lower in the age

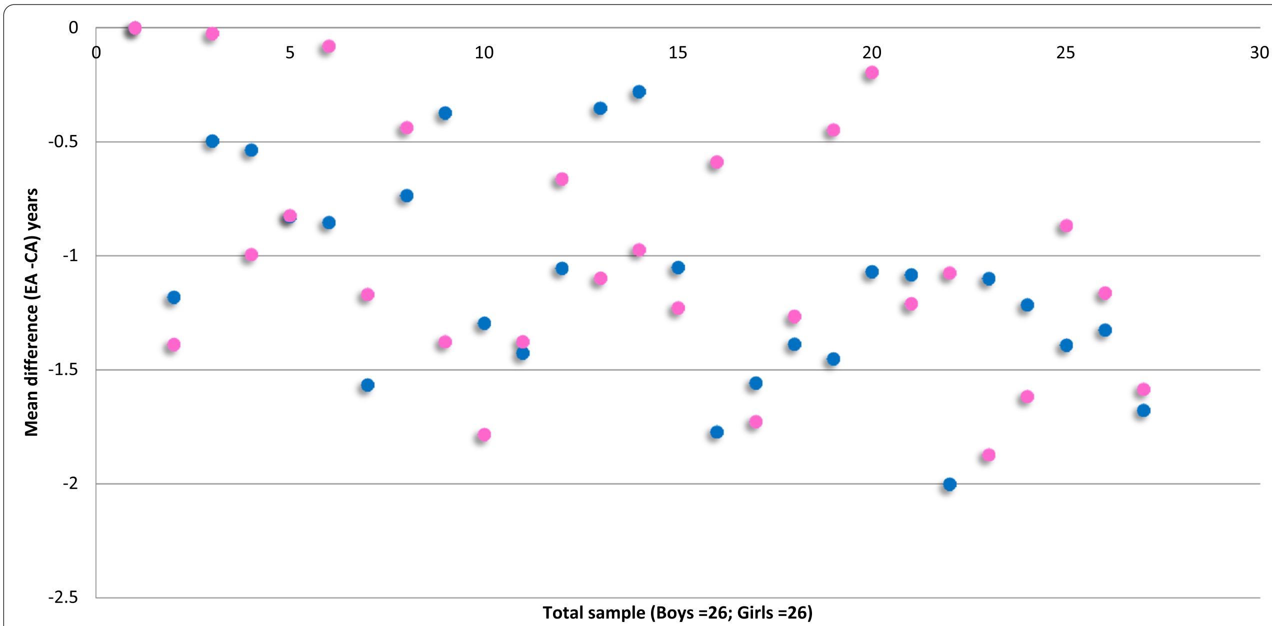

Fig. 5 Scatter plot showing the distribution of the test sample according to their mean difference between EA and CA

range of 10 to 13 years. This is the stage when the root formation is in the middle stage and the tooth eruption usually starts. The variations in the appearance of the calcification point at the root bifurcation area which usually occurs after the initial root formation are complete may also be the contributing factor. In 2006, Cameriere et al. presented a method of age estimation using the linear measurements of the root apices of the developing teeth based on its association with the calendrical age (Cameriere et al. 2006). Their method valued all the seven left mandibular teeth and measured the vertical height of the developing teeth and the near-horizontal measurements mesiodistally between the developing root tips. The present study used the area of the calcified part of the developing tooth and the area of the bony crypt of only one tooth, the mandibular left second molar. The ratio value gradually decreased as the tooth developed. A ratio value of 1 was given to that tooth where the root apex is fully closed and root formation is complete. Thus, the minimum value of CTR in the present study was 1.00. The initial evidence of calcification and the appearance of the cone-shaped calcified area are usually evident during the 4th year of life. Hence, the lower age limit of the sample was selected as 4 years in this study. The root apex closure also is usually completed by 16 years, and thus, this age was selected as the upper age limit of the sample. When the area, length, and width parameters were measured in digital radiographic images, they produce reliable and reproducible intra-observer measurements (Cameriere et al. 2004). The idea of using the crypt area and the tooth area and their ratio for age estimation was inspired from the works of Cameriere et al. (2004) wherein the pulp area and the tooth area were measured and a regression equation was derived for age estimation (Cameriere et al. 2004). Considering the ease of visualization and at the same time the presence of already completely developed first molar and the developing third molar in the near vicinity, the present study was designed on a preliminary basis using the second molar. Also, tooth development is recognized at the earliest, by the first appearance of the crypt on radiographs and during its development, and there is an established relationship between root formation and tooth eruption (Moorrees et al., 1969b; Smith and Buschang 2010). Studies similar to the Cameriere et al. study were also conducted on a representative sample of the Indian population using digital image analysis software like AutoCAD and Adobe Photoshop (Babshet et al. 2010; Babshet et al. 2011; Jeevan et al. 2011). Acharya developed a new digital technique for measuring the root dentinal translucency area using extracted, ground sectioned tooth, and derived regression formulae for translucency length and area (Acharya 2014). The present study applied the ImageJ tools to measure the area of mineralization in the developing mandibular second molar and the area of the bony crypt. Earlier, ImageJ tools were also used in dental age estimation using the pulp-to-tooth ratio parameters in maxillary canine tooth and also for counting the increment lines in cementum for age estimation (Juneja et al. 2014; Dias et al. 2010). ImageJ is a public domain, Java-based image processing program developed at the National Institute of Health, a biomedical research facility located in Maryland, USA. It can display, edit, analyze, process, save, and print images. It can calculate the area and pixel value statistics of user-defined selections (Rasband and ImageJ 1997). The use of such image analysis tools in analyzing the digital radiographic images avoids the bias inherent in observer subjectivity and improves the reliability of the data (Cameriere et al. 2001). Studies concentrating on the maturation stages of the third molar for age estimation are already available in the scientific literature (Mincer et al. 1993; Willershausen et al. 2001; Gunst et al. 2003). However, the present study was performed to introduce a novel methodology by using the crypt area and the developing tooth area for age estimation. The regression equation derived through the present study was also validated using a test sample from the same population and with the same age range. There was a significant negative correlation between CTR value and age. The correlation was comparatively lower in the age range of 10.00 to 13.00 years with a mean age of 11.5 years (±0.81) when compared with the other age range groups. This could be because of the variations in the appearance of the root bifurcation point in the radiographs. The mean difference between the calendric age and the estimated age gradually increased as the tooth development continues. In group 1, with the age range of 4.01 to 7.00 years the difference was lesser in girls than boys. If the age difference is less in a particular age group, it means that the tooth area is greater, and thus, the CTR value is reduced. Hence, it can be deduced that in age group 1, the mineralization of teeth in girls is ahead of those in boys. This age group is predominated by the crown formation with regards to the development of the mandibular second molar. However, in group 4, with the age range of 13.01 to 16.00 years, the root maturation is ahead in boys than in girls. It was also observed that a residual value between the calendric age and the estimated age was less than 1 year (−0.80) during the crown formation stage, i.e., till the age of 7 years. Thus, it may be concluded that this novel methodology of using the crypt to tooth ratio may be useful in estimating the age of when the tooth crown is under mineralization process. Though well-established age estimation methods are using the developing dentition, this novel methodology may be treated as an additional method and strictly not as a stand-alone method.

Conclusions

A new method of dental age estimation was attempted using the crypt area and the developing tooth area using digital radiographs. The novel CTR parameter may be useful to estimate the age during the crown formation stage of the mandibular second molar. This new method could be an additional measure to estimate the age in addition to the already well-established age estimation methods for children. However, large sample studies using several teeth need to be designed to further explore the potential application of CTR as a parameter in dental age estimation.

Limitations

The present study was a pilot study, conducted with limited sample size. Also, only one developing tooth, i.e., the mandibular second molar was considered in this study. This study may be extended to include the developing third molars also. The draw tool in ImageJ was used instead of the auto-selecting or wand tools. Hence, the observer needs technical training in using such image editing tools.

Abbreviations

CTR: Crypt to tooth ratio; OPG: Orthopantomogram; CA: Calendric age; EA: Estimated age.

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Authors' contributions

The study design was framed by the first author [JP]. He performed the analysis and the manuscript preparation. The second author [RB] guided the first author in the study and did the proof reading and corrections in the manuscript. Both authors have read and approved the manuscript before submission.

Author's information

The first author [JP] is a qualified forensic odontologist and a Fellow of Indian Board of Forensic Odontology [IBFO]. He handles medicolegal dental age estimation cases referred to his institute. He has a teaching experience of nearly 25 years in the subjects of dental anatomy, oral histology, oral pathology, and forensic odontology. The second author [RB] is an Associate Professor at School of Forensic science, National Forensic Sciences University, Gandhinagar. He is a recognized PhD guide for forensic science and also the head of International Centre for Humanitarian Forensics (ICHF), NFSU, Gandhinagar, India.

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Availability of data and materials

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

The Institutional Ethical Committee (IEC) of Govt. Dental College and Hospital, Ahmedabad, had approved the project wherein the study materials were used for this study (Ref. No. IEC-GDCH/S.1/2019). Informed consent was obtained from all individual participants included in the study. The parent's written consents were obtained in case of minors.

Consent for publication

Consent to publish the study results in scientific journals along with the consent to participate was obtained from the study subjects.

Competing interests

The authors declare that they have no competing interests.

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