Original Article

Age Estimation using Pulp/Tooth Area Ratio of Maxillary and Mandibular Canines on Digital Orthopantomographs in a Sample of Sri Lankan Population

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Abstract

Introduction: Reliable age estimation at the death of human remains is considered crucial to interpret osteological data. In addition to gender, age is an essential basic biological parameter which facilitates the identification of human remains in forensic and palaeodemographic contexts. It is also well established that the use of morphological characteristics of the teeth is considered to help more reliable age estimates than most of the other methods as the teeth are sometimes the only skeletal remains to be used for the estimation of age if the skeletons are highly damaged.

Aim: The aim of the present study is to propose a method for assessing the chronological age based on the relationship between age and measurement of pulp/tooth area ratio (AR) on canine teeth using digital orthopantomographs for a Sri Lankan population.

Materials and Methods: The sample consisted of orthopantomographs (OPGs) of 231 subjects (113 males and 118 females) aged 17–76 years. The AR of upper and lower canines was calculated according to the reported technique, and statistical analysis was performed to obtain multiple regression formulae for dental age calculation, with chronological age as the dependent variable, and gender, left and right side of upper and lower canines as independent variables.

Results: The AR between right and left canine teeth of maxilla and mandible shows no statistically significant difference and also no significant effect of gender on all regression models. Furthermore, as the intercept and slope of all regression equations show highly significant consistency in predicting the chronological age any permanent canine tooth of the dentition can be used with high reliability for the estimation of age. Mean prediction errors of the present study were 0.16 years and 0.21 years, respectively, for right and left mandibular canines and 0.10 years and 0.15 years, respectively, for right and left maxillary canines and confirms the high reliability and accuracy in the prediction of age.

Conclusion: The result of the present study shows that estimating the age using the formulae is highly reliable and accurate, and intercept and slope of the formulae are different in each population group.

Key Words: *Age estimation, chronological age, forensic anthropology, panoramic radiography, pulp/tooth ratio*

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Introduction

The craniofacial skeleton and teeth have been identified as indispensable in the identification of individuals, especially following mass disasters and estimation of age at death of skeletonized remains. Forensic odontology is an area of specialization in the field of dentistry that provides essential support in situations such as criminal investigations, disasters, accidents, and genetic research in a forensic context. The ability to provide an estimate of an individual's age using teeth is a vital element of forensic odontology that is often required in paleodemographic, anthropological, and archaeological research. Being the most indestructible bodily structures and can be easily inspected in living individuals the teeth are becoming more and more useful in forensic odontology.[1]

In young people, the estimation of age at death can be done by examining the teeth present in the oral cavity. This is

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a relatively simple method and the stage of development of the deciduous and permanent dentition can be studied using radiological or histological techniques or a combination of both. Age can be determined by comparing the findings with already established reference data on the chronology of tooth development. This method may be used to estimate the age until the third molar is completed at the age of 21 years.

In the past in Sri Lanka, when the need arose to determine the age of an individual, the norms established for Western populations have been used. The norms for the eruption of the permanent dentitions of Sri Lankans are now available[2] and the times of eruption have been found to be significantly different from those established for other Caucasian populations.

Since the estimation of age beyond young adulthood become considerably more difficult, a method was developed by Gustafson,[3] using the teeth of forty Caucasian adults. The method is based on six age-related changes in dental tissues. These included attrition, amount of secondary dentine formed, the level of the periodontal attachment, amount of secondary cementum formed, root resorption, and the extent of translucent dentine in the root. Although Gustafson's technique is considered a significant contribution toward forensic identification, the published articles contained many statistical errors.[4] Since then modifications to this method have been suggested by many investigators to improve this method of age estimation.

There are no such established criteria for the Sri Lankan population when it comes to estimating the age of over 21-year-old living or diseased individuals as well as skeletal remains of archaeological or forensic importance. Our department receives the number of cases for estimation of the age of such individuals from time to time and still relies on the norms established for other world populations. Further, estimation of the age of the above-mentioned groups must be done through nondestructive, inexpensive methods such as degree of attrition or radiological analysis of secondary dentine formation. Radiological methods of secondary dentine formation have the advantage of being nondestructive and can be applied to living as well as deceased individuals.[5]

According to literature, using radiological analysis of secondary dentine formation, researchers have developed a method for estimating the chronological age of an adult based on the relationship between age and pulp size measured on different types of radiographs[6-8] and also presented different regression formulae for different populations.[9-11] In those studies, canines have been chosen for a number of reasons such as they have the longest functional survival rate in the mouth, undergo less wear as a result of diet than posterior teeth, less likely than other anterior teeth to suffer wear as a result of particular work and the single-root teeth with the largest pulp area and thus the easiest to analyze.

It has been shown that the eruption times of the permanent and deciduous teeth and the sizes of the permanent and deciduous teeth (the length and the crown diameters) of Sri Lankans are significantly different from those of Caucasians.[2,12] As these factors have a definite effect on the age-induced changes in the dental hard tissues, it is essential that a method of age estimation needs to be developed for Sri Lankans. Therefore, it is worth of establishing our own method of estimating the chronological age using the tooth/pulp ratio of canines to estimate the age of an individual, both living and diseased.

Therefore, the objective of the present study is to propose a method for assessing the chronological age based on the relationship between age and measurement of pulp/tooth area (PA/TA) ratio on canine teeth (both upper and lower) using digital orthopantomographs (OPG).

Materials and Methods

Two hundred and thirty-one OPG available in the department of Oral Medicine and Periodontology University Dental (Teaching) Hospital, Peradeniya, Sri Lanka of 113 males and 118 females, were included in the present study. The selected OPGs of the patients' age ranged from 17 to 76 years with known chronological age. The age of the individuals whom we took the OPG categorized according to their chronological age to determine the best age range of accuracy in the determination of age. The selected teeth on the OPG were fully erupted into the oral cavity and the roots of the canines have fully formed. The teeth free from developmental or morphological abnormalities including dental caries, root resorption, enamel attrition, periodontal diseases, restorations, prosthesis, pulp stones, calcified canals, etc., were selected for the study. In addition, impacted, rotated or malaligned teeth and teeth with large areas of enamel overlapping between neighboring teeth were excluded from the study. Ethical approval was obtained from the Ethics review committee of the Faculty of Dental Sciences, University of Peradeniya, Sri Lanka (ERC/FDS/UOP/1/2019/20).

The radiographic images were saved as high resolution in JPEG size for further image analysis. Following the method of De Luca *et al*.,[13] the images of the canines were processed with a computer-aided drafting program (Adobe Photoshop CS4). A minimum of 20 points from each tooth outline and 10 points from each pulp outline were identified and connected with the line tool, also on the Draw Toolbox, and the area of both tooth and pulp were ascertained [Figures 1 and 2].

All measurements were performed by a single-blinded observer. To minimize intraobserver variations the points on the teeth and pulp surface selected in a way that they were yielded following specific measurements as shown in Figures 1 and 2. The area measured using Adobe Photoshop (CS4) image processing program's quick selection tool. Intra-observer reproducibility was assessed by having the observer re-evaluating 20 randomly selected radiographs after an interval of 1 month. All variables were entered separately for each tooth on a Microsoft Office Excel spreadsheet**.** The first and the second determinations were compared using paired student *t*-test to analyze any statistically significant difference.

Morphological variables and the chronological age of the individual were entered into Microsoft Excel spreadsheet

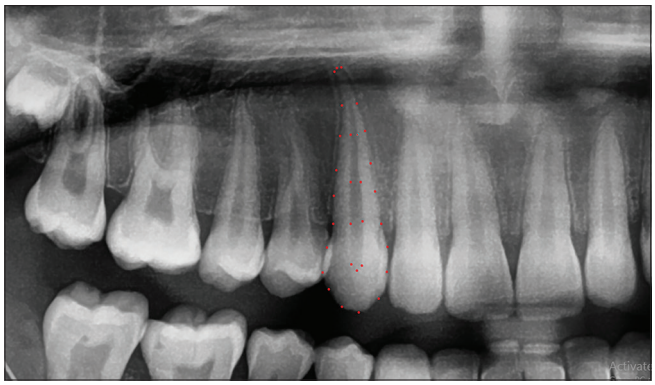

Figure 1: Plots of data and regression line (left panel) and residuals against fitted values (right panel) using a simple linear regression model (Eq.(1): Age = 89.439–466.41 ratio) to describe age as function of ratio.

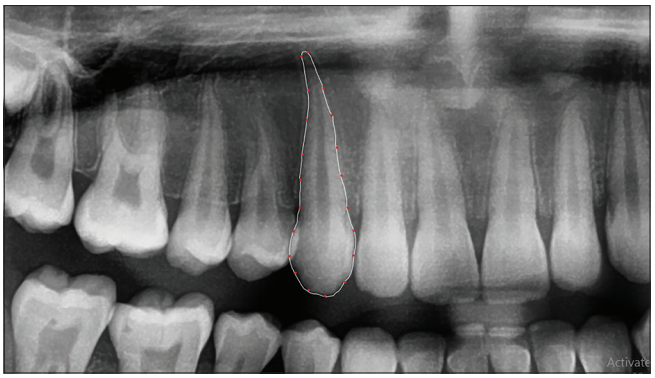

Figure 2: Plots of data and regression line (left panel) and residuals against fitted values (right panel) using a simple linear regression model (Eq.(2): Age = 87.260–449.63 ratio) to describe age as function of ratio.

for the use of age estimation. Correlation coefficient was evaluated between age and predictive variable (pulp/tooth surface area). A multiple linear regression model was developed to estimate the age of the individual. The estimated age was compared with the chronological age of the individual using the statistical software SPSS version 17 (AQ4: IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp).

Results

The paired Student's *t*-test between the first and second determination disclosed $P = 0.78$ indicating the negligible difference. Thus, the systematic measurement error is thought to give no effect on the statistical analysis.

The results of the Kolmogorov–Smirnov test and Anderson Darling Test confirmed that the data are distributed normally. The average age of the teeth was 19–76 years in males and 19–72 in females. PA/TA ratio of left and right canines did not show any statistically significant difference between male and female neither in maxilla nor mandible. Therefore, male and female data of PA/TA were combined for further analysis. Moreover, the ratio between the left and right sides of maxillary and mandibular canine did not show any statistically significant difference as well. The correlations between age with PA/TA ratio are shown in Table 1. All ratios showed highly significant negative correlations with the age.

Regression analysis

The linear regression equations that can be used to estimate the age using the PA/TA ratio of mandibular and maxillary left and right canines are presented in Table 2. Besides, the coefficients of determination (R^2), adjusted coefficients of determination (adjusted-R^2), standard error of the estimate, F-value and *P* values are also presented in Table 2. A significant linear regression was observed in relation to all canine teeth of the maxilla and mandible.

Furthermore, Table 3 shows the details of the fitted models with the corresponding t-value and its *P* value. Since all the $P < 0.05$, both the intercept and the slope of all four regression equations are significant at 95% confidence.

Table 1: Correlation of the age with pulp area/tooth area ratio

Variable	Pearson's correlation coefficient	*P*
Mandibular-right canine		
Tooth area	0.007	0.963
Pulp area	−0.088	0.557
Ratio	−0.999	<0.001**
Mandibular-left canine		
Tooth area	−0.175	0.323
Pulp area	−0.210	0.232
Ratio	−0.997	<0.001**
Maxillary-right canine		
Tooth area	−0.123	0.232
Pulp area	−0.176	0.087
Ratio	−0.999	<0.001**
Maxillary-left canine		
Tooth area	−0.251	0.079
Pulp area	−0.290	0.061
Ratio	−0.997	<0.001**

**Correlation is significant at the 0.01 level (two-tailed)

In addition, the regression plot along with its lower and upper confidence limits and predicted limits are shown in Figures 1-4. The residual plot [Figures 1 and 4] shows no obvious pattern, and the data points did not plot outside the expected boundaries. Besides, the regression line [Figures 1 and 4] shows that the regression model fits the data trend reasonably well. Hence, all four diagnostic plots support the chosen models.

Discussion

Reliable age estimation at the death of human remains is considered crucial to interpret osteological data. In addition to gender, age is an essential basic biological parameter which facilitates the identification of human remains in forensic and palaeodemographic contexts.[14]

The commonly used methods for estimation of the age of an individual are based on mainly bones and teeth. Analysis of macroscopic characteristics of various skeletal structures

Table 2: Linear regression equations for estimation of the Age using ratio between pulp area/tooth area

	Equation	R^2 (%)	Adjusted R^2 (%)	SSE	*F*	*P*
Mandibular right canine	Age=89.439-466.41 × ratio	99.72	99.72	31.90	16191.98	<0.001
Mandibular-left canine	Age=87.260-449.63 × ratio	99.46	99.45	29.77	5921.61	<0.001
Maxillary-right canine	Age=99.340-531.68 × ratio	99.73	99.72	49.90	35,041.60	<0.001
Maxillary-left canine	Age=99.342-530.91 × ratio	99.49	99.48	31.45	9433.50	<0.001

SSE: Standard error of the estimate

Table 3: Details of the regression equations and their significance of predicting chronological age

Coefficients	Value	SE	*t*	*P*
Mandibular-right canine				
Intercept	89.439	0.426	209.72	<0.001
Slope	−466.41	3.670	−127.25	<0.001
Mandibular-left canine				
Intercept	87.260	0.732	119.20	<0.001
Slope	−449.63	5.843	−76.95	<0.001
Maxillary-right canine				
Intercept	99.340	0.345	287.83	<0.001
Slope	−531.68	2.840	−187.83	<0.001
Maxillary-left canine				
Intercept	99.342	0.683	145.44	<0.001
Slope	−530.91	5.470	−97.13	<0.001

SE: Standard error

such as: the pubic symphysis,[15,16] the auricular surface of the ilium,[17] the sternal rib ends,[18] or the endo-and ectocranial sutures[19,20] are being frequently used. In addition, the ossification of bones of the hand and wrist using radiographic analysis[21] are also important parameters in age estimation.

It is also well established that the use of morphological characteristics of the teeth is considered to help more reliable age estimates than most of the other methods. The teeth are sometimes the only skeletal remains to be used for the estimation of age if the skeletons are highly damaged. Age estimation using teeth have a greater advantage over the bone as teeth are the strongest structure of the body and are highly resistant to mechanical, chemical, or physical impacts and also it can be clinically examined even in living individuals.[5,22] Furthermore, the nutritional, medical, environmental, and living conditions have minimal effect on the individual dental age predictors.[23]

Various macroscopical, microscopical, biochemical analysis, and radiological techniques have been developed to estimate the unknown age of individuals, both in living and at death using teeth. Perusal of literature reveals that many reference charts on the chronology of tooth development have been reported by many investigators for different population groups, taking into consideration the degree of crown development, the dates of emergence of tooth crowns in the oral cavity, and the degree of completion of roots of erupted teeth.[2,24-26] Using radiographs, Gleiser and Hunt[24] described in detail the stages of growth of the mandibular first molar from birth onward. They emphasized that the degree of tooth development is a better indicator of age than clinical tooth emergence dates. Although their use is limited to the ages <21 years, they appear to give useful and reasonably accurate results when estimating the age of an individual. Beyond the age of 21 years estimation of the age of an individual basically depends on the age changes of teeth due to several factors, such as periodontal disease, secondary dentin deposition, root translucency, cementum apposition, root resorption, color change, and increased root roughness.[3,27-29] However, some methods are destructive and are therefore may not be appropriate for living individuals. Two criteria at present be measured through nondestructive methods are tooth wear,[17,30] and secondary dentin deposition.[31] Although wear of tooth surface is affected by various external factors including masticatory function, diet, tooth form and position, and enamel thickness and hardness, the secondary dentin deposition is a regular, ongoing process that is modified only by caries or specific types of abrasion. And also, secondary dentine deposition can be studied radiologically.[7]

The secondary dentine is defined as the formation of dentine after the completion of the primary dentine, which is not affected by external stimuli. In addition, the rate of secondary dentine deposition for age estimation can be used to estimate age in living individuals using radiological images which is nondestructive. The study of the apposition of secondary dentine offers numerous advantages over other factors of age changes in teeth as it has been shown to provide a reliable age at death estimate and may help to narrow an estimate when used in combination with other morphological markers.[29] The assessment of PA/TA ratio and pulp/tooth volume ratio are methods to indirectly quantify secondary dentine deposition. A strong correlation between pulp width and age was first established in a study by Kvaal *et al*.[6] from measurements of pulp size on periapical radiographs. Furthermore, the authors believed that their formulae could be applied to different racial groups, and however, they suggested population-specific studies should be conducted to validate their methodology.[31] Moreover, the number of authors used different radiological methods for the estimation of age using pulp/tooth ratio.[5,23,32-39]

In the study done by Cameriere *et al*.,[5] two simple linear regression equations were obtained for age estimation on canines from the maxilla and mandible separately on the left and right side and they had concluded mean prediction errors of 2.68 years and 2.73 years for upper and lower canine tooth, respectively. They further suggested that future research should aim at acquiring larger sample sizes, to reduce standard errors of age estimation, at studying the effect of race and culture on model parameters, and at investigating the use of several teeth together, to improve dental age estimation.

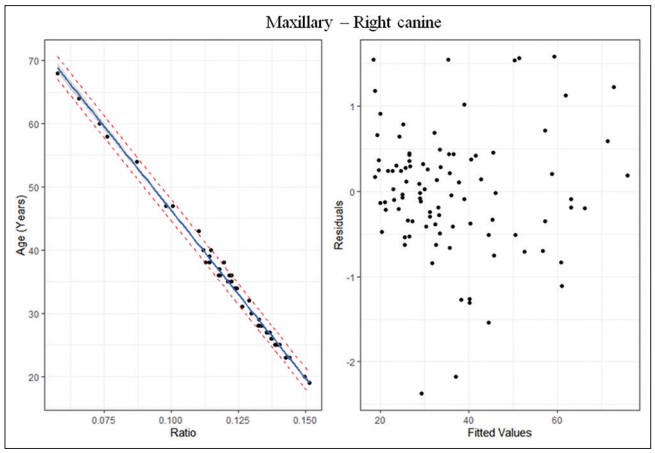

Figure 3: Plots of data and regression line (left panel) and residuals against fitted values (right panel) using a simple linear regression model (Eq.(3): Age = 99.340–531.68 ratio) to describe the age as function of ratio.

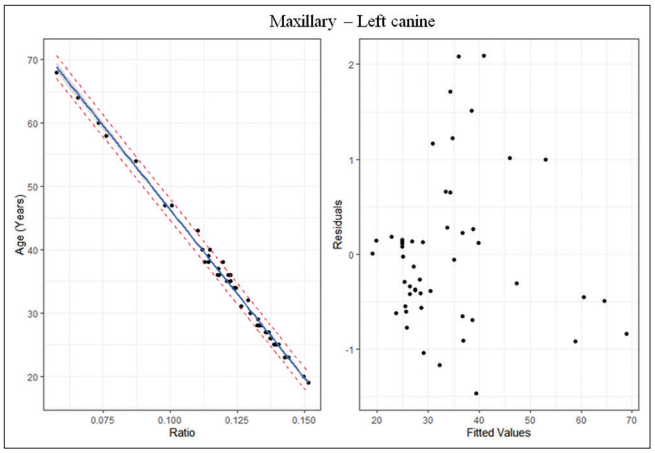

Figure 4: Plots of data and regression line (left panel) and residuals against fitted values (right panel) using a simple linear regression model (Eq.(4): Age = 99.342–530.91 ratio) to describe age as function of ratio.

A study[37] done on an Indian population to estimate the age using left and right maxillary canine PA/TA ratio on OPG concluded that there is a significant correlation between age and morphological variables; PA/TA ratio and pulp/root width. Based on these variables, they further affirmed that chronological age can be determined with an accuracy of 96% in a Karnataka population investigated. They presented the linear regression equation to estimate the age as Age = 87.305 − 480.455 (PA/TA ratio) + 48.108 (pulp/root width).

In a sample of the Iranian population,[38] the PA/TA ratio of upper canine was better correlated with chronological age than that of lower canine and it was showed that a significant and inverse correlation between age and PA/TA ratio of upper and lower canines (r = –0.794 for upper canine and r = –0.282 for lower canine; $P < 0.001$). The mean difference between actual and estimated age using upper canine was 6.07 ± 1.7.

In another study by Sakhdari *et al*.[39] in Iran for age estimation from maxillary right canine PA/TA ratio using digital OPG concluded that in males, the regression equation underestimated or overestimated the actual age. Moreover, in females, this equation accurately estimated the age in 16% of cases. They further concluded that according to the results, PA/TA ratio cannot be used for age estimation alone; but it can be used in combination with other indices for this purpose.

The result of the present study showed that TA and pulp area ratio between the right and left canine teeth of maxilla and mandible shows no statistically significant difference. Therefore, either the right or left canine tooth of the maxilla and mandible can be used to estimate the age using the present equation. Furthermore, the correlation of PA/TA ratio with age shows the significant negative correlation in maxillary and mandibular canines of left and right sides. It affirms that all four canines can be used with high reliability for the age estimation in the present sample. In addition, simple linear regression equations obtained for age estimation show no significant effect of gender on all regression models. Therefore, it seems that gender is not a limiting factor in estimating the age using the present regression equations. Furthermore, as the intercept and slope of all regression equations show highly significant consistency in predicting the chronological age, any permanent canine tooth of the dentition can be used with high reliability for the estimation of age. Mean prediction errors of the present study were 0.16 years and 0.21 years, respectively, for right and left mandibular canines and 0.10 years and 0.15 years, respectively, for right and left maxillary canines and further confirms the high reliability and accuracy in the prediction of age.

An earlier report on the application of PA/TA (rather than volume) ratio to estimate age concluded that the formula which had been derived for an Italian population could be applied to other populations as well.[32] However, the present study further supported the view held by other studies that population-specific formulae have to be adapted for different populations.[40]

There are certain limitations in the present study. Although the results of the present study showed a significant correlation between age and area of pulp in a Sri Lankan population, it cannot be generalized to other world populations. Even for the Sri Lankan population future studies are recommended using a larger sample which represents every province, ethnic group, etc., Similar studies on other populations are also suggested to carry out a meaningful inter-population comparison. The present study focused on maxillary and mandibular canine teeth. However, this radiographic method of age estimation cannot be applied to multirooted teeth, as accurate measurements are difficult to perform on these. Therefore in situations where canine teeth are missing the present method cannot be applied. On the other hand, other tooth types in the dentition of varying age groups can also be used in the future to estimate the age using more advanced radiographic techniques such as cone beam computed tomography (CBCT) and Micro CT taking into account the factors that influence the volume of pulp. CBCT and Micro CT technology offer 3D visualization and more accurate imaging with high resolution compared to analog and digital radiographs such as OPG. The advantages of using CBCT and Micro CT over conventional

radiographic methods include controlled magnification without distortion, lack of superimposition, and convenient multiplanar and 3D displays.

And also, there are reports in the literature concerning the precision and accuracy of the measurements when using digital measurements as it may be affected by distortions on panoramic images related to inadequate patient positioning, as the curved arch of the jaw is projected on to a flat film, can course certain amount of distortion when measuring the image presented there. However, measurement error can be minimized and the accuracy level of the measurements can be enhanced by using measurement software available with the CBCT and Micro CT techniques.

Conclusion

The result of the present study and previous studies show that estimating the age using the formulae is highly reliable and accurate, and intercept and slope of the formulae is different in each population group. Therefore, population-specific formulae may give more accurate estimation of age as a formula devised for one population may not be applicable for another.

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Conflicts of interest

There are no conflicts of interest.

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