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Age Estimation by Pulp/Tooth Ratio in Canines by Peri-Apical X-Rays

ABSTRACT: Estimation of age in individuals has received considerable attention in forensic science, in which it is a widely used method for individual identification, together with paleo-demographic analyses to establish mortality patterns in past populations. The present investigation, which is a continuation of a previously published pilot study, was conducted to examine the possible application of the pulp/tooth area ratio by peri-apical images as an indicator of age at death. A total of 200 peri-apical X-rays of upper and lower canines were assembled from 57 male and 43 female skeletons of Caucasian origin, aged between 20 and 79 years. They belong to the Frassetto osteological collection of Sassari (Sardinia) and are housed in the Museum of Anthropology, Department of Experimental and Evolutionistic Biology, University of Bologna. For each skeleton, dental maturity was evaluated by measuring the pulp/tooth area ratio on upper (x_1) and lower (x_2) canines. Very good agreement was found between intraobserver measurements. Statistical analysis was performed in order to obtain multiple regression formulae for dental age calculation, with chronological age as dependent variable, and gender, and upper and lower canines as independent variables. Stepwise regression analysis showed that gender did not contribute significantly to the fit ($p = 0.881$) whereas variables x_1 and x_2 and the first-order interaction between them did. These two variables explained 92.5% of variations in estimated chronological age and the residual standard error was 4.06 years. Lastly, two simple linear regression equations were obtained for age estimation using canines from the maxilla and mandible separately. Both models explained 86% of variations in estimated chronological age and allowed an age-at-death estimate with a residual standard error of about 5.4 years.

KEYWORDS: forensic science, age determination, forensic odontology, dental pulp, pulp/tooth area, stepwise linear regression

In many mass disasters, age estimation is a widely used method for identification, and it is also necessary in paleo-demographic analyses to establish mortality patterns in past populations.

Several methods of age estimation have been studied using bone, which changes as an individual grows. Bone modification is visible until a subject reaches adulthood, and subsequently in the degeneration process. A particular problem for age estimation is that premortem modifications may vary from subject to subject and, in addition, post-mortem changes and taphonomy are influenced by many factors (time, humidity, etc.). Of the various parts of the body used in age estimation, teeth are the least effected by the taphonomic process. Their durability means that they are sometimes the only body part available for study. Several age estimation methods apply the various forms of tooth modification, including wear (1–3), root dentine transparency (4–6), tooth cementum annulation (7,8), racemization of aspartic acid (9,10), and apposition of secondary dentine (11–13). Wear and the apposition of secondary dentine are the currently available nondestructive methods. Tooth wear is influenced by various external factors (masticatory function, type of food, timing and sequence of tooth eruption, tooth form, position of teeth, thickness and hardness of enamel, and predisposition to enamel hypoplasia (14)). However, the apposition of secondary dentine is a continuing, regular process, which is only modified by caries or particular abrasion. The difference between primary and secondary dentine is very difficult to observe and consequently an indirect technique is used which studies the reduction of pulp area. Secondary dentine has been studied using several methods, both by section and X-ray (12–16). One method used by us for age estimation involved orthopantomographs (OPG) of the maxillary canines to study the pulp/tooth area ratio (17). Canines were chosen for a number of reasons: they are often present in old age, they are less likely than other anterior teeth to suffer wear as a result of particular work and are the single-root teeth with the largest pulp area, and thus the easiest to analyze. This study was conducted on a sample of 200 peri-apical X-rays of upper and lower canines to examine the possible application of the pulp/tooth area ratio by peri-apical images as an indicator of age at death.

Materials and Methods

Peri-apical X-rays of 114 canines of males and 86 canines of females aged between 20 and 79 years were analyzed. The teeth were taken from the osteological collection of Sassari (Sardinia), preserved in the Museum of Anthropology, Department of Experimental and Evolutionistic Biology, University of Bologna. The collection was assembled by Prof. Fabio Frassetto in the first half of the 20th century and contains 606 skeletons—337 males and 269 females—of individuals born between 1828 and 1916 who died between 1918 and 1932 (Table 1). Fifty-seven upper and lower canines of males and 43 of females were studied (Table 2). The ages of subjects ranged between 20 and 77 years for males, and 20 and 79 years for females. Canines without pathologies were chosen and, if both were present, bilateral canines were studied. Peri-apical X-rays were taken digitally (FARO Production) at an exposure of 10 ma, 70 Kvp. It was possible to use normal X-rays which then had to be scanned (with a scanner of at least 300 dpi resolution) and stored digitally. Following Cameriere et al. (17) the radiographic images of the canines (RIC) were

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TABLE 1—*Summary of osteological collection of Sassari, Italy (Frassetto collection).*

Subjects	M	F	Total
Total	337	269	606
Known age	312	253	565
Range of ages	9–86	14–98	9–98
Range of dates of birth	1841–1913	1828–1916	1828–1916
Range of dates of death	1918–1932	1925–1932	1918–1932

TABLE 2—*Age and gender distribution of study sample.*

Age	M	F
20–29	11	8
30–39	14	9
40–49	12	10
50–59	13	9
60–69	5	2
>70	2	5
Total	57	43

processed using a computer-aided drafting program (AutoCAD2000, Install Shield 3.0, 1997). Twenty points from each tooth outline and 10 points for each pulp outline were identified and used to evaluate both tooth and pulp areas.

Statistical Analysis

For each skeleton, dental maturity was evaluated by measuring the pulp/tooth area ratio on upper (x_1) and lower (x_2) canines. All measurements were carried out by the same observer. To test intraobserver reproducibility, a random sample of 40 peri-apical X-rays was reexamined after an interval of two weeks. Intraobserver reproducibility of measurements was studied using the concordance-correlation coefficient. The two morphological variables, x_1 and x_2, and subject's gender were entered in an EXCEL file for use as predictive variables for age-at-death estimation in the subsequent statistical analysis. Actual age at death was also recorded. Correlation coefficients were evaluated between age at death and predictive variables. In order to obtain an estimate of age at death as a function of variables x_1, x_2, and gender, a multiple linear regression model with first-order interactions was developed by selecting those variables, which contributed significantly to age-at-death estimations using the stepwise selection method. Analysis of covariance (ANCOVA) was then applied to study possible interactions between significant variables and gender. Besides evaluating age at death using measurements of canines from both jaws, we also evaluated separate predictions restricted exclusively to either maxillary or mandibular canines. Statistical analysis was performed with S-PLUS 6 statistical programs (S-PLUS 6.1 for Windows Professional Edition Release 1). The significance threshold was set at 5%.

Furthermore, to evaluate the accuracy of the regression model, the age at death of the skeletons (Age_i, $i = 1, \ldots, n$; with $n = 100$) were compared with the estimated ones ($Age_{\mathrm{est},i}$, $i = 1, \ldots, n$; with $n = 100$) using the mean prediction error:

$$ME = \frac{1}{n}\sum_{i=1}^{n} E_i = \frac{1}{n}\sum_{i=1}^{n} \left|Age_i - Age_{\mathrm{est,i}}\right|,$$

where each E_i, ($i = 1, \ldots, n$) is the absolute value of the difference between the age at death of the ith skeleton.

Finally, to validate the regression model, we randomly chose a new sample of 10 skeletons and peri-apical X-rays of their canines were analyzed. Hence the ages at death of these skeletons were compared with the ages estimated using the regression equation.

Results

There were no statistically significant intraobserver differences between the paired sets of measurements carried out on the reexamined peri-apical radiographs. Pearson's correlation coefficients 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. Age at death was modelled as a function of the morphological variables (predictors) and, to optimize the model, a stepwise regression procedure was applied. Statistical analysis showed that gender did not contribute significantly to the fit ($p = 0.881$) whereas variables x_1 and x_2 and the first-order interaction between them did (Table 3), so that only these variables were included in the regression model, yielding the following regression formula

$$\mathrm{Age} = 114.624 - 431.183x_1 - 456.692x_2 + 1798.377x_1x_2 \quad (1)$$

In Eq. (1), sexual dimorphism does not significantly change with age. This model had the lowest Akaike information criterion (AIC) value among the considered multiple regression models, as there was a modest improvement in model fit when gender or terms of order of three or greater were included in the model. Indeed, the full model explained 92.8% of total variance, whereas model (1), with the selected variables, explained 92.5% ($R^2 = 0.925$). The residual standard error was 4.06 years and the medians of the residuals were -0.772 years, with IQR = 6.16 years. The accuracy of the method was ME = 3.36 years.

The residual plot (Fig. 1, left) shows no obvious pattern and only three observations appeared to be possible outliers. The observed versus predicted plot (Fig. 1, right) shows that the regression model fits the trend of the data reasonably well. Hence, both diagnostic plots support our chosen model. Separate parameter estimates are given in Table 3 for canines from maxilla and mandible, respectively.

When only the lower canine is considered, the regression equation may be written as follows:

$$\mathrm{Age} = 89.456 - 461.873x_1 \quad (2)$$

TABLE 3—*Stepwise regression analysis predicting chronological age from chosen predictors.*

	Value	Standard Error	t Value	p
Lower and upper canines				
Intercept	114.624	4.87	23.5	<0.001
x_1	-431.184	58.39	-7.4	<0.001
x_2	-456.692	58.40	-7.8	<0.001
x_1x_2	1798.377	483.33	3.7	<0.001
Lower canine				
Intercept	89.456	1.914	46.742	<0.001
x_1	-461.873	18.578	-24.861	<0.001
Upper canine				
Intercept	99.937	2.324	43.009	<0.001
x_2	-532.775	21.454	-24.834	<0.001

x_1x_2 specifies interaction between variables x_1 and x_2.

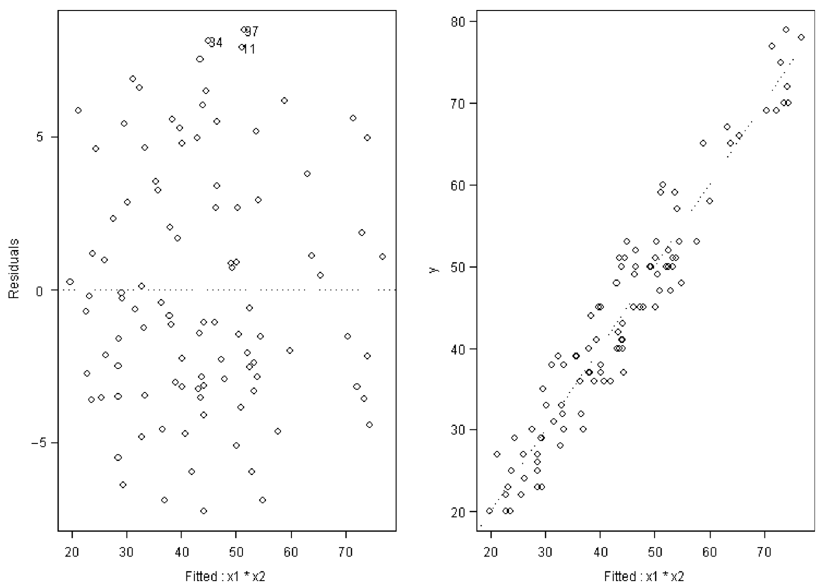

FIG. 1—*Plots of residuals against fitted values (left) and of observed against predicted values (right) using regression model (1).*

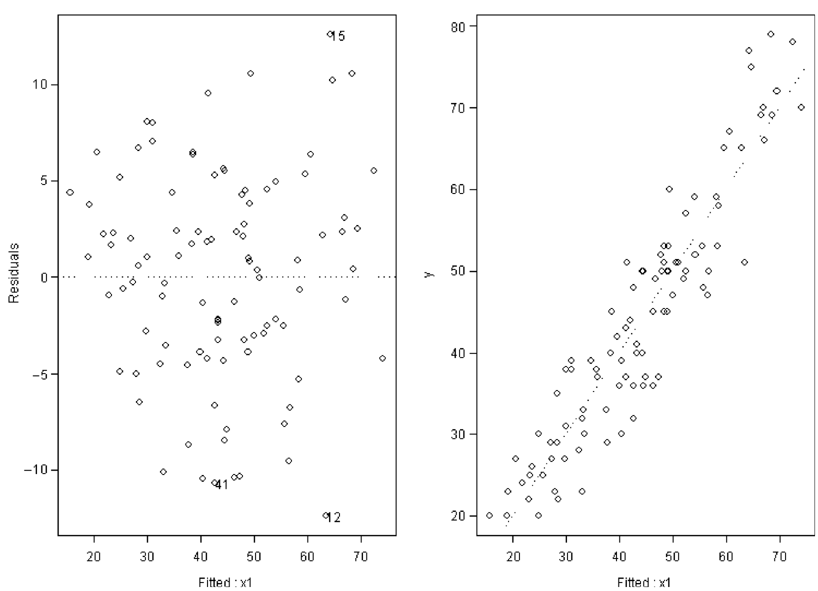

FIG. 2—*Plots of residuals against fitted values (left) and of observed against predicted values (right) using regression model (2).*

When only the upper canine is included, the equation becomes

$$\text{Age} = 99.937 - 532.775x_2 \qquad (3)$$

The coefficient of determination for both regressions (2) and (3) was $R^2 = 0.86$, which was slightly weaker than the coefficient of determination when both upper and lower canines were included in the model. However, it still remained highly significant.

When predicted age was obtained using Eq. (2), the residual standard error was 5.44 years and the medians of the residuals were 0.512 years, IQR = 7.54 years (Fig. 2). The accuracy of the method was ME = 4.38 years. Using Eq. (3), the residual standard error was 5.45 years and the medians of the residuals were −0.161 years, IQR = 9.41 years (Fig. 3). The accuracy of the method was ME = 4.46 years.

The residual plots (Figs. 2 and 3, left) show no obvious pattern and only three observations appeared to be outside the expected boundaries. The observed versus predicted plots (Figs. 2 and 3, right) show that the regression models fit the trend of the data reasonably well.

Only a small increase in the residual errors could be seen when the simplest linear models [Eqs. (2) and (3)] with only upper or lower canines as covariates were used. It should also be noted that, although it was not the best fit, this simple linear model explained 86% of total variance.

An ANCOVA was performed, to study the possible effect of gender on the linear regression models with only one canine as covariate, According to the regression with both canines, gender did not significantly influence regression models (2) and (3), which can be used to estimate chronological age for both female and male groups.

Discussion

The need to estimate age in skeletons of adult people is an important problem in forensic and anthropological sciences. Although several parts of the body can be used for age estimation,

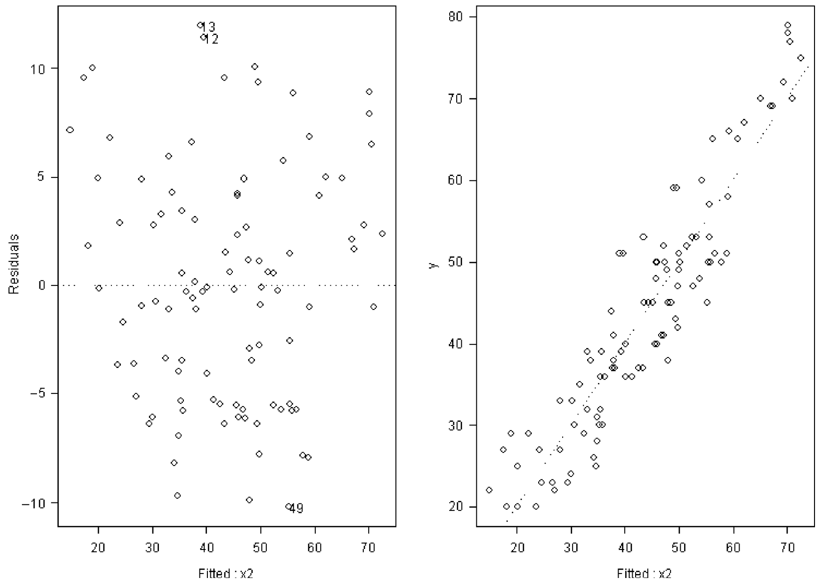

FIG. 3—*Plots of residuals against fitted values (left) and of observed against predicted values (right) using regression model (3).*

the poor condition of the remains, often prevent their use. In particular, crashes and fires in the case of the recently dead, and dampness and burial conditions in the case of elderly subjects, make many parts of the body unusable. For this reason, the teeth are that part of the body most frequently used for identification and age estimation when skeletal remains are in poor condition.

Although there are many dental methods, some are very complex or destructive and are not therefore normally used in anthropology. Dental wear is the method most widely used by anthropologists but, as attrition is related to diet, habits, and culture, this method has little application except for the population from which the sample was collected.

The apposition of secondary dentine is often taken into account, because the pulp is surrounded not only by harder tissue such as enamel, but also by dentine, which changes during an individual's life.

This study follows the previous paper (15) in which the pulp/tooth area ratio of upper canines as a method for age estimation was evaluated by orthopantomography. When pulp and tooth areas were measured on digitized peri-apical images of canines, the concordance correlation coefficient showed that there were no significant intraobserver differences. It was concluded that, in order to estimate age in skeletons of adult people, the RIC technique produces reliable and reproducible intraobserver measurements. According to previous studies, analysis of covariance shows that gender has no significant influence on age estimation when canine measurements are used. Hence, gender was not included as a factor in any of the above model equations.

TABLE 4—*Recorded and estimated ages applying Eqs. (1)–(3) to a set of 10 randomly chosen skeletons.*

Recorded Age	Estimated Age (Eq. [1])	Estimated Age (Eq. [2])	Estimated Age (Eq. [3])
24	26	22	30
53	55	56	53
58	60	59	59
78	77	70	73
22	26	23	23
39	35	40	33
37	38	36	42
57	54	56	52
25	24	26	21
36	38	36	40

Regression Eq. (1), with both canines as covariates, showed higher R^2 and lower IQR than regression Eqs. (2) and (3). Hence, the age-at-death estimates obtained using model (1) turned out to be better than those obtained using only one canine. Nevertheless, both models (2) and (3) allow accurate estimations of age (Table 4). These results indicate the appropriateness of using canines as morphological variables to predict individual ages, especially when comparing the obtained standard deviation with other skeletal age calculation techniques based on, for example, cranial suture, os pubis, auricular surface of the ilium, or sternal rib. Future research should aim at acquiring even larger sample sizes in order to reduce standard errors of estimates, and at investigating the effect of race and culture in model parameters.

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