Original Article

Stereomicroscopic study on unsectioned extracted teeth

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Abstract

Introduction: Age has been considered as a reliable marker for establishing the identity of a person in the field of forensic medicine. Teeth are useful skeletal indicators of age at death since it can survive for decades. Nondestructive methods ensure the evident preservation of dental hard tissues that reflect age changes from the cradle to the grave. Therefore, an attempt was made for estimating the age using the nondestructive method. **Aims and Objectives:** The aim of this study to assess whether physiological changes of the teeth allow possible correlation for accurate age estimation and to establish a graduation standard by microscopic observation for a better age correlation. **Materials and Methods:** The study was carried on 209 teeth samples extracted for orthodontic treatment or periodontal diseases comprised both maxillary and mandibular teeth across different age groups. The assessment of these changes was carried out by well-established standard methods with some proposed modifications. **Results:** Pearson correlation analyses revealed root dentin translucency with the highest correlation ($r = 0.97$) followed by periodontal ligament attachment ($r = 0.95$), root dentin color ($r = 0.95$), and attrition being the least correlated ($r = 0.90$). All the parameters taken for the study contributed to stepwise linear regression analysis ($R = 0.98$; $P < 0.01$) indicating a strongly positive relationship between age and the changes observed. A regression formula was obtained with mean error age difference ±1.0 years. **Conclusion:** The present study showed that extracted tooth is highly significant in identifying the age without being sectioned or further processed and also signifies the use of microscope for observation of these changes, thus reducing the errors of calibrating the age.

Key words: Age estimation, nondestructive method, physiological changes, stereomicroscope, unsectioned teeth

Introduction

Over many decades, the assessment of age and sex of the victim or remains had been considered as a reliable aspect for establishing the identity of the person in the field of forensic science, particularly in the legal and criminal investigations. Age can be determined from a variety of general physical factors such as height and weight which

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is applicable in early periods of life, dental development, and changes occurring at puberty like the appearance of hair and their growth and secondary sexual characteristics.

Dental age is one of the measures of physiological development that are uniformly applicable from infancy to adulthood. Teeth which can sustain postmortem decomposition are the most reliable tool in the identification of bodies in mass disasters and natural calamities. The age estimation of persons younger than 20 years is usually more accurate by taking a radiograph of the individual's jaw and comparing it to a chart showing the stage of the development of the dentition. In individuals older than 20 years, when the dentition's development has been completed, age estimation becomes more complicated.[1]

Various authors developed morphological, histological, biochemical, and radiographic approaches to quantify dental age changes. Gustafson in 1950 suggested the use of six regressive changes as a predictor of age.[2] Johanson in 1971 in his research used same six criterions but different ranking scale to estimate the age of an individual. Solheim in 1980 used *in situ* teeth and eight variables to estimate the actual age. None of the changes took singly proved to estimate age more accurate than when these were studied collectively.[3]

However, in morphological, histological, and biochemical techniques extracted tooth were sectioned due to which the tooth cannot be preserved as evidence, wherein preservation of tooth conveys supreme importance in the field of forensic sciences. To overcome the drawback of low correlation and high error rates, a relatively simple evaluation technique with modifications was followed in the present study to assess these variables along with the help of visualizing tooth under stereomicroscope.

Aim and objective

The aim of the present study is to assess whether physiological changes of the teeth allow possible correlation for accurate age estimation and to establish a graduation standard by microscopic observation for a better age correlation by precise evaluation under stereomicroscope through which we may overcome errors ensued on visualizing teeth through naked eye.

Materials and Methods

The sample composed of 209 teeth extracted from randomly selected population comprising 86 males and 123 females. The age distribution of the individuals was ensured to be relatively equal across the different age groups although first, second, and eighth decades of life were slightly underrepresented. The teeth were extracted for orthodontic purposes or periodontal diseases and composed of anterior and posterior teeth representing both maxillary and mandibular dentition. The unsectioned teeth obtained were observed for four alterations of the teeth, namely, attrition, periodontal ligament (PDL) attachment, and root dentin translucency followed by root dentin color under stereomicroscope.

Attrition was evaluated under stereomicroscope by Li and Ji's 10 stage criteria (Scores 0–9) adapted for assessing average stage of attrition in the posterior teeth (premolars, molars) because of its accuracy and convenience of use.[4] Johanson's seven-stage criteria (Scores A0–A3) primarily proposed for grading anterior sectioned tooth[5] were modified in accordance with ten-stage criteria (Scores 0–9) proposed by Li and Ji subsequently applied to assessing anterior (incisors, canines) unsectioned teeth as well to avoid computing errors by relating two different methods [Figures 1 and 2].

PDL attachment and root dentin translucency grading were observed under stereomicroscope by combining two standard methods – for age group below 40 years subjective grading by Johanson method[5] and for age group above 40 years Lamendin *et al.* method[6] was followed. Although the formulae were primarily intended for single rooted and sectioned tooth, same formulae were employed for multirooted and sectioned teeth as well similar to studies by various authors. For age group above 40 years of age, three variables specifically the height of periodontal attachment from the cementoenamel junction (CEJ) (P), the root dentin translucency (T), and the height of the root (H) was considered. PDL attachment level was obtained from the CEJ and the maximum root dentin translucency length from the apex, both on the labial aspect with the help of vernier calliper scale [Figure 3]. Only one formula was computed for all types of teeth and scores were given accordingly.[6] For age group below 40 years of age, PDL attachment and root dentin translucency were calculated separately by Johanson method (Scores 0-3) [Figure 4].[5]

The **c**olor of the root dentin was assessed by modified Solheim's method under stereomicroscope.[7] As an

Figure 1: Dentin attrition anteriors

alternative of stereomicroscope true bite colour scale, a dental shade guide (VITA tooth guide 3D master, VITA Zahnfabrik, Germany) though used as an indicator of crown color was employed for observing root dentin color and grading's were based on 11 stages (Scores 1–11) given by Acharya and Kumar[3] [Figure 5].

Statistical analysis

After collecting the samples, the modified scores for each physiological alteration based on the modified method followed for individual teeth as described in Table 1 was given, and total scores were calculated. The statistical analysis was carried out using Statistical Package for Social Sciences (SPSS Inc., Chicago, IL, USA, version 15.0 for Windows). Pearson's correlation coefficient was calculated to assess the correlation between individual parameters in the study with the actual chronological age. The data obtained was subjected to stepwise linear regression analysis with dependent variable of age and physiological alterations as individual independent variables. The paired *t*-test was applied to see the difference between actual age and estimated age.

Results

The sample composed 209 teeth extracted from randomly selected population comprising 86 males and 123 females with age group of predominantly 41–60 years of age and least being age group of about 71–75 and 11–20 years of age with a mean age of 46.3 years [Table 2].

The Pearson's correlation coefficients (r/R) of each of the dental variables along with their significance levels were estimated. Among the parameters, root dentin translucency produced the strongest correlation ($r = 0.97$) to actual age irrespective of Johanson method for age group below 40 years of age or Lemendin method for age group above 40 years of age. Root dentin color and PDL attachment gave similar correlation of estimated age to the actual age ($r = 0.95$) while attrition produced the lowest correlation to age ($r = 0.90$), all of which are statistically significant ($P < 0.01$) [Table 3].

Stepwise linear regression analysis was used to find correlation between the age and continuous variables obtained in the study. The variables fit the linear model using age as a dependent variable were: (a) Root dentin translucency, $R^2 = 0.94$; standard error = 3.76 years; $P < 0.005$; (b) root dentin translucency, root

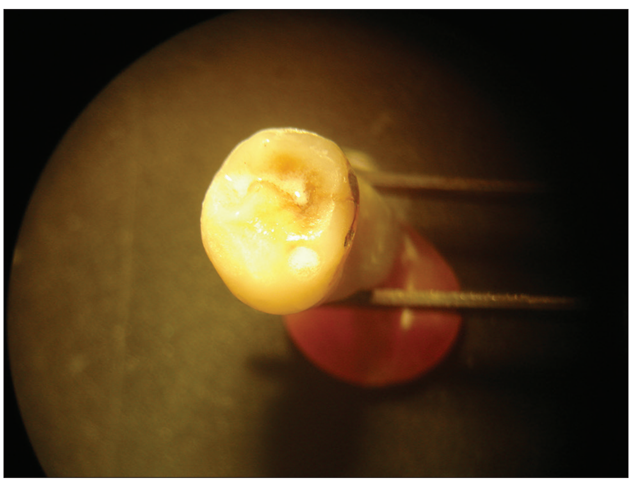

Figure 2: Dentin attrition posteriors

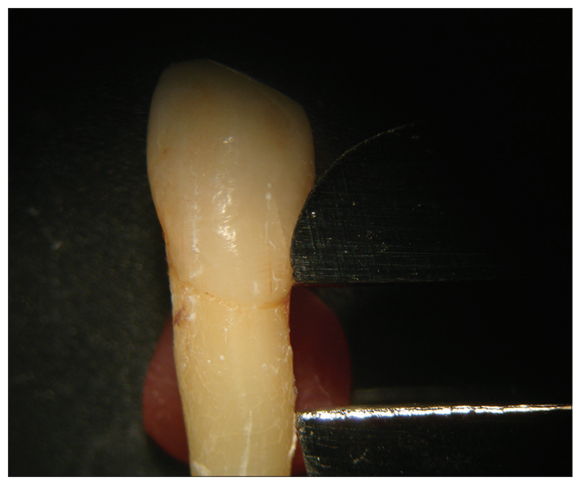

Figure 3: Periodontal attachment

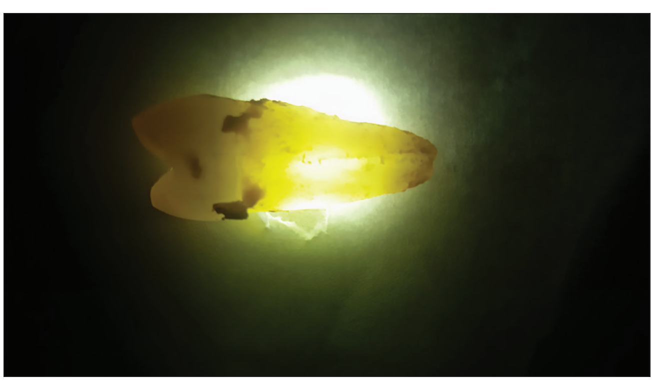

Figure 4: Dentin transluency

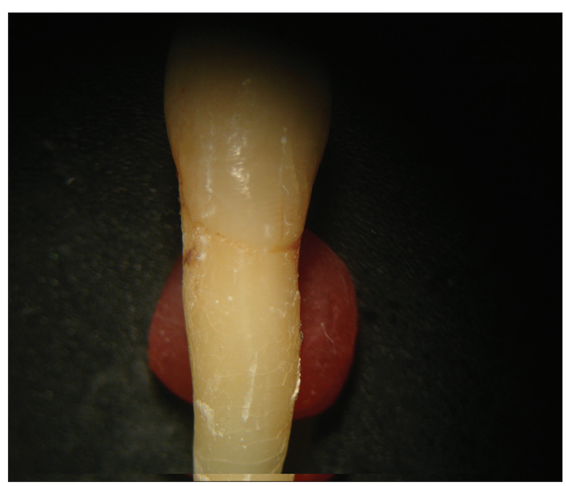

Figure 5: Root color

Table 1: Scores allotted to all the parameters evaluated based on the degree of change as per described methods for each parameter

Parameters evaluated	Method studied and modified criteria		Scores
	Posteriors (Li and Ji method)	Anterior (modified Johanson method)	
Attrition	No attrition. Sharp cusp	No attrition	0
	Slight attrition on top ridge of cusp	Slight attrition in incisal edges	1
	Cusp appears obtuse or a limited oblique facet	-	2
	Greater part of cusp worn away	Greater part of enamel worn away	3
	Dentin appears spot (<1 mm)	Dentin appears on incisal edges (<1 mm)	4
	Dentin appears spot (>1 mm)	Dentin appears clearly (>1 mm)	5
	Dentin spot coalesces with one other cusp	-	6
	Dentin spot coalesces with one or more cusp	Dentin appears exposed along with secondary dentine	7
	Exposed dentin appears as circle	-	8
	Exposed dentin completely	Completely exposed dentin	9
Parameters evaluated		**Age group**	
	<40 years	**>40 years (lamendin method)**	
Periodontal ligament attachment	0 - no periodontal disease	P=Periodontosis height × 100/root height	
	1 - periodontal disease without bone loss		
	2 - bone loss involving more than one-third of root		
	3 - bone loss more than two-thirds of the root		
Root dentin translucency	0 - no transparency	T=Transparency height × 100/root height (the arbitrary values are calculated and correlated with the scores obtained by Johanson)	
	1 - beginning of transparency		
	2 - more than one-third of the apical root		
	3 - more than two-thirds		
Root dentin color	Alphanumeric values - 1M-1, 2L-2, 2M-3, 2R-4, 3L-5, 3M-6, 3R-7, 4L-8, 4M-9, 4R-10, 5M-11		

Table 2: Age distribution of the study sample

Age wise distribution	
Age group (years)	***n* (%)**
11-20	12 (5.7)
21-30	38 (18.2)
31-40	14 (6.7)
41-50	55 (26.3)
51-60	55 (26.3)
61-70	32 (15.3)
71-75	3 (1.4)
Mean age	46.3

Table 3: The Pearson's correlation coefficients (*r*/*R*) of each of the dental variables along with their significance levels

Parameter	Correlation coefficient	Significance (*P*)
Attrition	0.90	<0.001*
Periodontal ligament attachment	0.95	<0.001*
Root dentin translucency	0.97	<0.001*
Root dentin color	0.95	<0.001*

*Values with high significance

dentin color; $R^2 = 0.97$; standard error = 2.62 years; $P < 0.005$, (c) root dentin translucency, root dentin color, PDL attachment level $R^2 = 0.98$; standard error = 2.29 years; $P < 0.005$, and (d) root dentin translucency, PDL attachment level, attrition; $R^2 = 0.98$; standard error = 2.16 years; $P < 0.005$). All the variables showed significant correlation independently as well as dependently with each other variables with the actual age [Table 4].

Finally, multivariable analysis with linear regression model was done. The scatter diagram prepared from the data showed increase in the values of the points with increasing age. The regression equation was obtained. The average point values, calculated ages, and difference between actual age and calculated age in each sample were calculated using paired *t*-test. The coefficient of correlation (r) was calculated from the equation.

Formula derived for age estimation:

$$\text{Age} = 0.2*X^1 + 1.1*X^2 + 0.3*X^3 + 0.7*X^4 + 17.9$$

(X^1 – Root dentin translucency; X^2 – Root dentin color; X^3 - PDL attachment; X^4 - Attrition).

Coefficient of correlation = $r/R = 0.98$.

Analysis between the actual age and estimated age in the total of 209 samples using Student's paired *t*-test showed mean age of 45.3 years with standard deviation of ±14.5 years. The mean difference between the actual age and estimated age was found to ±1.0 years with significance of $P < 0.01$ [Table 5].

Discussion

Age estimation can demonstrate serious measure in victim documentation process. In case of unknown deceased bodies, age estimation becomes necessary if there is no antemortem information available, and a personal profile has to be recreated. In the recent years, various methods were adapted using skeletal and dental structures to estimate the age precisely.[8] The tooth is the one of the hardest substance in the human body that shows resistant to external environmental factors such as local pathological factors and physiological factors such as very high temperatures. Numerous age estimations methods have been developed for adults. The simple visual method based on the clinical experience without using recognized methods may be possible but unacceptable without validation.[9]

Among the most recognized method, morphological methods for age estimation from teeth have been developed is widely accepted and applied to samples without taking the postmortem interval into consideration. The problem with morphometric method being not always been successively validated. Pillai and Bhaskar showed in India population Gustafson's method of age determination using attrition, root dentin color, root dentin translucency, cementum apposition, and secondary dentin though showed higher values from human skeletal remains than in freshly extracted teeth are under the influence of external factors such as race and culture.[10] Although various authors have shown a significant change in the correlation between male and female, the present study concentrated on the most accurate and precise method for each parameter which is not influenced much by external factors such as age, sex, and race.

The degree of attrition is reflected by the condition of the enamel and the exposed dentin in corresponding with the pulp cavity or the cusp height. The grading was given

Table 4: Step wise linear regression analysis to find correlation between the age and continuous variables

Stepwise linear regression model summary			
R	***R*²**	**Adjusted *R*²**	**SE of estimated**
0.970[a]	0.94	0.94	3.76
0.986[b]	0.97	0.97	2.62
0.989[c]	0.98	0.98	2.29
0.991[d]	0.98	0.98	2.16

[a]Root dentin translucency, [b]Root dentin translucency, dentin color, [c]Root dentin translucency, dentin color, periodontal ligament attachment, [d]Root dentin translucency, root dentin color, periodontal ligament attachment, attrition. SE: Standard error

Table 5: Analysis between the actual age and estimated age using Students paired *t*-test

Estimation of mean error between the actual age and estimated age in the study sample using Students paired *t*-test						
Variables	***n***	**Mean±SD**	**Minimum**	**Mean difference**	***t***	***P***
Actual age	209	46.3±15.6	1.1	1.0	6.412	<0.001*
Predicted age	209	45.3±14.5	1.0			

*Statistically significant. SD: Standard deviation

from no attrition (Score 0) to dentine exposed on the entire occlusal surface and the secondary dentine being exposed (Score 9). The present study showed a coefficient value of 0.90 which is slightly lower than the actual study by Li and Ji method performed only on molars ($r = 0.95$) but significantly higher than Solheim's correlation ($r = 0.47$)[11] and Acharya and Kumar's correlation ($r = 0.16$) obtained using Johanson's method on various tooth types.[3] Monzavi *et al*. in his study mentioned two major series of methods for age estimation based on dental parameters and specified Helm *et al*. used the severity of attrition of molar teeth to estimate age which showed moderated accuracy ($r = 0.70$). Lovejoy in 1985 obtained a correlation coefficient of 0.96 relatively larger to the one derived in the present study, and their errors were considerably lower than our study. Monzavi *et al*. in 2003 stated Kambe *et al.* in 1991 devised a simple method to assess dental attrition in terms of its area and number of sites on each tooth demonstrated a significant positive correlation with age with multiple correlation coefficient value of 0.93.[12]

The lower correlation was obtained in our study than few other studies as a result of assessing attrition in a combination of multiple tooth types. However, various studies showed in more recent times, the causes of attrition have involved other factors such as bruxism, diet, environment, and medication. Although helping in age estimation from attrition, this method has its own weaknesses and limitations in age estimation by examination of dental attrition as the sole indicator.[11]

In the oral environment, periodontal attachment level is subjected to action of various physical and chemical changes. Degeneration of the soft tissue surrounding the teeth is termed as gingival regression. The teeth appear yellow, smooth area under stereomicroscope below the enamel and darker, and clearer than rest of the root.[13] The PDL attachment level was calculated on the labial surface since it is less subjected to pathological changes as stated by Johanson *et al*. and Lamendin *et al*. However, Lamendin *et al*. showed a higher correlation than Gustafson's, Johanson, and Solheim method by considering only two criteria (PDL attachment and root dentin translucency) with more precision for cases of individuals above 40 years of age.[6]

Taking these factors into consideration, PDL attachment and root dentin translucency grading were observed under stereomicroscope by combining two standard methods – for age group below 40 years subjective grading by Johanson method which had proved to be better method (Score 0–3) and for age group above 40 years Lemendin method was followed. Although the formulae were primarily intended for single rooted and sectioned tooth, same formulae were employed for multirooted and unsectioned teeth. The labial aspect was measured using vernier callipers under normal magnification of stereomicroscope. The labial aspect was favored by most of the authors in various previous studies as it is least exposed to the environment and least susceptible to pathological alterations.[14] Only one formula was computed for all types of teeth and scores were given accordingly.

Acharya *et al*. in his study showed highest correlation of PDL attachment to age among all the parameters. He obtained a correlation of 0.38 by Johanson method and correlation of 0.40 by Lamendin method. Johanson *et al*. obtained correlation coefficient of 0.49 significantly higher than method followed by Solheim (r = 0.32).

Root dentin translucency starts in the apical portion of the root and increases with the age in coronal direction. This particular change is least affected by environmental factors and the pathological processes. It always shows symmetrical distribution on both the sides of the jaw.[15] According to Maple, the amount of secondary dentine present and the translucency of the dentine of the root were the best indicators of an individual's age.

Lamendin *et al.* stated that root dentin translucency never appears before the age of 20 years and is due to the deposit of crystals within the dentinal tubules. In the study, translucency is measured from the apex of the root to the maximum height of translucency on the labial aspect. The translucency of dentin as noted in the ground section than in the unsectioned tooth is due to an increase in intra tubular mineralization which has same refractive index as that of peritubular dentin giving translucent appearance within dentin. This translucency is first noted in the apical part of the tooth because of lesser diameter of dentinal tubules in the root dentin compared to the coronal part.[6]

Bang and Ramm used root dentine translucency in their study and found a mean error of estimation of ±4.7 years in 58% cases and ±10 years in 79% of individuals.[16] In the study by Wegener and Albrecht, the correlation coefficient was 0.67 and concluded the best range of age was 30–60 years using translucency factor.[17] Hopp and Blick used length of translucency zone so that the mean error of estimation was ±5 years with 90% reliability. Johanson obtained a correlation of 0.86 for this parameter while Solheim who showed correlation between 0.68 and 0.86 in different measurements and 0.57–0.83 in different teeth.[18] Singhal *et al*. in his study determined using the length and the area of translucency or translucency found in the root's apical section and obtained a correlation of 0.81.[19]

In the present, we obtained a correlation of 0.97 by combining both the age groups signifies much higher than the previous study though the differences can be due to the nature of sample where Indian studies showed higher correlation and also on sectioned teeth showed lower correlation than the unsectioned teeth. However, better accuracy but with more precision was obtained by combining both Lamendin and Johanson method. Kashyap and Koteswara Rao, who modified Gustafson's method claimed an average error in age estimation as low as 1.59 years in his study was considered slightly superior to Johanson method followed in the present study.[20]

Bommannavar and Kulkarni in his comparative study using dentin translucency as indicator for age estimation showed the better efficiency of the digital method to estimate age when compared with the calliper method. The age estimated was within ±5 years in 70% of the cases, where only 24% of cases were estimated to within ±5 years with the caliper method.[21]

The color of tooth is still considered as interesting criteria for age. Solheim believed that color was better than most other regressive changes. He conducted study on both intact as well as sectioned teeth to determine the correlation between color and age. High accuracy with high precision was obtained in sectioned tooth than intact tooth.[7] Estimation of tooth root color was performed with the use of stereomicroscope true bite color scale. In the recent years, dental shade guide has been used extensively by several authors and reported the teeth tend to darken with age. The present study uses VITA tooth shade guide similar to study by Acharya *et al*. By use of VITA shade guide, the color of root is assessed, and grading was given from scores 1 to 11 from 1 M to 5 M, respectively.[3] Martin-de Las Heras *et al*. in his study under spectroradiometry showed correlations ranging from 0.53 to 0.75.[22] Solheim obtained a summative of r = 0.44 for all types of teeth combined whereas in the present study showed correlation of 0.95 which is shown to be higher than results obtained in any other studies. Acharya and Kumar showed a correlation of 0.21.[3] Ten Cate *et al*. used color as a sole indicator for the estimation of age founded a higher correlation similar to the present study with an estimated acceptable error of <10 years of mean differences. The lower correlation in the past studies probably because of observations of tooth color specimens buried for prolonged intervals that has been highly influenced by the soil components.[23]

The mean age difference between actual and calculated age was ±1.0 years comparatively higher than ±3.63 years by Gustafson's original work. Singh and Mukharjee used same six criteria of secondary changes and concluded the maximum differences in the estimated age from multiple roots of the same tooth are 11.4 years using Gustafson's method, 16.7 years by Johanson and 12.9 years by Bang and Ramm. The maximum differences in the age estimates for the same individual using multiple teeth are 13.7, 19.5, and 13.8 years, respectively.[24]

However, Johanson's sectioned method under stereomicroscope showed highest accuracy and precision with mean age error of ±0.48 years less than mean age error obtained in the present study. The mean difference between the actual age and estimated age was significant, but the difference was less compared to those reported by the previous studies by Gustafson[2] in 1950; Bang and Ramm's[18] in 1970; Pillai and Bhaskar[10] in 1974; Singh and Mukharjee[24] in 1985; Kashyap and Koteswara Rao[20] in 1990; Lamendin *et al*.[6] in 1992; Solheim[25] in 1993; Lucy *et al*.[14] in 1996, and Singh *et al*.[1] in 2014 [Table 6].

Valenzuela *et al*. in found that in fresh extracted teeth, the variables that made the greatest contributions to predictions of age were dental attrition, dentin color, and translucency width and in teeth from human skeletal remains, the variables that made the greatest contributions to age calculation were cementum apposition, pulp length followed by dental attrition, root translucency, and dental color. Thus, they recommended the use of different regression models to calculate age depending on the postmortem interval.[26]

Summary and Conclusion

The formulae derived in the present study only yields the predicted populations mean age. Further studies can be carried out to assess the reliability and validity of this method by evaluating inter- and intra-observational differences of the parameters possibly resulting in a better age correlation than the correlation obtained in the present study. In the present study, the difference between the actual age and estimated age could have been reduced by considering factors such as gender, inclusion of more precise objective measurements, and type of tooth in specific for all the study samples. Various pathological conditions and quality of oral hygiene influence adversely the different dental features which may affect the secondary changes in the teeth for estimation of age.

The use of stereomicroscope for observation of these changes had not only given significant results but also have reduced the mean age difference between actual and estimated age to ±1.0 years significantly lesser compared to most studies done in the past. Each dental age estimation method provides a different combination of accuracy, precision, procedures, and requires different equipment. It is essential not only to create methods for age estimation but also to test their reliability using independent data and observers.

Table 6: Comparison of mean age difference between actual and estimated age in our study and various previous studies

Various studies	Mean age difference between actual and estimated (years)
Gustafson (1950)	±3.63
Bang and Ramm (1970)	±10.07
Pillai and Bhaskar (1974)	±8.13
Singh and Mukharjee (1985)	±4.9
Kashyap and Rao (1990)	±1.59
Lamendin *et al*. (1992)	±8.4
Solheim (1993)	±3.4
Lucy *et al*. (1996)	±7.0
Singh and Gorea (2004)	±2.16
Shrigiriwar and Jadhav (2013)	±4.43
Singh *et al*. (2014)	±2.64
Present study (2016)	±1.0

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Conflicts of interest

There are no conflicts of interest.

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