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Age Estimation in Iranian Population Using Tooth/Pulp Ratio in Mandibular Premolars by Panoramic Radiography

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Abstract

Background: Age estimation is a fundamental part of forensic science. Considering the fact that pulp size decreases throughout the life because of secondary dentine apposition, many researchers have used it as a method for age estimation with great results. This study aimed to determine the applicability of tooth to pulp ratio in mandibular second premolars in an Iranian population using panoramic radiographs.

Methods: The sample consisted of 153 digital panoramic images of male and female Iranians aged 13-70 years, and the tooth to pulp ratio of both left and right mandibular second premolars was obtained using AutoCAD software. Analysis of variance (ANOVA), Pearson's correlation, and regression analysis were used to analyze the data. A total of 20 images were reexamined after 30 days to test intra-observer error.

Results: ANOVA test showed that gender had no effect on age estimation, and there was a significant correlation between age and tooth/pulp ratio (r=0.679 for right premolar, r=0.712 for left premolars, and r=0.736 for both premolars). Linear regression formulae were created based on the results and the mean differences between the estimated and actual age were reported to be 10.35±0.51, 9.17±0.54, and 8.83±0.52 for right, left, and both premolars, respectively. Intra-observer error showed no significant difference between repeated measurements.

Conclusions: Tooth/pulp ratio of mandibular second premolars is a reliable and repeatable method for age estimation in Iranian population and using premolars of both sides yields the best results.

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Background

Age estimation is a fundamental component of forensic medicine. It is used to predict the age of an unidentified person whether dead or alive helps in a lot of situations such as creating a profile and identifying victims in criminal cases and large-scale fatalities due to disasters or accidents. Moreover, it can be used in social cases such as refugees with no identification to grant civil rights (1-3). Therefore, a growing demand for age estimation has been shown.

Currently, the most commonly used methods are based on the analysis of skeletal remains but bones are subject to taphonomic processes and only have acceptable results in individuals in their early adulthood (4-6). Recently, numerous dental methods have been proposed for age estimation because unlike bones, teeth are much less affected by environmental or systemic factors such as drugs or endocrine problems. Additionally, tooth development patterns are less diverse than other developmental symptoms and are preserved for a long time after death; therefore, teeth are excellent parameters for age estimation (4, 7-10). Some methods are based on dental development but they do not yield reliable results after the age of 21 (4,11). Methods that are used in adults

Highlights

► Pulp/tooth ratio of premolars on panoramic is useful to predict chronological age.
► Using both premolars' ratio was better correlated with chronological age than one of them.
► Upper canine ratio was better correlated with chronological age of premolars.

are based on degenerative processes of the teeth but a lot of them require extraction of the teeth and studying them which can be both expensive and unethical (12-17). However, some changes like secondary dentin deposition in teeth happen during the entire lifespan of a subject and except for caries and specific abrasion, no other factor affects it. Additionally, it can be analyzed using radiographs which can be easily done in living individuals with a low cost. Therefore, it has become a popular parameter for age estimation in recent years (18-23).

Kvaal et al first introduced a method for age estimation based on secondary deposition by taking radiographs of teeth and measuring parameters like width and length of different parts of tooth and pulp. Based on the analysis of the data, they concluded that indeed secondary has a correlation with chronological age and can be used to

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estimate age, producing acceptable results (19, 24). In 2004, Cameriere et al proposed a new method based on the analysis of pulp to tooth area ratio (AR) in radiographs. This method was originally tested on maxillary canines using peri-apical x-rays and showed acceptable results (20). Since Cameriere's method was introduced, multiple researchers have tested and validated its repeatability and reliability in different cultures and different teeth such as incisors and mandibular premolars (4,18,25-38). The mandibular premolars alongside maxillary canines have shown to be the most appropriate teeth to be analyzed for age estimation (28,29). Panoramic radiographs have also been tested for age estimation with great success (28,39,40). They are taken routinely for clinical examination of teeth and offer a great field of all quadrants of the mouth and unlike peri-apical x-rays, which can differ based on the technique and technician's experience, they have high repeatability with little or no error (12).

The aim of this study is to determine the accuracy of Cameriere's method for age estimation in an Iranian population in Ahvaz using panoramic radiographs and to create a formula for age estimation specific to this region. If the results are satisfactory, it can be recommended for future use in forensic science.

Materials and Methods

The sample consisted of 153 digital panoramic radiographs belonging to patients who had taken them for other purposes. They were selected randomly from the archive of the Department of Oral and Maxillofacial Radiology at Ahvaz JondiShapur University of Medical Sciences, Faculty of Dentistry. Panoramic images belonged to 65 male and 88 female patients aged 13-70 years. The subjects were divided into 6 equally distributed groups based on their age with an interval of 10 years each. The patients' gender, date of birth, and date of x-ray were recorded according to their files, and the chronological age of the patients was obtained by subtracting date of image from date of birth. All panoramic radiographs were taken using a Cranex D X-ray unit (Sordex, Helsinki, Finland). Radiographs with the following criteria were included in the study: having good image quality with visible contours of tooth and pulp and the presence of both mandibular left and right second premolars with fully formed roots. The exclusion criteria were the presence of one of the following conditions: root fillings, crown restorations, severe caries, rotations, severe attrition, large areas of overlap with neighboring teeth, or other developmental anomalies.

All panoramic radiographs were obtained from the imaging software as high-resolution JPEG images. Images were attached to a worksheet in Autodesk AutoCAD 2019. For each of the mandibular second premolars, at least 20 points were selected and connected to determine the outline of the teeth using the polyline tool and at least 10 points were selected to identify the pulp area (Figure 1). Tooth/pulp AR was calculated by dividing tooth area by
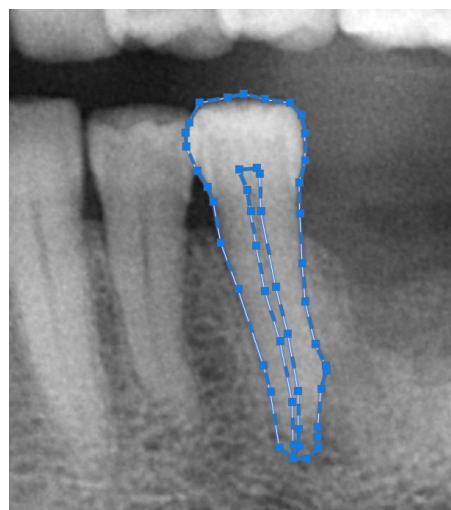
Figure 1. A Cropped Digital Panoramic Image Showing at Least 20 and 10 Points, Respecitvely, for Tooth and Pulp Area Connected to Show Periphery of the Mentioned Areas Using AutoCad Software.

pulp area.

In order to blind the observer, after entering each subject's chronological age and gender into a Microsoft Office 2013 Excel spreadsheet (Microsoft Corp., Redmond, WA, USA), every image was given a unique identification number and the measurements were performed randomly based on ID numbers. A total of 20 images were randomly selected and measured after 30 days in order to test intra-observer error using a paired sample t-test.

All statistical analyses were performed by Statistical Package for the Social Sciences (SPSS version 23.0) and $P < 0.05$ was considered statistically significant.

Analysis of variance (ANOVA) test was performed to determine whether any significant difference existed between male and female subjects in terms of AR. Pearson's correlation coefficient between age and AR for right, left and, and both premolars together was calculated to determine if any relationship existed. Linear regression was developed and used to estimate age based on AR for left, right, and both premolars.

Results

The present study was conducted on 153 digital panoramic radiographs which were divided into 6 age groups of roughly the same size (25±2). Table 1 shows the number

Table 1. Gender and Age Distribution of Subjects

Age Group	N	Males	Females
Under 20	24	11	13
20-29	27	8	19
30-39	27	11	16
40-49	26	19	7
50-59	24	9	15
Over 60	25	7	18

of cases and gender distribution in 6 age groups.

Paired sample *t* test showed no significant difference in intra-observer error between first and second measurements for premolars ($P=0.586$ for the right side, $P=0.429$ for the left side).

ANOVA test showed no statistically significant difference between AR and gender ($P=0.221$, $P=0.896$).

Tooth to pulp AR increased with age and ranged from 4.78 to 10.76 for right second premolar and from 4.42 to 10.67 for left second premolar. Figure 2 shows AR for the right and left premolars by age group.

Pearson's correlation coefficient showed a strong and direct relationship between age and tooth to pulp AR (r=0.679 for right premolar, r=0.712 for left premolars, and r=0.736 for both premolars). Table 2 shows Pearson's correlation coefficient for different teeth and Figure 3 shows the scatter diagram of the correlation between age and AR in the right and left second premolars.

Linear regression formulae were created to estimate the age based on AR of different teeth according to regression model analysis (Tables 3 and 4).

Results of the difference between estimated age using formulae and actual age can be seen in Tables 4 and 5. Best results were found when using both premolars for age estimation (8.83±0.52) compared to the right (10.35±0.51) and left (9.17±0.54).

Discussion

Age estimation is an indispensable factor in forensic medicine for creating the profile of living and dead unidentified persons. Estimating the age of a living person

Table 2. Pearson's Correlation Coefficient Between Age and Tooth to Pulp Area Ratio (AR) for Different Teeth

| Tooth	R	R Square	Standard Error of the Estimate
Right premolar	0.679	0.461	11.951
Left premolar	0.712	0.506	11.438
Both premolars	0.736	0.542	11.055

has a lot of challenges because it needs to be ethical, cost-effective, and simple. Secondary dentine apposition in teeth is an excellent biomarker for age estimation because it happens throughout the lifespan of a person and can be analyzed in dental radiographs such as peri-apical and panoramic x-rays thus making it widely acceptable and executable (19,41,42). Cameriere et al have proposed the use of pulp to tooth AR to determine the age and previous studies have shown great acceptability and validity in different ethnic groups (18,20-22,25-28,42).

In the current study, digital panoramic radiographs of Iranians living in Ahvaz were used to determine age based on the tooth to pulp AR. One of the concerns for measurement error in panoramic radiographs is that some observers may have trouble identifying anatomical structures. However, in this study, all images had high image quality with great contrast and tooth and pulp had visible outlines so the issue was resolved. In addition, insignificant statistical value for intra-observer error showed that the method had high repeatability.

Mandibular second premolars were chosen for the assessment of age because previous studies have shown that maxillary canines and mandibular premolars have the
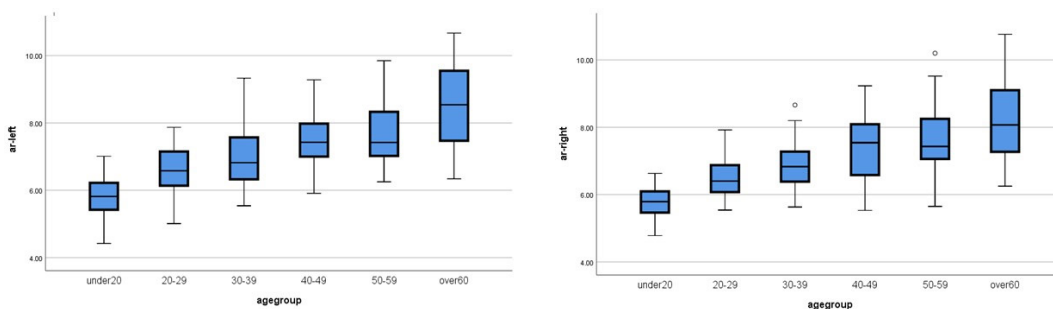
Figure 2. Boxplots Showing Tooth to Pulp Area Ratio of Right and Left Premolars in Different Age Groups.
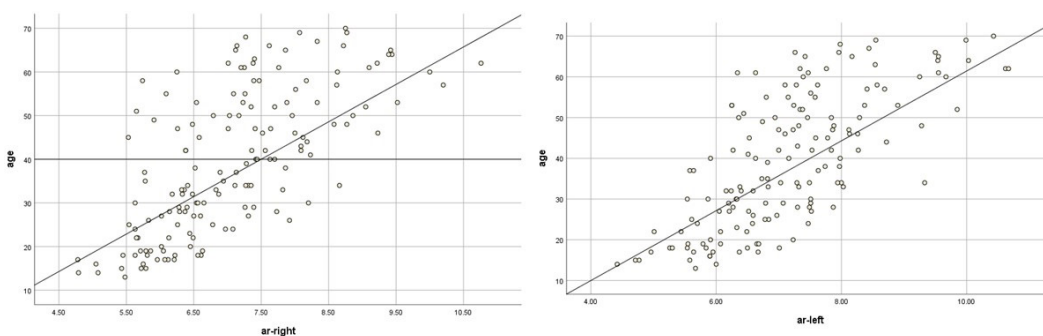
Figure 3. Scatter Diagram of Correlation Between Age and AR in the Right and Left Second Premolars.

Table 3. Regression Model Analysis Used to Create Formulae for Age Estimation

Model		Unstandardized Coefficients		t	P Value
		B	Standard Error		
Right premolar	(Constant)	-27.500	5.974	-4.604	0.000
	Area ratio-right	9.480	0.834	11.364	0.000
Left premolar	(Constant)	-26.899	5.415	-4.967	0.000
	Area ratio-left	9.271	0.745	12.443	0.000
Both premolars	(Constant)	-34.552	5.693	-6.069	0.000
	ar-left	6.075	1.180	5.146	0.000
	ar-right	4.321	1.265	3.416	0.001

Table 4. Formulae Created for Age Estimation

Tooth	Formula
Right premolar	(9.48*ARR) - 27.5
Left premolar	(9.271*ARL) – 26.889
Both premolars	(4.321*ARR) + (6.075*ARL) -34.552

Table 5. Mean Difference Between Estimated and Actual Values of Age

Age Group	Right Premolar	Left Premolar	Both Premolars
Under 20	12.38±0.88	9.77±1.14	8.87±0.94
20-29	10.92±1.12	8.93±1.09	8.30±0.93
30-39	7.48±1.13	6.60±1.14	5.57±1.08
40-49	8.29±0.96	5.77±0.80	5.93±0.87
50-59	10.37±1.47	11.70±1.33	11.57±1.72
Over 60	12.99±1.61	12.74±1.84	13.22±1.72
Total	10.35±0.51	9.17±0.54	8.83±0.52

highest accuracy for age estimation (18,28,29,36,37,42). Mandibular premolars were chosen instead of canines because no studies have been conducted in Iranian population using premolars. Teeth on both sides of the mandible were analyzed to see if the results improved.

The study showed that gender had no effect on age estimation and tooth to pulp AR, as has been published by other researchers in previous studies (10,18,26,39).

Data showed that tooth to pulp AR increased with age which means that the pulp area decreased with age due to secondary dentine apposition, as has been published in previous studies (27-32,34). Pearson correlation coefficient showed a significant relationship between chronological age and tooth/pulp AR which suggests that the pulp/tooth AR can be a good parameter for age estimation. Tooth to pulp AR was used in the current study unlike other studies which used pulp to tooth AR; therefore, the correlation coefficient was direct as opposed to the inverse correlation reported in other studies. However, the value of regression coefficient was in the same range as reported in other studies, indicating that premolars are significantly correlated with age (28,29). Canines have also been reported to have a strong correlation with age. Dehghani et al reported that the regression value of maxillary canines was r=−0.794 which is close to the regression value reported in our study (ranging from 0.679 to 0.736), which means that premolars have a high correlation with age in Iranian population (25).

Regression coefficient of both premolars (r=0.736) was slightly higher than each premolar alone (r=0.679 for right and r=0.712 for left), showing that using both premolars can produce improved results although not with a high margin.

The mean difference between chronological age and estimated age using AR of both premolars had the highest accuracy (8.83±0.52). According to Cameriere et al (18,20), the mean error of equal or less than 10 years is acceptable for forensic science, and the mean difference in this study is close to the residual standard error reported by Cameriere et al (7.42 years) when using a single type of premolars (28). However, this value decreased to 5.75 when they used two different premolars which could mean that the use of two different teeth would yield better results as opposed to one tooth on both sides. Yet, this conclusion needs to be tested in an Iranian population in the future.

It was observed that the mean difference between chronological age and estimated age obtained via regression formulae yielded the best results in ages between 30 and 50 years (5.57±1.08 in 30-39, 5.93±0.87) which means premolars have the best accuracy for age estimation in adults compared to younger or older individuals.

Conclusions

The results of the study showed that using tooth/pulp ratio of mandibular second premolars can be a simple and cost-efficient method with acceptable results for age estimation in Iranians especially when both right and left premolars are used. The formulae created can be used to determine the age of individuals in a short period of time. The use of panoramic radiographs with higher image quality can improve the results especially if they are used to determine the formulae. Further studies are needed to be done on Iranian population to determine the effects of other teeth or combinations of them.

Conflict of Interest Disclosures
The authors declare that they have no conflict of interests.

Ethical Statement
The protocol of the study was reviewed and approved by the Ethics Committee of Research Deputy of Ahvaz JondiShapur University of Medical Sciences, Ahvaz, Iran, and the study was conducted in accordance with the ethical standards of the Declaration of Helsinki.

Authors' Contribution
MK and AD conceived the idea of the research. MK carried out the experiment and wrote the manuscript with help and supervision of AD.

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