Original Article

A Comparative Study to Evaluate Age Using Pulp to Tooth Area Ratio Using Radiography and Tooth Cemental Annulations Using Phase Contrast Microscopy

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Abstract

Background: Age estimation is important in forensic medicine and odontology for identification of deceased victims and also for crimes and accidents. Various studies have compared the different morphological methods, biochemical methods and radiological methods of age estimation. However, no study has compared the accuracy of a nondestructive (radiographic) method with a destructive (histologic) method.

Aim and Objectives: Aim of the study was to evaluate and compare the efficacy of the age estimation using pulp to tooth area ratio by radiography and tooth cemental annulations by phase contrast microscopy.

Materials and Methods: Eighty permanent single-rooted extracted teeth were utilized in the study. The radiographic age was estimated using the pulp/tooth area ratio; histologic age was estimated using tooth cemental annulations. The results of both the methods were compared to evaluate the more accurate method.

Results: The correlation between actual age and estimated radiographic age was found to be $r = 0.92$, $P = 0.00$ ($P < 0.001$) and correlation between actual age and estimated histologic age was found to be $r = 0.97$, $P = 0.00$ ($P < 0.001$).

Conclusion: Both the radiographic method and histologic methods can be efficiently used to estimate age, however; we found that the histologic method had better accuracy in age estimation.

Key Words: *Histologic age, radiographic age, tooth cemental annulations*

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Introduction

Forensic odontology or forensic dentistry, as defined by Keiser–Nielsen in 1970, is that branch of forensic medicine which in the interest of justice deals with the proper handling and examination of dental evidence with proper evaluation and presentation of the dental findings.[1] Among the various aspects of forensic odontology, age is important in most cultural and judicial hierarchies and has assumed increasing importance for the assessment of both criminal culpability and legal/social categorization.[2] Age estimation may also help in other situations such as; determining the legal liability of teenagers and adults of unknown age, assist adoption processes, release retirement funds for adults of unknown age as well as support research in archeology, and paleodemography.[3]

Various studies have compared the different age estimation techniques. However, no studies have been done with the aim to compare between the accuracy of a radiological method and a histological method for evaluation of age and correlation of this estimated age value with the actual age. With this background, we carried out a study to evaluate the efficacy of the age estimation methods using pulp to tooth area ratio by radiography and tooth cemental annulations by phase contrast microscopy.

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Materials and Methods

The present study was carried out in the department of oral pathology, department of oral surgery, and department of oral medicine and radiology of a health institute. After obtaining Institutional Ethical clearance, 80 extracted teeth were included in the study. Permanent single-rooted extracted teeth with fully formed roots; which were extracted due to orthodontic or prosthetic reasons from patients of age group 21 years to 70 years were included in the study.

Multirooted, carious, exhibiting severe attrition or abrasion, with any developmental anomalies, endodontically treated teeth, restored, impacted teeth or teeth from patients with any systemic conditions, and bone anomalies were excluded from the study.

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Each tooth in the study was first assessed by radiological method and then studied for cemental annulations method for age estimation and then compared with actual chronological age.

Chronological age estimation

The chronological age of the patient was calculated by subtracting the date of extraction from the date of birth of the patient.

Radiographic method of age estimation

The extracted tooth was placed along its long axis on the radiographic sensor of RVG. A digital radiograph of the tooth was taken using paralleling technique and viewed using the dental imaging software and converted to high resolution life size JPEG images for importing to AutoCAD 2016 software program. The pulp and tooth areas were measured using the point and line tools on the draw toolbox and the pulp/tooth area ratio calculated. The ratios obtained were utilized for subsequent statistical analysis.

These ratios were subjected to linear (single tooth) regression analysis using SPSS statistical package version 20 (IBM Company Chicago, IL, USA). Linear regression equations were performed to calculate the regression equations. The regression correlation coefficients® were compared to ascertain which of them had a better relationship with age.

All measurements were carried out by the same observer with ample experience of this technique.

Histologic method of age estimation

Once the radiograph was taken, the tooth was sectioned for histologic analysis. The ground sections of the teeth of 100 µm thickness were prepared based on the method described by stott *et al*. (1982).[4]

The sections were examined under binocular Olympus microscope (BX-43) in phase-contrast mode. Micrographs were taken with a 10× objective with the help of Olympus Camedia C-5060 digital camera. Incremental lines were identified as dark and light bands. The cementum thickness was measured in three different regions where the lines seemed to run approximately parallel, and the average between the three measurements was utilized for calculations as "X," i.e., the total width of cementum (from DEJ to cementum surface).

Then, the measurement was made of the width occupied by the two adjacent incremental lines (i.e., one dark and one light) which were most easily recognizable, as "Y." The number of incremental lines in the total cementum width was calculated as follows:

Number of incremental lines (n) = X/Y.[5]

By adding average age of eruption in years for each tooth as presented in Grey's Anatomy in the counted number of incremental lines, the chronological age of the individual was obtained as follows:

Histologic estimated age (E) = number of incremental lines (n) + eruption age of tooth (t)

Results

The study sample included 48 teeth from females and 32 from males. The age range of the studied sample was from 21 years to 70 years. The sample consisted of 30 maxillary teeth and 50 mandibular teeth.

Comparison between estimated radiographic age and chronologic age

Pearson's correlation was used to compare the estimated radiological age and chronological age of the sample teeth and the $P = 0.0$ (<0.001) and $r = 0.93$ [Table 1].

Since r value (0.93) was found to be >0.8 it was concluded that there is a strong correlation between radiographic estimated age and chronological age confirming that estimating the age using pulp tooth area ratio is relatively accurate.

Comparison of estimated radiographic age and chronologic age in maxillary teeth

A scattered plot showing a significant direct correlation between the actual age and estimated radiographic age in maxillary teeth is shown in Graph 1. Among all the maxillary teeth examined in our study, the maxillary canine was most closely related to actual chronological age ($r = 0.62$, $P = 0.0$).

Comparison of estimated radiographic age and chronologic age in mandibular teeth

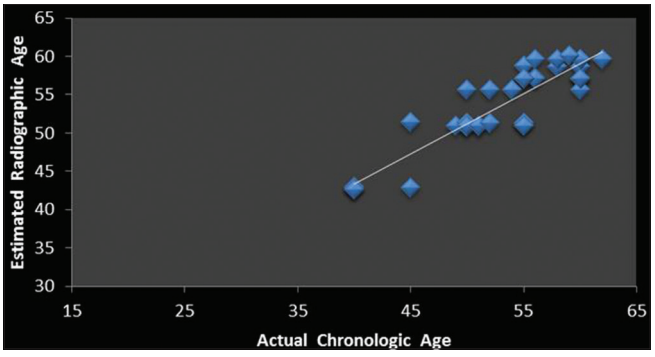
A scattered plot showing a significant direct correlation between actual age and estimated radiographic age in mandibular teeth is shown in Graph 2. Among all the mandibular teeth examined in our study, the mandibular canine was most closely related to age ($r = 0.83$, $P = 0.0$).

Comparison of estimated radiographic age and chronologic age with gender

The effect of gender on age estimation was determined in this study. The correlation coefficient r in males was 0.86 and in females was 0.97. Thus indicating that in both the

Table 1: Comparison of actual chronological age and radiographic age of all the teeth in the sample

Age	Sample size	Correlation coefficient
Actual chronological age	80	0.93
Estimated radiographic age	80	



Graph 1: A scattered plot showing correlation between the actual chronologic age and estimated radiographic age in maxillary teeth

genders there is a strong correlation; however, the values in females are more closely related as compared to the males.

Comparison between estimated histologic age and chronologic age

Pearson's correlation was used to compare the estimated histological age and chronological age of the sample teeth and the $P = 0.0$ ($P = 0.001$) and $r = 0.97$ [Table 2].

Since r value (0.97) was found to be >0.8 it was concluded that there is a strong correlation between histologic estimated age and chronological age. This confirms that estimating the age using tooth cemental annulations is relatively accurate.

Comparison of estimated histologic age and chronologic age in maxillary teeth

A scattered plot showing a significant direct correlation between estimated histological age and chronological age in the maxillary teeth is shown in Graph 3. The correlation coefficient $r = 0.77$, $P = 0.0$.

Comparison of estimated histologic age and chronologic age in mandibular teeth

A scattered plot showing a significant direct correlation between the estimated histological age and actual age in mandibular teeth is shown in Graph 4. The correlation coefficient $r = 0.84$.

Comparison of estimated histologic age and chronologic age with gender distribution

The effect of gender on age estimation was determined in this study and found that the correlation coefficient r in females was 0.88 and in males was 0.86. Thus indicating that in both the genders there was a strong correlation.

Comparison between estimated radiographic age, estimated histologic age with chronologic age

After the age evaluation, the estimated radiographic age and estimated histological age was compared with the actual chronologic age. It was found that correlation between actual age and estimated radiographic age was found to be $r = 0.92$, $P = 0.00$ ($P < 0.001$) and correlation between actual age and estimated histologic age was found to be $r = 0.97$, $P = 0.00$ ($P < 0.001$).

Thus, the histologic age was found to be more closely related than radiographic age.

Discussion

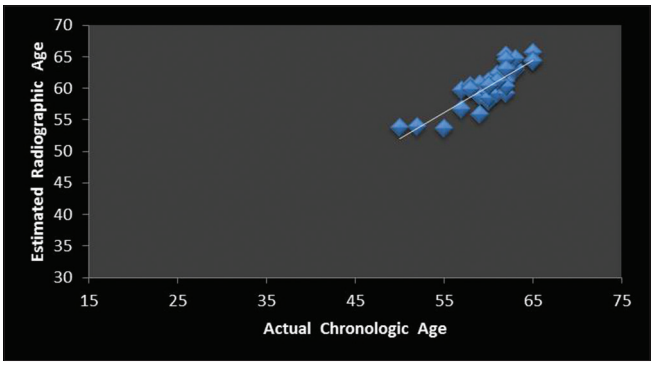
Different methods of age estimation have been studied individually and in combination. This study is first of its kind to evaluate and compare two different methods (one destructive and other nondestructive) for age estimation, namely, age estimation from pulp to tooth area ratio and age estimation from tooth cemental annulations.

Assessment of pulp to tooth area ratio is an indirect quantification of secondary dentin deposition. This radiographic method of age estimation was utilized in our study.

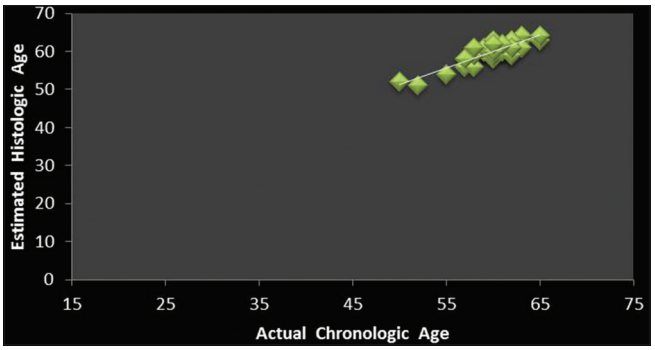
In our study, the correlation coefficient r between the two variables that is, the chronological age and the estimated radiological age was found to be 0.93, which indicated that the two variables are linearly related to each other. This was similar to results by Singaraju *et al*.[6]

Table 2: Comparison of actual chronological age and histologic age of all the teeth in the sample

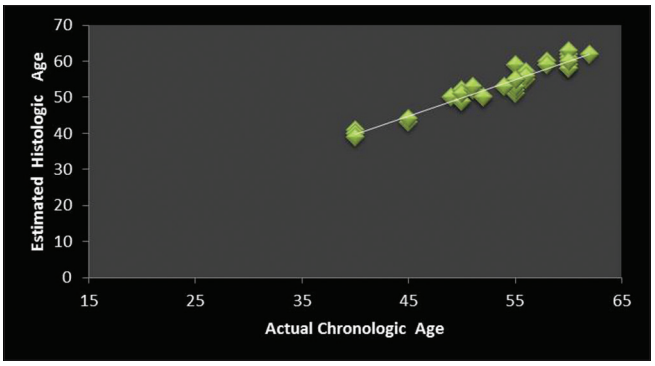
Age	Sample size	Correlation coefficient
Actual chronological age	80	0.97
Estimated histologic age	80	



Graph 2: A scattered plot showing correlation between the actual chronologic age and estimated radiographic age in mandibular teeth



Graph 3: A scattered plot showing correlation between the estimated histological age and actual chronologic age in maxillary teeth



Graph 4: A scattered plot showing correlation between the estimated histological age and actual chronologic age in mandibular teeth

In our study, it was found that the mandibular teeth gave a higher coefficient compared to maxillary teeth and hence found to be significant for age estimation. This was in accordance to the study done by Chandramala *et al*.[7]

The comparison of the volume ratio average among age groups also showed a significant difference between sexes. The regression analysis of correlation between aging and decrease in the pulp chamber volume showed a good correlation in both genders, but stronger correlation was seen in females than in males, suggesting a greater decrease in the pulp chamber in females than in males of the same age.[6]

Agematsu *et al*. examined the correlation in both the genders by performing a regression analysis for age estimation based on the correlation between aging and decrease in the pulp chamber volume and reported that a higher correlation was observed in females than in males. The results of the present study are similar to these observations by Agematsu *et al*.[6]

After radiographic age estimation, one longitudinal ground section from each tooth was utilized for histologic age determination using tooth cemental annulations. In our study, a strong positive correlation between the two variables of estimated age calculated from cemental lines and the actual chronological age was found.[8]

Present investigation showed a strong linear correlation $r = 0.97$ between cemental annulations and chronological age. This supports the findings of Dias *et al*., Aggarwal *et al*., and Lipsinic *et al*. Thus, in our study estimated histologic age of all the teeth proved to be closely correlated with chronological age.

In our study, we also determined the effect of gender on histologic age estimation. Both the genders were found to be closely correlated to each other. This was in accordance to a study by Nagalaxmi *et al*. However, other researchers have found to have a better correlation in females as compared to males in similar studies.[9]

Thus, from our study, it can be inferred that both the radiographic method ($r = 0.93$) and histologic method ($r = 0.97$) can be efficiently used to estimate age however, we found that the histologic method had slightly better accuracy in age estimation. Even though comparable results have been obtained from both the age estimation methods, the choice of technique should also weigh in its advantages and limitations.

The radiographic method of age estimation is a noninvasive, quick, economic technique[10] and can be applied reliably and accurately in living individuals.[11] However, it cannot be used in multirooted teeth, as accurate measurements of such teeth are difficult to perform and errors in angulations and magnification could affect measurements.[10,11]

The histologic method of age estimation is a relatively reliable and acceptable method and good reproducibility of results are achieved with advanced techniques of sectioning, use of phase contrast microscopy and digitalized images. However, a major disadvantage of this method is the necessity to extract or section the tooth. It is not practical among living individuals.[12]

The future scope of this study lies in analyzing larger sample sizes to reduce the standard errors of the estimates and investigate the effect of race, culture, para-functional habits, diet, enamel hypoplasia, and other parameters on age estimation methods.[11]

Conclusion

Age estimation may be necessary for living and deceased and may apply to children, adolescents or adult age group.[13] In our study, it can be inferred that although both the radiographic and histologic methods can be efficiently used to estimate age, the histologic method had slightly better accuracy in age estimation than the radiographic method. The most important aspect of dental age estimation for the forensic odontologist is that one should not restrict to only one age estimation technique but apply the different techniques available and perform repetitive measurements and calculations to establish maximum reproducibility.[11]

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Conflicts of interest

There are no conflicts of interest.

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