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Relationship of the Pulp-to-Enamel Volume Ratio of Impacted Mandibular Third Molars with Chronological Age in an Iranian Population

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A B S T R A C T

Objectives: This study aimed to examine the relationship of pulp-to-enamel volume ratio of third molars on cone-beam computed tomography (CBCT) images with chronological age, in order to create a regression equation that can be used to estimate age in both males and females.

Materials and Methods: A total of 122 impacted mandibular third molars of patients over the age of 18 years, who were referred to the dental clinic of Tehran University of Medical Sciences were analyzed in this cross-sectional study. CBCT images in DICOM format were imported into 3D Slicer software for calculation of pulp and enamel volumes. Data were analyzed by multiple linear regression (backward method) and Pearson's correlation coefficient (alpha=0.1 in regressions, and 0.05 in correlations).

Results: There was a significant inverse correlation between the pulp-to-enamel volume ratio and chronological age in both males (-0.55, P<0.001) and females (-0.517, P<0.001), as determined by the Pearson's correlation coefficient. The mean absolute error (MAE) was lower in females (4.67 years) than in males (8.02 years) when using the pulp-to-enamel volume ratio as the sole independent variable. The regression formula that utilized the pulp-to-enamel volume ratio as a sole independent variable was able to estimate age in both sexes.

Conclusion: The results showed a statistically significant correlation between age and pulp-to-enamel volume ratio of impacted mandibular third molars, and this ratio may be applied as a useful index for age estimation.

Keywords: Age Determination by Teeth; Cone-Beam Computed Tomography; Dentin, Secondary

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INTRODUCTION

Age estimation is applicable to both living individuals and deceased bodies [1]. With the surge in global immigration, there has been an increasing need for determining the age of living individuals who often lack valid identification documents. Accurate age determination is crucial during various legal, medical, and social processes. By establishing the factual age of a person, it becomes possible to determine whether they have reached a specific legal age [2]. Typically, age estimation

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in living individuals is expected to be more precise than in deceased individuals, despite the availability of fewer methods for age estimation in living subjects. When estimating the dental age of adults, it is important to consider age-related changes that occur in teeth. Scientific methods must take into account factors such as periodontal attachment loss, erosion, root translucency, cementum deposition, root resorption, secondary dentin formation, discoloration, and increased root roughness [3]. However, these parameters are also influenced by function and pathological conditions, making them less accurate than assessing developmental stages of teeth [4]. For example, the severity of attrition is significantly impacted by environmental factors like occlusion, diet, and individual habits, which may lead to inaccurate estimations [5]. Dental measurements can be taken using two-dimensional (2D) radiographs such as panoramic and periapical, or three-dimensional (3D) radiographs like cone-beam computed tomography (CBCT) and micro-computed tomography [6]. However, the use of 2D radiographs is controversial because they cannot show the 3D morphological changes in the pulp cavity [7]. In contrast, CBCT provides precise anatomical details in three dimensions and allows for investigation of a tooth from coronal, axial, and sagittal views. Therefore, CBCT has been utilized in various studies to analyze the relationship between age and secondary dentin deposition by examining different variables such as pulp area-to-tooth ratio, pulp volume, and pulp-to-tooth volume ratio [7-9].

The pulp-to-enamel volume ratio of impacted third molar teeth has also been suggested for age estimation as the distribution of enamel and secondary dentin in these teeth is not impacted by physical or chemical stimuli or chewing habits [10]. Thus, this study aimed to examine the relationship between pulp-to-enamel volume ratio of impacted third molars on CBCT images and chronological age, in order to create a regression equation that can be used to estimate age in both males and females.

MATERIALS AND METHODS

This cross-sectional study was approved under the ethical approval code of IR.TUMS.DENTISTRY.REC.1399.135. CBCT images of impacted mandibular third molars were retrieved from the archives of the Radiology Department of the Faculty of Dentistry, Tehran University of Medical Sciences. Radiographs were selected by convenience sampling. Accordingly, all eligible radiographs that met the eligibility criteria were enrolled. A total of 122 eligible CBCT images taken between 2020 and 2022 were collected. The CBCT scans belonged to patients over the age of 19 years that had an impacted mandibular third molar. Since in the real world, when estimating the age of an adult, we often encounter a case where we do not have their actual age, we did not group individuals by age in this study.

The sample size was calculated using multiple regression analysis based on Zhang et al. [10]. With alpha=0.05, beta=0.20 (power=80%), five independent variables, and an anticipated R^2=0.10, the minimum required sample size was 122 teeth.

The CBCT images had been obtained with Whitefox CBCT scanner (Acteon, Italy) with 105kVp tube potential, 9mA tube current, and an exposure time of 17 seconds, and had a nominal diameter of 150mm and a nominal height of 130mm.

All CBCT scans had been requested for diagnostic or therapeutic purposes, and no additional radiation was given to the patients for the purpose of this study. Age and sex information was collected from the personal details entered into the system. The inclusion criteria included the presence of an impacted mandibular third molar, acceptable image resolution, no artifacts caused by metal restorative materials in adjacent teeth, and no pulp calcifications. The exclusion criteria were absence of age information, lack of root formation, and any anatomical complications in the roots. The highest resolution DICOM files were saved using Romexis software (Planmeca, Helsinki, Finland) and imported into 3D Slicer (The Slicer Community, USA), a free, open-source software that enables voxel

counting, dividing images into various parts, and measuring the volume of 3D photographs. This tool was used for calculating, analyzing, and visualizing the images. The measurement tool of the software was calibrated before measuring each patient's CBCT image.

In this study, the 3D Slicer software was used to perform 3D reconstruction of enamel and pulp chamber. The software has several tools with distinct functions, and the specific tool employed for this task was the Segment Editor. Once the images were imported into 3D Slicer and the desired tooth was selected, the Segment Editor offered various automatic and semi-automatic methods for identifying image components based on their radiodensity. For the purpose of reconstructing the dental pulp in three dimensions, the 'Grow from Seeds' tool was utilized. This particular tool employs an algorithm capable of partitioning images into foreground and background elements.

To obtain the 3D reconstruction of the pulp, lines were drawn on the desired area in sagittal, axial, and coronal sections. The foreground, which refers to the pulp, was marked by drawing lines, while the rest of the image was labeled as the background. By applying the 'Grow from Seeds' tool, the image was then separated into two parts: the pulp and the background. If the segmentation was correct, a 3D pulp image could be generated. Additional tools such as erasers, scissors, and smoothing were available for manual and automatic editing of the 3D image created using the 'Grow from Seeds' algorithm, with the aim of improving the accuracy of the calculations. Figures 1–3 show the measurement process.

To determine the 3D reconstruction of enamel, the threshold tool was utilized due to its radiopaque nature. This tool enabled the selection of a specific opacity range, and then all points with that opacity were automatically identified by the software to create a 3D image. Various manual and automatic tools such as cut, eraser, and smoothing could then be used for editing. Subsequently, the volume of 3D images obtained from the pulp and enamel of the mandibular third molar was calculated using the Segment Statistics Module. The volume ratio of pulp to enamel was also determined. Information concerning age and sex for each tooth was entered into Excel software and subsequently imported into IBM SPSS Statistics for Windows (version 25.0) for statistical analysis.

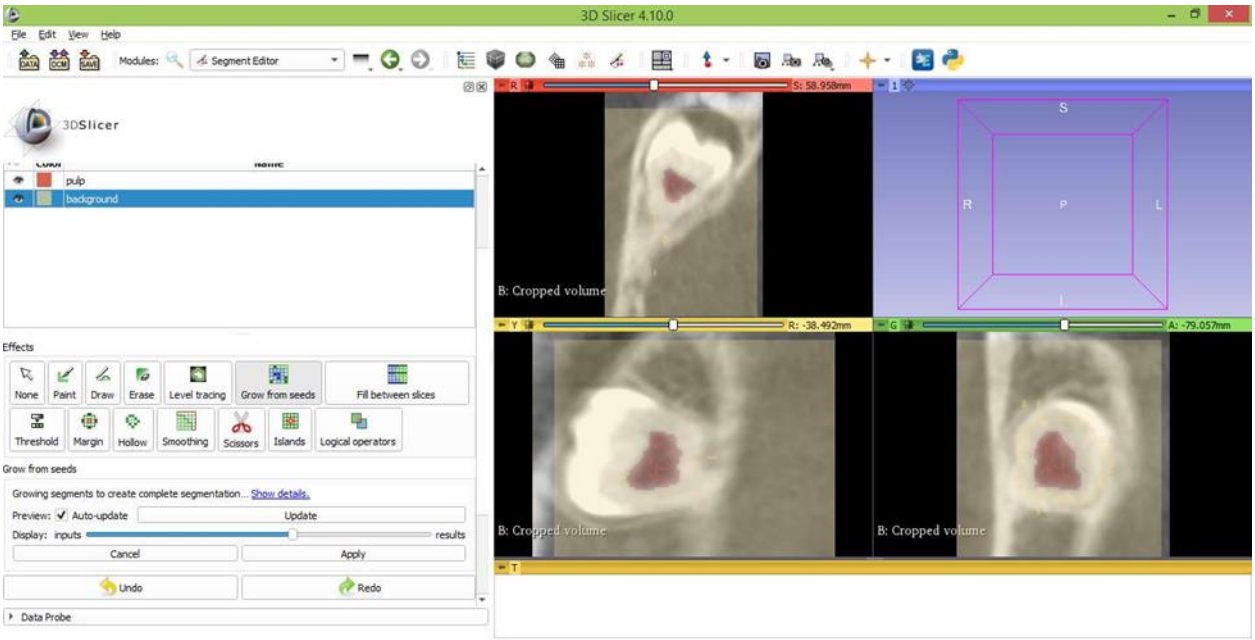


Figure 1. Segmentation of pulp using the Grow from Seeds tool in 3D Slicer.

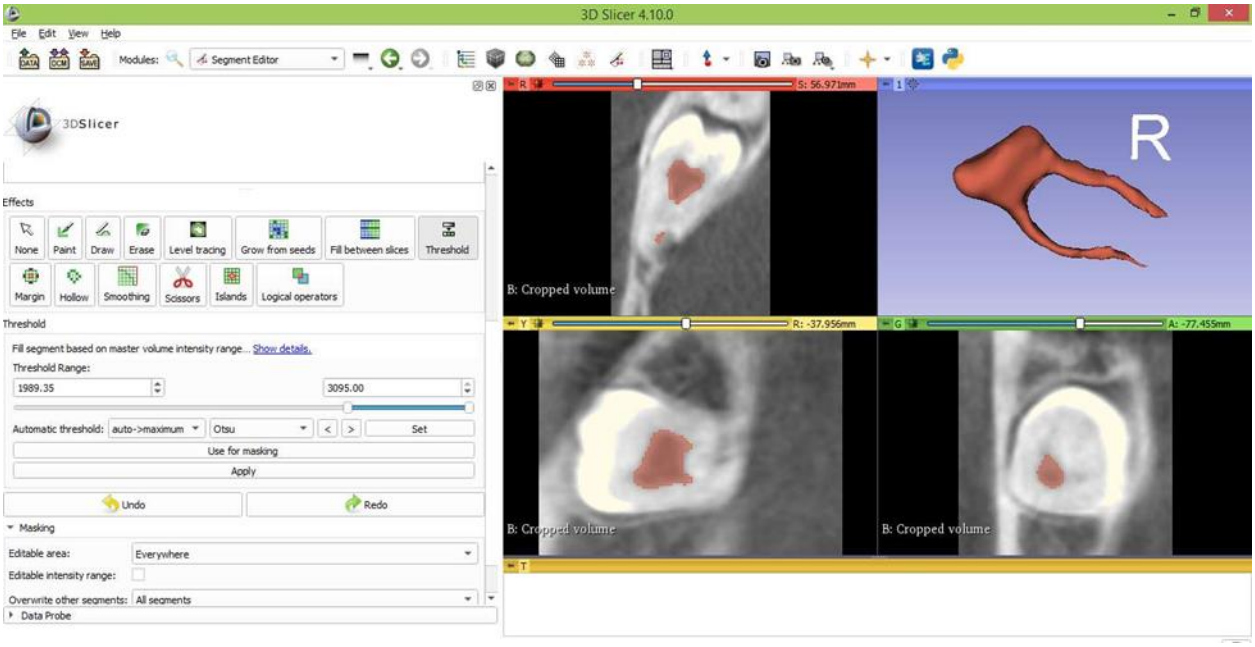


Figure 2. Enamel identification using the Threshold tool; the 3D pulp obtained by Grow from Seeds is also visible.

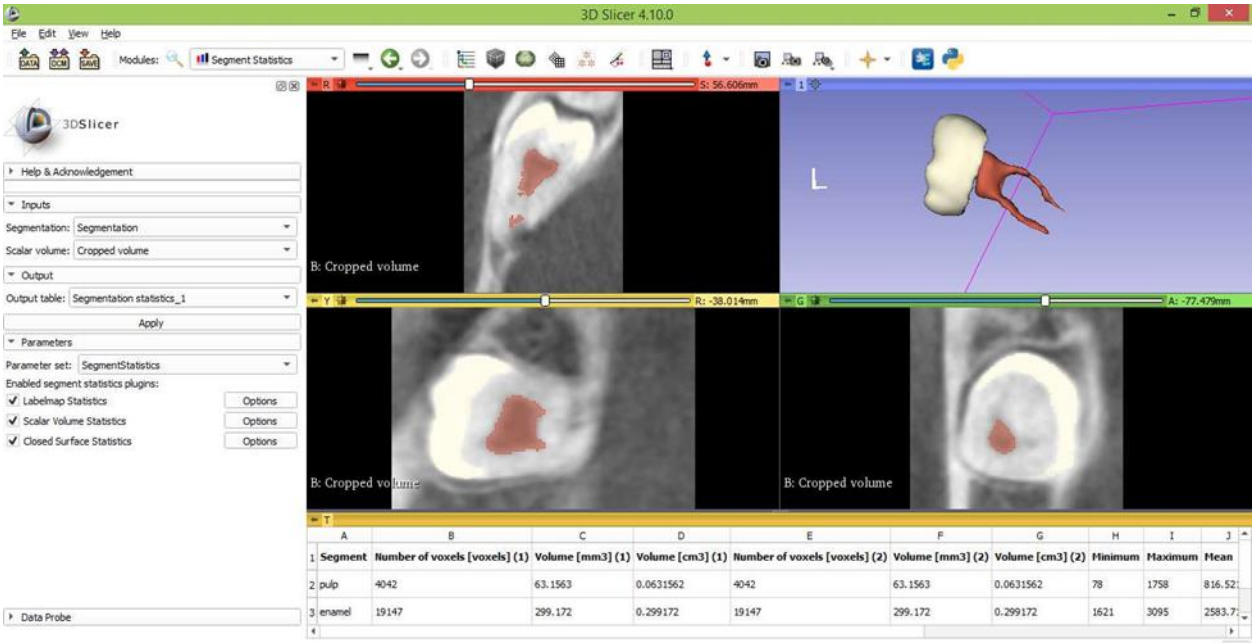


Figure 3. Calculation of pulp and enamel volumes using the Segment Statistics Module.

Statistical analysis:

The statistical analysis was performed using IBM SPSS Statistics for Windows version 25.0 (IBM, USA) In order to establish the relationship between the volume ratio of pulp to enamel and age, multiple linear regression, backward method was employed. The Pearson's correlation coefficient was calculated to determine the association between age and the volume ratio of pulp to enamel in impacted mandibular third molars. The resulting relationship was expressed as two mathematical equations (one for each sex) that could estimate age as a dependent variable, with the volume ratio of pulp to enamel serving as the independent variable for both males and females ($P<0.10$ in regression, and $P<0.05$ in correlations). All measurements were performed by two trained examiners. The primary examiner

performed the segmentations. A second examiner reviewed the segmentations for accuracy. Because the study was retrospective and based on archived CBCT images, no repeated measurements were obtained; therefore, formal intra- and inter-examiner agreement testing could not be performed.

RESULTS

This study examined CBCT images of 122 impacted third molar teeth. The sample consisted of 60 left impacted third molars and 62 right impacted third molars, with 84 teeth belonging to females and 38 teeth belonging to males. Descriptive information concerning age, pulp volume, enamel volume, and volume ratio of pulp to enamel was recorded for both male and female patients in Table 1.

Table 2 presents the results of the Pearson's correlation coefficient, which was used to examine the relationship between age and the volume ratio of pulp to enamel in both females

Table 1. Descriptive information related to age of participants, pulp volume, enamel volume, and pulp-to-enamel volume ratio

		Minimum (mm^3)	Maximum (mm^3)	Mean (mm^3)	Standard Deviation
Enamel volume	Female	145.2600	327.6560	227.695655	41.1656425
	Male	159.7660	322.2810	239.104342	39.7694695
Pulp volume	Female	24.9480	118.7040	52.297032	16.0753412
	Male	20.1406	103.8280	63.636053	18.8329605
Pulp-to-enamel volume ratio	Female	0.1204	0.3980	0.230719	0.0571186
	Male	0.1235	0.4152	0.265660	0.0683663
Age	Female	19	58	28.65	7.315
	Male	19	83	29.26	13.805

Table 2. Calculation of Pearson's correlation coefficient to investigate the correlation of age with pulp-to-enamel volume ratio

		Sex	Age	Enamel volume (mm^3)	Pulp volume (mm^3)	pulp/enamel volume ratio
Age	Pearson's correlation coefficient	Female	1	−0.057	−0.444	−0.517
		Male	1	-0.400	-0.596	-0.550
	P value	Female	–	0.608	<0.001	<0.001
		Male	–	0.013	<0.001	<0.001
Enamel volume	Pearson's correlation coefficient	Female	−0.057	1	0.516	−0.102
		Male	-0.400	1	0.575	0.044
	P value	Female	0.608	–	<0.001	0.356
		Male	0.013	–	<0.001	0.795
Pulp volume	Pearson's correlation coefficient	Female	−0.444	0.516	1	0.785
		Male	-0.596	0.575	1	0.827
	P value	Female	<0.001	<0.001	–	<0.001
		Male	<0.001	<0.001	–	<0.001
Pulp-to-enamel volume ratio	Pearson's correlation coefficient	Female	−0.517	−0.102	0.785	1
		Male	-0.550	0.044	0.827	1
	P value	Female	<0.001	0.356	<0.001	–
		Male	<0.001	0.795	<0.001	–

and males. The analysis revealed a significant correlation between age and the volume ratio of pulp to enamel, as well as between age and pulp volume and enamel volume.

For both females and males, a regression equation was derived to predict age based on the volume ratio of pulp to enamel as an independent variable. In addition, separate regression equations were developed using three variables: pulp-to-enamel volume ratio, pulp volume, and enamel volume. Table 3 shows the regression coefficients for predicting age in males and females using all three variables.

The following equations were derived to predict age using the volume ratio of pulp to enamel and all three variables (pulp volume, enamel volume, and pulp-to-enamel volume ratio) separately for females and males:

-*Females:* Age = −0.144 × (Enamel volume − 0.26) × (Pulp volume − 193.077) × (Pulp-to-enamel volume ratio + 78.352) (R^2 = 0.312, P<0.001)

-*Males:* Age = −0.591 × (Enamel volume − 1.801) × (Pulp volume − 506.305) × (Pulp-to-enamel volume ratio + 190.505) (R^2 = 0.595, P<0.001)

Table 4 displays the regression coefficients for predicting age in males and females using only the variable of the volume ratio of pulp to enamel. The regression equations to predict age using only the volume ratio of pulp to enamel as a variable were as follows:

-*Females:* Age = −66.223 × (Pulp-to-enamel volume ratio + 43.934) (R^2 = 0.267, P<0.001)

-*Males:* Age = −110.993 × (Pulp-to-enamel volume ratio + 58.75) (R^2 = 0.302, P<0.001)

The mean absolute error (MAE) between the predicted age and actual age was 4.67 years in females for the regression equation using only the volume ratio of pulp to enamel, and 4.73 years for the regression equation that includes the volume of pulp and enamel as well.

When using the volume ratio of pulp to enamel as the only variable, the MAE between the predicted age and actual age in males was 8.02 years. However, when including the volume of pulp and enamel as additional variables, the MAE decreased to 6.87 years.

The accuracy of the regression equations in predicting age was compared to the actual

Table 3. Calculation of the regression equation to estimate age in patients using three variables: pulp-to-enamel volume ratio, pulp volume, and enamel volume

	Sex	Unstandardized Coefficients B	Standardized Coefficients Beta	P-value
Multiple correlation coefficient (R)	Females	78.352	-	<0.001
	Males	190.505	-	<0.001
Enamel volume	Females	−0.144	−0.808	0.032
	Males	−0.591	−1.703	<0.001
Pulp volume	Females	0.526	1.157	0.055
	Males	1.801	0.505	<0.001
Pulp-to-enamel volume ratio	Females	−193.077	−1.508	0.004
	Males	−506.305	−2.507	<0.001

Table 4. Calculation of the regression equation to estimate the age using the variable of the volume ratio of pulp to enamel

	Sex	Unstandardized Coefficients B	Standardized Coefficients Beta	P value
Multiple correlation coefficient (R)	Women	43.934	-	<0.001
	Men	58.750	-	<0.001
Pulp to enamel volume ratio	Women	−66.223	−0.517	<0.001
	Men	−110.993	−0.550	<0.001

age of the participants. The following results were obtained:

For females, when using the regression equation that only included the volume ratio of pulp to enamel as a variable, the estimated age differed from the actual age by more than 9 years in only 13.1% of the cases (8.3% underestimation and 4.8% overestimation). For males, this percentage was 23.7% (10.5% underestimation and 13.2% overestimation) when using the same regression equation.

However, when including all three variables (pulp-to-enamel volume ratio, pulp volume, and enamel volume) in the regression equation, the percentage of the cases where the estimated age differed from the actual age by more than 9 years decreased. For females, this percentage was 11.9% (8.3% underestimation and 3.6% overestimation), while for males, the percentage increased to 31.6% (18.4% underestimation and 13.2% overestimation).

DISCUSSION

Dental age estimation can be conducted using dental radiographs. While 2D radiographs were once the norm, the introduction of CBCT in the late 1990s brought about a new method for 3D volumetric measurement of the pulp cavity [11-14]. Unlike 2D radiographs, CBCT does not suffer from issues such as low resolution or dimensional changes due to magnification and distortion.

Various teeth have been used in dental age estimation studies using CBCT, including single-rooted and multi-rooted teeth [13, 14]. However, some studies have only examined the coronal pulp and neglected the radicular pulp [15]. Mandibular third molars were selected for this study due to their independence from external factors like physical and chemical effects, eating habits, and mastication. The total volume of the pulp was also considered in this study, which included both the pulp chamber and radicular pulp volume.

Some studies have used the pulp-to-total tooth volume ratio in their analysis [16, 17]. However, dietary habits and chewing can cause errors when enamel changes have occurred. To avoid this, Agematsu et al. [18] used the volume ratio of pulp to whole teeth except enamel as an independent variable to prevent bias due to enamel changes.

Since the teeth in our study were impacted, eating habits and chewing did not affect the samples, and detection of enamel was easier due to its high radiodensity. This is why we used the volume ratio of pulp to enamel in our study to avoid the problems mentioned earlier. Our findings showed an inverse correlation between the volume ratio of pulp to enamel and age, similar to previous studies conducted using both 2D [19, 20] and 3D [12, 21, 22] radiographs.

The Pearson's correlation coefficient was used to examine the relationship between chronological age and independent variables such as pulp volume or volumetric pulp to tooth ratio. In our study, the Pearson's correlation coefficient for the pulp-to-enamel volume ratio was -0.55 for males and -0.517 for females, indicating a significant correlation between age and the pulp-to-enamel volume ratio. While the findings are similar to previous studies, there were slight differences in results due to varying study populations and tooth types [10, 11, 13, 23, 24]. Our study found that the accuracy of age estimation differed between males and females, with higher accuracy observed in females. Therefore, different age estimation formulas were needed for each sex. This differs from the findings of Sakuma et al. [5] Zhang et al. [10] and Jagannathan et al. [25] where sex did not appear to have an impact on age estimation. However, it is consistent with the results of Biuki et al. [26] Porto et al. [24] and Someda et al. [27].

The determinant coefficient was employed to examine the relationship between chronological age and independent variables such as pulp chamber volume or volume ratio of pulp to tooth in this study. Our study found that using regression equations that included all three variables (enamel volume, pulp volume, and volume ratio of pulp to enamel) for age estimation resulted in more accurate results for females, with the estimated age differing from the actual age of people by over 9 years in only 11.9% of the cases. Conversely, when using only the volume ratio of pulp to enamel as a variable,

the estimated age differed by over 9 years from the actual age in 13.1% of the cases.

For males, it is better to use the regression equation that only utilizes the pulp-to-enamel volume ratio for age estimation, as it yielded more accurate results, with the estimated age differing from the actual age by over 9 years in 23.7% of the cases. On the other hand, when using the regression equation that included both the pulp and enamel volume ratios, as well as pulp volume and enamel volume as variables, the estimated age differed from the actual age by over 9 years in 31.6% of the cases. The MAE between the estimated age and the actual age within 10 years is considered acceptable for estimating the age of adults in legal medicine. In our study, the MAE was considered acceptable, with 4.67 years for females and 8.02 years for males. However, this study has several limitations. It was a cross-sectional, single-center, retrospective study that used archived CBCT images and convenience sampling. Only impacted mandibular third molars were evaluated, the sample size was relatively small, and the sex distribution was unequal. Formal intra- and inter-examiner agreement testing was not performed, and the derived regression equations were not externally validated. Therefore, the findings should be generalized with caution. Future multicenter prospective studies with larger, balanced samples and formal calibration assessment are recommended. External validation in independent Iranian and other populations, inclusion of erupted and multi-rooted teeth, and comparison with automated segmentation or artificial intelligence-based methods are also suggested.

CONCLUSION

Our study found a significant correlation between age and the volume ratio of pulp to enamel in impacted mandibular third molars in both Iranian males and females. Additionally, our findings showed that accuracy of age estimation using regression equations was higher for females than males. The pulp-to-enamel volume ratio may serve as a useful index for age estimation in the Iranian population, but external validation is needed.

CONFLICT OF INTEREST STATEMENT

None declared.

GENERATIVE AI IN SCIENTIFIC WRITING

None declared.

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