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ORIGINAL ARTICLE

Estimation of Age Based on Tooth: Pulp Area Ratio of Maxillary Central Incisor assessed in an Indian Sample—An Intraoral Periapical Radiographic Study

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ABSTRACT

Radiographic study of teeth is a quick, simple, and a nondestructive process that can be applied to living and deceased persons. Further, procedures, such as computer-assisted image analysis avoid the bias inherent in observer's subjectivity and improve reliability, accuracy, and precision. This study intends to assess indirect digitization of intraoral periapical radiograph of maxillary central incisors by using standardized, specially designed software "DentalCAD" to estimate the area of dental pulp, which will be correlated with different age groups.

Keywords: Age estimation, Forensic science, Intraoral periapical radiographs, Pulp/tooth area, Stepwise linear regression.

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INTRODUCTION

We are all biologically unique and become diverse as we age. Age is a progressive inevitable change among living beings and estimation of age is required for identification, which is necessary for legal forensic purpose. Teeth are the hardest bodily structures and are least affected by the taphonomic process.[1] Because of their individuality and specificity, dentition and finger prints are two of the most scientifically reliable methods of identification. Teeth, the hardest bodily structures and the most durable part of skeleton, are useful in forensic science and anthropology and can act as biomarker of aging.[2,3] As age advances, many changes occur in tooth structures. The enamel does not show age-related changes except for loss in permeability and increase in brittleness. The remaining structures, such as dentin, cementum, and dental pulp show age-related physiologic and pathologic changes.[2,4] One such change is the deposition of secondary dentin throughout the life,[5] which results in reduction of root canal length and width.[6] Age estimation of individuals older than 21 years of age still constitutes a great challenge for medicolegal research.[7] Age estimation of dental pulp is usually done using radiographic and digital methods. Any tooth can be used to assess age. Maxillary central incisors that are used for age estimation are single-rooted teeth and thus are easier to analyze. The main aim of the study was to estimate the age of an adult individual based on the relation between age and measurement of the pulp/tooth area ratio obtained from intraoral periapical radiographs beyond 21 years of age.

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MATERIALS AND METHODS

The present study was conducted in the Department of Oral Medicine and Radiology, Kamineni Institute of Dental Sciences, Narketpally, Nalgonda district, Andhra Pradesh, India, and approved by the "Ethics Committee" of the institution. The study group comprised 200 subjects; of which, 100 were males and 100 females in the age range of 21 to 60 years, categorized into 4 age groups of 10 years of difference. Each group of 10-year interval had 50 subjects; out of which, 25 were males and 25 females. The maxillary central incisor free from caries, fracture, restoration, abrasion, and erosion was selected for the study. An intraoral X-ray machine with 65 KvP and 8 mA attached with a long cone was used to take radiographs with an exposure time of 0.8 seconds. All the films were processed manually in a well-equipped, light-proof dark room.

The intraoral periapical radiographs were scanned in the HP scanjet G2 410 with 1200×1200 dpi resolution and the images were stored in a computer file in the JPEG format.

For the image analysis, Vision AMD processor with 11 inch monitor Windows 7 basic operating system 2 GB RAM, 320 GB hard disk with the software "Dental CAD" was used.

The radiographic images of maxillary central incisor were processed using standardized specially designed software "DentalCAD" (Fig. 1).

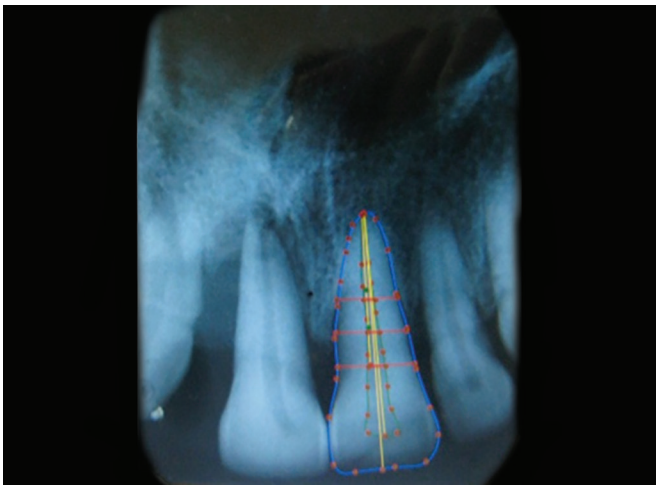


Fig. 1: Scanned IOPA radiographic image in "Dental CAD"

Twenty points from each tooth outline and fifteen points for each pulp outline were identified. The tooth outline was marked with the points; the cementoenamel junction (CEJ) was identified. The measurements of the tooth area and pulp area from the radiographic images of the maxillary central incisor were evaluated. The tooth length, pulp length, and root length were measured. The width of the root and pulp at three different levels, one at the CEJ, second at the midroot level, and third at the midpoint level between the CEJ and mid root level, was measured. Ratios between the length and width measurements of the same tooth were calculated in order to avoid measurement errors due to differences in magnification of the image on the radiograph, such ratios are the morphological variables (Fig. 2).

The morphological variables were:

p = pulp/root length
r = pulp/tooth length
a = pulp/root width at the CEJ level
c = pulp/root width at the midroot level
b = pulp/root width at the midpoint level between the CEJ level and midroot level.
AR = pulp/tooth area ratio

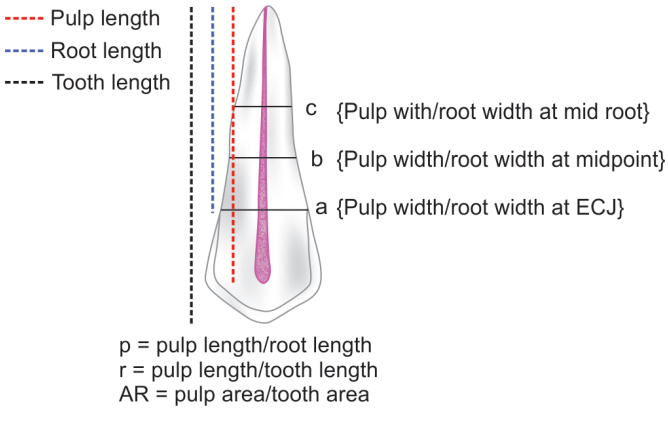


Fig. 2: Morphological variables

The morphological variables, age, and the subject's name and date of birth were entered in Microsoft Excel spreadsheet.

The chronological age was calculated by subtracting the patient's date of birth from the date of radiograph taken. Then the measurements were statistically analyzed.

Statistical Analysis

Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS). Karl Pearson's correlation coefficient was calculated to determine the association between age and PT ratios as well as analysis of covariance (ANCOVA) to study the possible interaction between age and sex and maxillary central. Linear regression equations were performed to calculate the regression equations. Regression equations were also computed for estimating age. The standard error of estimate (SEE) was calculated to predict the deviation of the estimated age from the actual. The significance of the difference between chronological and dental age and intra-examiner reproducibility were tested by paired Student's t-test.

RESULTS

Table 1 describing the morphological variables for 100 males and 100 females did not show any difference between the genders as p and t-values were not significant. The above observation shows that gender does not influence the regression model used to estimate chronological age in both males and females.

Pearson's correlation coefficient was found to assess the nature and degree of relation of different morphological variables with chronological age. Pearson's correlation coefficients between age and predictive morphological variants in the total sample were significant and inversely correlated for all variables. Among them, AR (pulp/tooth area ratio) and c (pulp/root width at midroot level) were highly significant ($p < 0.001$), whereas p (ratio of pulp/root length) and r (ratio of pulp/tooth length) showed a low level of relation with age as shown in Table 2.

The subject's age was modeled as a linear function of the morphological variables (predictors). So a stepwise regression procedure was applied to optimize this model.

Table 3 shows regression analysis in 100 males, Table 4 in females, and Table 5 in the total sample.

Regression analysis showed a total standard error of 9.727 and an explained variance of 41.8% in predicting chronological age in males from all the predictors. In the females, regression analysis showed a total standard error of 9.235 and an explained variance of 42.7% in predicting chronological age from all the predictors and in the total subjects of 200, the regression analysis showed a total standard error of 9.423 and an explained variance of 40.9% in predicting chronological age from all the predictors.

Table 1: Descriptive information on morphological variables for males and females

Variable	*Gender*	*Mean*	*SD*	*t-value*	*p-value*	*Sig.*
AR (pulp/tooth area ratio)	Male	0.18270	0.042208	1.511	0.313	NS
	Female	0.17740	0.031095			
p (pulp/root length)	Male	1.40450	0.130681	–0.904	0.367	NS
	Female	1.41990	0.109355			
r (pulp/tooth length)	Male	0.83510	0.058921	–0.658	0.511	NS
	Female	0.84010	0.048000			
a (pulp/root width at CEJ)	Male	0.24870	0.046616	1.532	0.127	NS
	Female	0.23890	0.043829			
c (pulp/root width at midroot level)	Male	0.17290	0.043036	0.734	0.464	NS
	Female	0.16890	0.033429			
b (pulp/root width at midpoint b/w a & c)	Male	0.20550	0.040486	–0.457	0.648	NS
	Female	0.21020	0.094644			

Student (unpaired) t-test; $p < 0.05$ significant; $p > 0.005$ nonsignificant

Table 2: Correlation between age and predictive morphological variables

		Correlation between age with			
Sample	*Morphological variables*	*r-value*	*t-value*	*p-value*	*Sig.*
Total	AR (pulp/tooth area ratio)	–0.411**	–6.33643	0.000	S
	p (pulp/root length)	0.452**	–1.9548	0.000	S
	r (pulp/tooth length)	0.680**	–3.75136	0.000	S
	a (pulp/root width at CEJ)	0.610**	–4.3089	0.000	S
	c (pulp/root width at mid root level)	0.383**	–2.82894	0.000	S
	B (pulp/root width at midpoint b/w a & c)	0.346**	–1.33856	0.000	S
Male	AR (pulp/tooth area ratio)	–0.449**	–5.29288	0.000	S
	p (pulp/root length)	0.438**	–1.98232	0.000	S
	r (pulp/tooth length)	–0.139	–1.39472	0.166	NS
	a (pulp/root width at CEJ)	0.623**	–5.74874	0.000	S
	c (pulp/root width at mid root level)	0.397**	–2.67561	0.000	S
	b (pulp/root width at midpoint b/w a & c)	0.646**	–5.12626	0.000	S
Female	AR (pulp/tooth area ratio)	–0.362**	–4.96934	0.000	S
	p (pulp/root length)	–0.144	–1.44440	0.151	NS
	r (pulp/tooth length)	–0.179	–1.80851	0.075	NS
	a (pulp/root width at CEJ)	0.595**	–2.76928	0.000	S
	c (pulp/root width at mid root level)	0.354**	–2.98625	0.000	S
	b (pulp/root width at midpoint b/w a & c)	0.265**	0.042214	0.008	S

Pearson's correlation coefficient (r-value). $p < 0.05$, $p < 0.01$ significant; $p > 0.05$ nonsignificant; *Correlation is significant at the 0.05 level (2-tailed); **Correlation is significant at the 0.01 level (2-tailed)

Table 3: Regression analysis predicting chronological age from all the predictors (male samples)

Independent variables	*Regression coefficient*	*SE of regression coefficient*	*t-value*	*p-value*	*Sig.*
Intercept	107.523	21.116	5.092	0.000	S
AR (pulp/tooth area ratio)	–20.525	49.191	–0.417	0.677	NS
p (pulp/root length)	–0.352	8.671	–0.041	0.968	NS
r (pulp/tooth length)	–38.533	25.946	–1.485	0.141	NS
a (pulp/root width at CEJ)	–31.211	31.498	–0.991	0.324	NS
c (pulp/root width at mid root level)	–145.428	26.051	–5.582	0.000	S
b (pulp/root width at midpoint b/w a & c)	11.861	36.193	0.328	0.744	NS

Explained variance $R^2 = 41.8\%$, SE = 9.727

Table 4: Regression analysis predicting chronological age from all the predictors (female samples)

Independent variables	*Regression coefficient*	*SE of regression coefficient*	*t-value*	*p-value*	*Sig.*
Intercept	72.192	21.467	3.596	0.001	S
AR (pulp/tooth area ratio)	–89.209	56.904	–1.568	0.120	NS
p (pulp/root length)	2.681	10.720	0.250	0.803	NS
r (pulp/tooth length)	7.551	30.758	0.246	0.807	NS
a (pulp/root width at CEJ)	–2.688	28.456	–0.094	0.925	NS
c (pulp/root width at mid root level)	–202.789	30.242	–6.705	0.000	S
b (pulp/root width at midpoint b/w a & c)	22.767	10.324	2.205	0.030	S

Explained variance R^2 = 42.7%, SE = 9.235

Table 5: Regression analysis predicting chronological age from all the predictors (total sample)

Independent variables	*Regression coefficient*	*SE of regression coefficient*	*t-value*	*p-value*	*Sig.*
Intercept	93.954	14.703	6.390	0.000	S
AR (pulp/tooth area ratio)	–130.6887	42.3351	–3.0870	0.0023	S
p (pulp/root length)	0.677	6.569	0.103	0.918	NS
r (pulp/tooth length)	–19.583	19.332	–1.013	0.312	NS
a (pulp/root width at CEJ)	–16.014	20.117	–0.796	0.427	NS
c (pulp/root width at mid root level)	–167.770	19.275	–8.704	0.000	S
b (pulp/root width at midpoint b/w a & c)	19.689	9.979	1.973	0.050	S

Explained variance R^2 = 40.9%, SE = 9.423

Table 6: Regression analysis predicting chronological age from selected predictors (male samples)

Independent variables	*Regression coefficient*	*SE of regression coefficient*	*t-value*	*p-value*	*Sig.*
Intercept	78.465	4.952	15.844	0.000	S
AR (pulp/tooth area ratio)	–74.844	25.073	–2.985	0.004	S
c (pulp/root width at mid root level)	–139.740	24.591	–5.683	0.000	S

Explained variance R^2 = 40.1%, SE = 9.664

Table 7: Regression analysis predicting chronological age from selected predictors (female samples)

Independent variables	*Regression coefficient*	*SE of regression coefficient*	*t-value*	*p-value*	*Sig.*
Intercept	85.595	6.194	13.820	0.000	S
AR (pulp/tooth area ratio)	–63.629	32.084	–1.983	0.050	S
c (pulp/root width at mid root level)	–194.776	29.844	–6.526	0.000	S

Explained variance R^2 = 39.6%, SE = 9.284

Table 8: Regression analysis predicting chronological age from selected predictors (total sample)

Independent variables	*Regression coefficient*	*SE of regression coefficient*	*t-value*	*p-value*	*Sig.*
Intercept	81.048	3.813	21.254	0.000	S
AR (pulp/tooth area ratio)	–69.695	19.575	–3.560	0.000	S
c (pulp/root width at mid root level)	–160.792	18.855	–8.528	0.000	S

Explained variance R^2 = 39.3%, SE = 9.455

The results (Table 5) show that only variables AR and c together contributed significantly.

Table 6 shows the regression analysis from the selected variables AR and c to predict the chronological age for males with standard error of 9.664 and the explained variance of 40.1%.

Table 7 shows the regression analysis from the selected variables AR and c to predict the chronological age for females with standard error of 9.284 and the explained variance of 39.6%.

Table 8 shows the regression analysis from the selected variables AR and c to predict the chronological age in the total sample with standard error of 9.455 and the explained variance of 39.3%.

So the regression model utilizing AR and c [Tables 6 to 8] was used,which yields the following linear regression formula to predict the chronological age as shown in Table 9:

$$\text{Age} = 81.048 - 69.695\ (\text{AR}) - 160.792\ (\text{c})$$

In the total subjects of 200, the standard error obtained was 9.455 and the explained variance was 39.3%. For an unknown person without the sex identification, the above linear regression formula can be utilized. For a person whose sex is known and if the unknown individual is male, the formula for age prediction is

$$\text{Age} = 78.465 - 74.844\ (\text{AR}) - 139.740\ (\text{c})$$

Table 9: Regression model utilizing predictor variables for predicting chronological age

Group	*Prediction of age*	*Standard error*	*Explained variance R^2*
Male	Age = 78.465 – 74.844 (AR) – 139.740 (c)	9.664	40.1%
Female	Age = 85.595 – 63.6298 (AR) – 194.776 (c)	9.284	39.6%
Total	Age = 81.048 – 69.695 (AR) – 160.792 (c)	9.455	39.3%

In the present study with 100 males, the standard error was 9.664 and the explained variance was 40.1%. If the unknown individual is female, the regression formula for the age prediction is

Age = 85.595 – 63.6298 (AR) – 194.776 (c)

In the present study with 100 females, the standard error was 9.284 and the explained variance was 39.6%. The full model explained 40.9% of total variance, whereas the model with AR and c variables explained 39.3%.

The observed *vs* predicted plot (Graph 1) shows that the regression model fits the trend of data reasonably well with few observations appeared to be outside the boundary.

Graph 2 shows that the predicted age is more precise in the groups II and III and the difference increases as the age advances in the group IV.

DISCUSSION

Age estimation by apposition of secondary dentin is a quantitative method; more controllable scientifically and is less dependent on technical ability. The study of morphological parameters of the teeth on radiographs is considered to be more reliable than most other methods of age estimation.[8] Assessment of pulp/tooth area ratio, in particular, is an indirect quantification of secondary dentin deposition.[9] Secondary dentin has been preferred since it is encased not only by harder tissue, such as enamel and cementum, but also by primary dentin.[1] Evaluation of this parameter is considered an "internal examination," with the potential to eliminate the effect of environmental factors on human remains.[10] In addition, earlier studies have indicated that the amount of secondary dentin is correlated with chronological age[11–13] and can be measured indirectly by radiographs.[14–17]

The methods of measurements can be standardized and reproducible with newer technology, such as AutoCAD 2000, AutoCAD 2004, Photoshop 6, SEMPER'6, Geotech ®USA, EPI INFO.

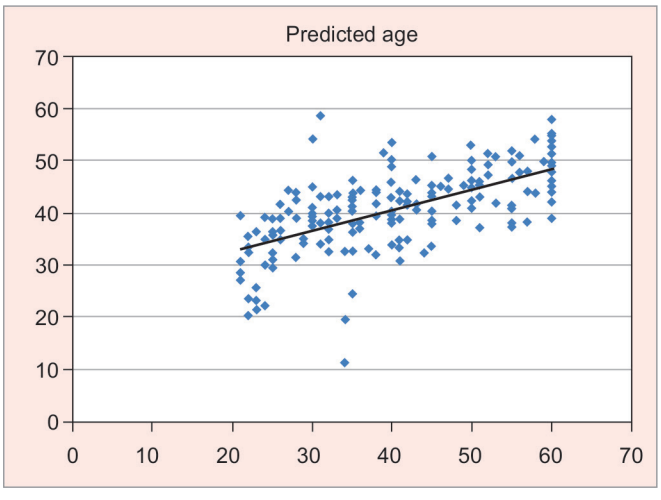
In the aforementioned technologies, we have to be dependent on a computer operator who is a specialist in AutoCAD to carry out the markings and calculate the measurements of the various parameters needed to arrive at the results. To avoid being dependent on a computer operator who is also an AutoCAD specialist, a software was specially designed that empowers an oral radiologist to make the measurements on his own.

The ratio between the tooth and pulp measurements was calculated and used in the analysis of this study in order to reduce the effect of a possible variation in the magnification and angulations of the radiographs.[14]

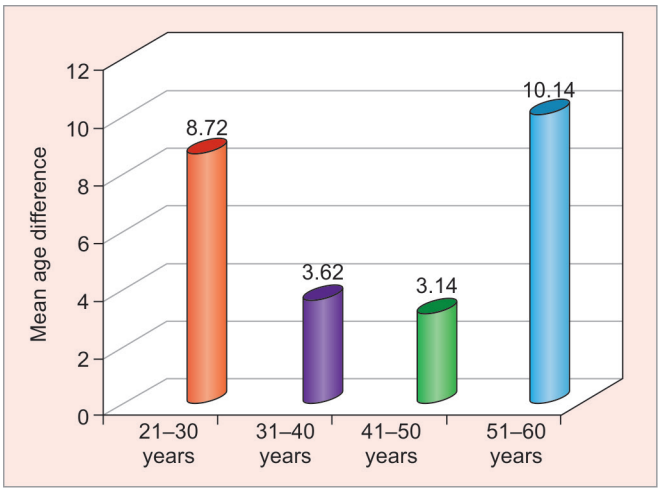
Propanpoch et al [17] tried a radiographic method of age estimation by morphometric analysis of the dental pulp chamber in adult human beings.

Kvaal et al[14] devised a radiographic method, which could be used to estimate the chronological age of an adult from measurements of the size of pulp on full-mouth radiographs.

In the present study, maxillary teeth were used as they are more convenient for age determination than mandibular teeth as Brkic et al, in 2006, found that although the teeth of both jaws are reliable for the dental



Graph 1: Predicted age



Graph 2: Mean: Age difference

age estimation, the correlation coefficient was stronger for all of the types of teeth in the upper jaw.[18]

In addition, Fancy, in 1980, stated that growth layers of maxillary teeth are more regular and distinct than those of mandibular teeth.[19]

Kvaal et al[14] stated that there were no significant differences between permanent teeth from the left and right sides of the jaw. Consequently, in the present study, teeth were chosen either from the left or from the right side, whichever were best suited for measurement.

The aim of this study was to attempt establishing a correlation between the chronological age of the individuals (21–60 years males and females) and the pulp/tooth ratio and width of the pulp chamber of maxillary central incisors as visualized and measured by periapical radiographs.

The morphological variables taken into consideration in the present study are "p" (pulp/root length), "r" (pulp/tooth length), "a" (pulp/root width at CEJ level), "b" (pulp/root width at mid-point level between CEJ level and midroot level), "c" (pulp/root width at midroot level), and "AR" (pulp/tooth area).

Ratios between the length and width measurements of the same tooth were calculated in order to avoid measurement errors due to differences in magnification of the image on the radiograph, such ratios are the morphological variables.

The present study showed that "gender" has no significant influence on age estimation when incisor measurements are used. This finding is similar to the previous studies conducted on 100 patients using OPGs with 45 males and 54 females[8] and another study done using intraoral periapical IOPA radiographs including 114 canines of males and 86 canines of females.[1]

In the present study, Pearson's correlation coefficient between age and morphological variables showed that all of them were significantly correlated with age and all correlation coefficients between age and morphological variables were significant and negative, comparing the above results with one of the study done on digitizing IOPA radiographs with 114 canines of males and 86 canines of females,[1] which showed the same result.

In the present study, the ratio between pulp and root length of the total samples was mildly significant. Whereas in a previous study done on OPGs by Cameriere et al[8] the variable p was positive and not significant. This may be attributed to the difference in sample sizes of the two studies.

Incidentally, in the present study, the variable p was positive and statistically not significant when the females alone were considered and, therefore, p was excluded as a predictor of age.

In the present study, "r" was positive and statistically not significant in both males and females when the statistical analysis was done separately for males and females and as such this was also excluded as a predictor of age. The ratios between the length measurements ("p" and "r") in the present study showed low level of relation with age and this finding is consistent with previous studies on canines in Orthopantomography radiograph[8] and also with another study done using mandibular lateral incisor, mandibular canine, mandibular first premolar, maxillary central, and lateral incisor and maxillary second premolar in OPG radiograph, which showed that there was no significant correlation between the age of the individuals and the ratios of the length measurements.[7]

The present study showed that the width ratios of the teeth (a–c) has a stronger correlation with age than the length ratios (p and r), and this finding is consistent with the previous studies done by digitizing OPGs using maxillary right canine[8] and another study done on 100 patients with IOPA radiographs on 6 types of teeth from each jaw and also confirms that on the pulpal morphology, the width of the pulp is a better indicator of age than length.[14]

This indicates that secondary dentin deposition is built on the walls of pulp and there is resultant obliteration of the pulp as the age advances and formation of secondary dentin reduces the pulp chamber width.

In the present study, the Pulp/tooth area ratio (AR) and width of the pulp at midpoint between the width of pulp at CEJ and the width of the pulp at midroot level (c) showed a high degree of correlation with age.

A statistically significant correlation between this sample aged 21 to 60 years and pulp/tooth area ratio was found in this study for the central incisors.

These results are in agreement with studies on maxillary and mandibular incisors done by Bosmans et al[20] and also are in line with studies using cone beam CT done by Yang et al.[21]

The study done by Someda et al[22] on pulp/tooth volume ratios of Japanese mandibular incisors revealed high correlations, who attributed accuracy of age estimation to the fact that mandibular central incisors have the lowest morphological diversity among human permanent teeth.

In our study, the analysis on regression models show that AR is better correlated with chronological age than the linear measurement ratio.

Similarly, the pulp/root width ratio is a better indicator of age than length of the tooth. This confirms similar studies in the past.[1,8,10]

With aging, the pulp cavity gradually becomes smaller because of secondary dentin deposition, as a consequence of this deposition there is tendency toward pulp obliteration.[4]

Accordingly formulae have been evolved based only on these 2 predictors, i.e., AR and c that showed better accuracy.

The formulae that were ultimately arrived are:

(a) Age = 81.048 – 69.695 (AR) – 160.792 (c) for individuals whose gender cannot be established.

(b) Age = 78.465 – 74.844 (AR) – 139.740 (c) for an adult male.

(c) Age = 85.595 – 63.6298 (AR) – 194.776 (c) for an adult female.

To generalize the study, every data set in the study was treated as belonging to an individual whose gender cannot be established.

The formula is fairly accurate when it comes to individuals within the age group of 31 to 50 years, i.e., groups II and III.

The accuracy is borne by the fact that the mean difference of the actual and the predicted ages is 3.62 for group II and 3.14 years for group III.

This compares extremely well when compared to a known SEE that has been as much as < 10 years in a study done previously.[23]

In the present study, the full model, i.e., using all the predictors ("AR", "p", "r", "a", "b", and "c") explained 40.9% of total variance. On the other hand, using a model with only the two most significant variables, i.e., AR and c, we found that the prediction was favorable since in this case the variance was found to be as good as only 39.3%. Two earlier studies: (a) Done on 100 OPGs explained 85.1% of total variance and the model with AR and c variables explained 84.9%[22] and (b) and another study done on IOPA radiographs showed 92.8% of total variance and 92.5% when selected variables were used.[1]

Lastly, the results of this study confirm the validity of dental methods, together with other methods for assessing biological age in the field of Forensic Sciences.

It would be an interesting study/research if the sample size is extended to include large variations in the demography/regions of the world, including not only age and gender but also race and culture parameters.

CONCLUSION

The purpose of this study was to estimate the age of an adult individual based on the relationship between age and the measurement of pulp/tooth area ratio obtained from intraoral periapical radiographs. Every data set for age estimation can be treated as belonging to an individual whose gender cannot be established. It is established that gender is absolutely insignificant for the prediction of age as per this study. It is significant to note that the formula evolved in this study is fairly accurate when it comes to individuals within the age group of 31 to 50 years. The accuracy is borne by the fact that the mean difference of the actual and the predicted ages is 3.62 for group II and 3.14 years for group III. The "pulp tooth area ratio" method is a useful technique to assess chronological age of an adult individual and seems promising in the age estimation.

The accuracy of this method depends on the precision of the measurements and the quality of the IOPA Radiographs.

In future, studies have to be conducted whereby the inter- and intraobserver variability can be reduced by image analysis program, which can recognize pulp and tooth outlines in a radiographic image and will be very useful in minimizing human manual measurement of morphological parameters. Further studies in this direction may be necessary with a larger sample size in order to reduce standard errors of estimates and also at investigating the effect of race and culture in the model parameters.

As accuracy of age prediction is the closeness of estimated age to chronological age, consequently, it is concluded that this research showed promising results for dental age estimation in a noninvasive manner using dental radiographs of maxillary incisor teeth among Narketpally (Nalgonda dist.) population.

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