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Age estimation by pulp/tooth area ratio in canines: Study of a Portuguese sample to test Cameriere's method

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ABSTRACT

Age estimation in adults is an important problem in both anthropological and forensic fields, and apposition of secondary dentine is often used as an indicator of age. In recent papers, Cameriere et al. studied the pulp/tooth area ratio of canines for this purpose. The present study examines the application of the pulp/tooth area ratio by peri-apical X-ray images as an age indicator in a Portuguese identified sample. The statistical model was then compared with results from an Italian identified sample, to establish whether a common regression model for both samples could be developed. The Portuguese sample consisted of 126 canines of male and 132 of female from subjects 20 to 84 years old, from the osteological collection of the Museum of Anthropology at Coimbra University. The Italian sample consisted of 114 canines of male and 86 of female from subjects 20 to 79 years old, analyzed in Cameriere et al. (2007) [20], and came from the Frassetto osteological collection of Sassari (Sardinia), now housed in the Museum of Anthropology, Department of Experimental and Evolutionistic Biology, University of Bologna. Statistical analysis was performed in order to obtain multiple regression formulas for dental age calculation, with chronological age as dependent variable, and gender and pulp/tooth area ratio on upper (RA_u) and lower canines (RA_l) as independent variables. ANCOVA analysis showed that gender was not significant but that variables RA_u and RA_l were. The regression model for the Portuguese sample yielded the following equations: Age = 101.3–556.68 RA_u (upper canines) and Age = 92.37–492.05 RA_l (lower canines). Both models explained about 97% of total variance, and mean prediction errors were ME = 2.37 years and 2.55 years, respectively. Comparisons between the equation referring to the Portuguese sample and the equivalent linear equations proposed by Cameriere et al. for the Italian sample did not reveal significant differences between the linear models, suggesting that a common regression model could be applied for both samples.

The common regression model, describing age as a linear function of RA_u and RA_l, yielded the following linear regression formulas: Age = 100.598–544.433 RA_u; Age = 91.362–480.901 RA_l, and explained 86% and 93% of total variance, respectively. Mean prediction errors were ME = 2.68 years and 2.73 years, respectively.

1. Introduction

Age estimation in adults is an important problem in both anthropological and forensic fields and, almost always concerns skeletal remains. For this purpose, various parts of the body are examined and several techniques are employed. Some age estimation methods apply various forms of tooth modification, including wear [1–4], root dentine transparency [5–9], tooth cementum annulation [10–12], racemization of aspartic acid [13–16], and apposition of secondary dentine [17–22]. The last is one of the most frequently used methods for age estimation in adults, since it is an ongoing, regular process, only modified by caries or particular abrasion. Since 2004, Cameriere et al. have published four papers on a new method for age estimation using apposition of secondary dentine in canines. Canines were chosen for a number of reasons: they have the longest functional survival rate in the mouth [1], undergo less wear as a result of diet than posterior teeth, 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. The aim of the present study was to test the Cameriere method on samples from the osteological collection of the Museum of Anthropology at

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Coimbra University, and to provide a specific formula. The equation was subsequently compared with the linear equation in [20], to establish whether a common regression model for both Italian and Portuguese samples could be developed.

2. Materials and methods

Peri-apical X-rays of 126 canines from male subjects, and 132 from female aged 20–84 years were analyzed. The teeth came from the osteological collection of the Museum of Anthropology at Coimbra University, which houses three main and important identified osteological collections: the Medical School Skull Collection, the Coimbra Identified Skeletal Collection (CISC) and the International Exchange skull collection [23]. While the latter two originate from the main local cemetery, the first one come from medical schools in Lisbon, Coimbra and Oporto Medical. This collection is composed of 585 complete skulls, collected between 1895 and 1903 by Bernardino Machado, and was the first identified osteological collection to be amassed in Coimbra. The International Exchange skull collection was probably initiated in 1915 by E. Tamagnini, who actively participated in accessions until 1942. The CISC was also made by Tamagnini and consists of 1075 complete skulls, collected between 1932 and 1942. Sixty-six upper and lower canines from male skulls and 63 upper and lower canines from female skulls were studied from CSIC (Table 1). A common element among the three collections is the low socioeconomic status of the individuals [23,3].

The age range was 20–78 years for males and 21–84 years for females. The majority of the old individuals were toothless which turned difficult to find canines for the higher age groups [4]. Canines without pathologies were chosen and, if both teeth were present, bilateral canines were studied. Peri-apical digital X-rays were taken using NOMAD hand-held dental X-ray device (Aribex, USA) combined with a digital sensor (DSX, Anthos, Italy) linked to a portable pc. All radiographs were taken with a Rinn-type digital sensor holder with 0.05 s exposure time and 60 kV. Following Cameriere et al. [20], the X-ray images were photo edited using Adobe Photoshop [5]. Twenty points from each tooth outline and 10 for each pulp outline were identified and used to evaluate both tooth and pulp areas.

The Italian sample, made up of 114 canines of males and 86 canines of females aged between 20 and 79 years were analyzed [Table 1]. 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. Fifty-seven upper and lower canines of males and 43 of females were studied. 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.

2.1. Statistical analysis

For each skull in the Portuguese sample, dental maturity was evaluated by measuring the pulp/tooth area ratio on upper (RA_u) and lower canines (RA_l). All measurements were carried out by the same observer. To test intra-observer reproducibility, a random sample of 40 peri-apical X-rays was re-examined after an interval of 2 weeks. Intra-observer reproducibility of measurements was studied with the concordance correlation coefficient. The two morphological variables, RA_u and RA_l, and subject's gender were entered in an EXCEL file for use as predictive variables for age-at-death estimation in later statistical analysis. Actual age at death was also recorded. Analysis of covariance (ANCOVA) was then applied to study possible interactions between age, upper and lower canines, and gender. Subsequently, the Portuguese sample was compared with the Italian one, previously evaluated in [20].

Statistical analysis was performed with S-PLUS (Windows Professional Edition, Release 6.1). The significance threshold was set at 5%.

In addition, in order to evaluate the accuracy of the regression model, age at death (Age_i, $i = 1,\ldots, n$; with $n = 100$) was compared with estimated ages ($Age_{est,i}$, $i = 1,\ldots, n$; with $n = 100$), with the mean prediction error:

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

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

Table 1
Age and gender distribution of Coimbra and Bologna samples.

Age	Males		Females		Total
	Coimbra	Bologna	Coimbra	Bologna	
20–29	10	11	10	8	39
30–39	12	14	10	9	45
40–49	10	12	10	10	42
50–59	14	13	12	9	48
60–69	10	5	11	2	28
>70	10	2	10	5	27
Total	66	57	63	43	229

Table 2
ANCOVA table for Portuguese sample.

	df	SS	MS	*F* value	*P*
Upper canines					
RA_u	1	35046.93	35046.93	4153.528	<0.001
g	1	6.55	6.55	0.776	0.380
Residuals	123	1030.57	8.38		
Lower canines					
RA_l	1	34836.32	34836.32	3503.206	<0.001
g	1	33.96	33.96	3.415	0.067
Residuals	123	1213.76	9.87		

Table 3
Regression analysis predicting chronological age from Portuguese canines.

Coefficients	Value	Std. error	*t* value	*P*
Upper canines				
Intercept	101.30	0.85	119.27	<0.001
Slope	−556.68	8.60	−64.73	<0.001
Lower canines				
Intercept	92.37	0.79	116.81	<0.001
Slope	−492.05	8.36	−58.84	<0.001

3. Results

There were no statistically significant intra-observer differences between the paired sets of measurements carried out on the re-examined peri-apical X-rays. With the measurements of the pulp/tooth area ratio of the Portuguese upper canines (RA_u), the ANCOVA (Table 2) did not show any significant differences in either intercept or slope between males and females. Similar results were obtained when RA_l measurements were examined (Table 2). The parameter estimates of the linear model with equal slope and intercept for male and female individuals are listed in Table 3. The regression model, describing age as a linear function of the Portuguese RA_u yielded the following linear regression formula, which explained 97.1% of total variance ($R^2 = 0.971$):

$$Age = 101.3 - 556.68\,RA_u \tag{1}$$

The median of the absolute values of residuals (observed age minus predicted age) was 2.1 years, with a quartile range of 2.14 years and a standard error of estimate of 2.89 years. The accuracy of the estimates was ME = 2.37 years. The residual plot (Fig. 1, right panel) shows no obvious pattern, and the data points did not plot outside the expected boundaries. The regression line (Fig. 1, left panel) shows that the regression model fits the data trend reasonably well. Hence, both diagnostic plots support the chosen model. When lower canines were taken into account, the regression model, describing age as a linear function of the RA_l of the Portuguese sample, yielded the following linear regression formula (Fig. 2, left panel):

$$Age = 93.27 - 492.05\,RA_l \tag{2}$$

Like Eq. (1), this model explained about 97% of total variance ($R^2 = 0.971$). The median of the absolute values of residuals (observed age minus predicted age) was 2.36 years, with a quartile range of 2.48 years and a standard error of estimate of 3.17 years. The accuracy of the estimates was ME = 2.55 years. Again like Eq. (1), the regression line (Fig. 2, left panel) shows that the regression model fits the data trend reasonably well, and the residual plot (Fig. 2, right panel) shows no obvious pattern.

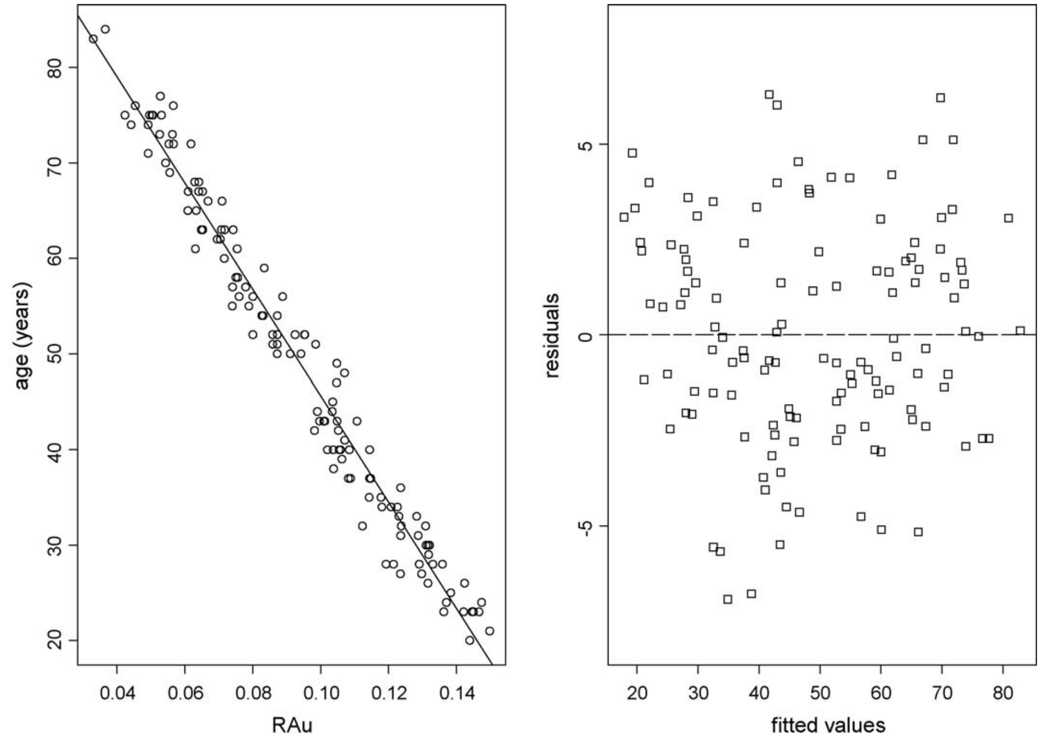

Fig. 1. Plots of data and regression line (left panel) and residuals against fitted values (right panel) using a simple linear regression model (Eq. (1)) to describe age as function of RA_u.

Comparison between equations describing age as a linear function of RA_u and RA_l and the equivalent linear equations in [20] did not show any significant differences between intercepts or slopes. These results indicated looking for a single linear regression model for both RA_u and RA_l in both Italian and Portuguese samples (Table 4).

As ANCOVA analysis confirmed that neither nationality (Portuguese or Italian nationality) nor gender affected the considered model, these factors were dropped. The estimated parameters are listed in Table 5.

This regression model, describing age as a linear function of the pulp/tooth area ratio in RA_u, yielded the following linear regression

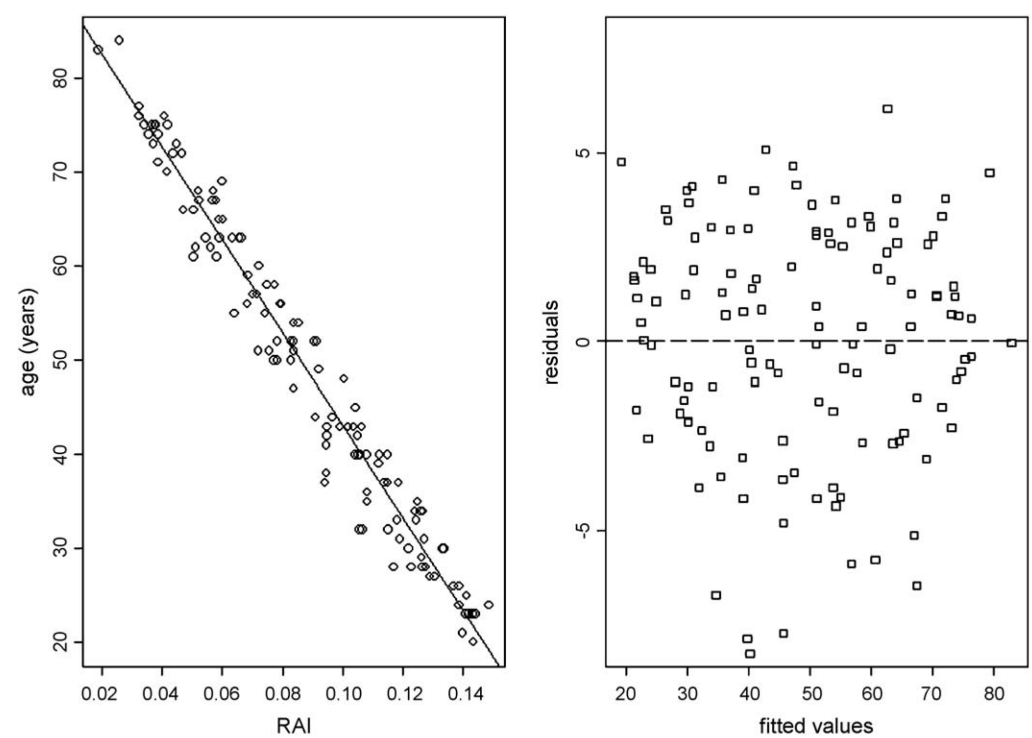

Fig. 2. Plots of data and regression line (left panel) and residuals against fitted values (right panel) using a simple linear regression model (Eq. (2)) to describe age as function of RA_l.

Table 4
ANCOVA table for Italian and Portuguese samples.

	df	SS	MS	*F* value	*P*
Upper canines					
RA_u	1	54689.13	54689.13	3080.66	<0.001
Nationality	1	59.88	59.88	3.373	0.068
g	1	26.05	26.05	1.468	0.227
Residuals	222	3941.04	17.75		
Lower canines					
RA_l	1	54518.22	54518.22	2902.489	<0.001
Nationality	1	0.13	0.13	0.007	0.935
g	1	27.88	27.88	1.484	0.224
Residuals	222	4169.88	18.78		

Table 5
Regression analysis predicting age from canines for Italian and Portuguese samples together.

Coefficients	Value	Std. error	*t* value	*P*
Upper canines				
Intercept	100.598	1.018	98.853	<0.001
Slope	−544.433	9.871	−55.155	<0.001
Lower canines				
Intercept	91.362	0.877	104.145	<0.001
Slope	−480.901	8.9161	−53.936	<0.001

equation:

$$\text{Age} = 100.598 - 544.433\,\text{RA}_u \tag{3}$$

This model explained 93% of total variance ($R^2 = 0.931$). The median of the absolute values of residuals (observed age minus predicted age) was 2.68 years, with a quartile deviation of 3.73 years and a standard error of estimate of 4.24 years. The accuracy of the estimates was ME = 3.32 years (Figs. 3 and 4).

When the pulp/tooth area ratio in RA_l was taken into account, the regression model for both Italian and Portuguese individuals yielded the following linear regression equation:

$$\text{Age} = 91.362 - 480.901\,\text{RA}_l \tag{4}$$

This model explained 93% of total variance ($R^2 = 0.9285$). The median of the absolute values of residuals (observed age minus predicted age) was 2.73 years, with a quartile deviation of 2.91 years and a standard error of estimate of 4.33 years. The accuracy of the estimates was ME = 3.39 years.

4. Discussion

Age estimation is essential in both forensic and anthropological sciences, and the apposition of secondary dentine is the most frequently used method of ascertaining age. Preliminary results by Cameriere et al. [18], applying orthopantomography, revealed a linear regression between age and RAu.

As in [20], this new paper was organized following the above preliminary study but using peri-apical X-rays instead of orthopantomography. The resulting images had greater contrast and less chance of overlap.

Measurements of pulp and tooth areas on digitized peri-apical images of canines produce more reliable and reproducible data. In addition, when extracted teeth are examined, peri-apical images of single canines makes data analysis even easier.

Two simple linear regression equations were obtained for age estimation on canines from the maxilla and mandible separately, with no significant effect of gender on the regression models. Dental maturity was then evaluated by measuring the pulp/tooth area ratio according to labio-lingual and mesial X-rays on upper and lower canines [21]. Results obtained with labio-lingual X-rays and mesial X-rays were similar to those obtained with single labio-lingual X-rays of canines. Instead, age-at-death estimates, obtained with the pulp/tooth area ratio on both canines and both

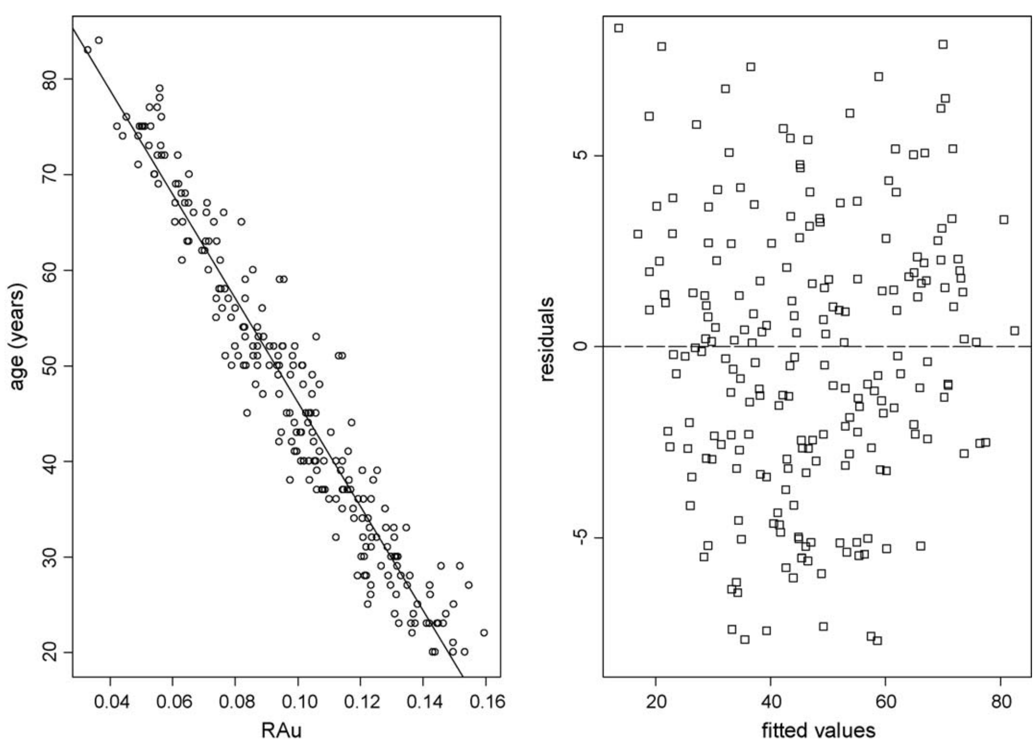

Fig. 3. Plots of data and regression line (left panel) and residuals against fitted values (right panel) using a simple linear regression model (Eq. (3)) to describe age as function of RA_u for both Italian and Portuguese individuals.

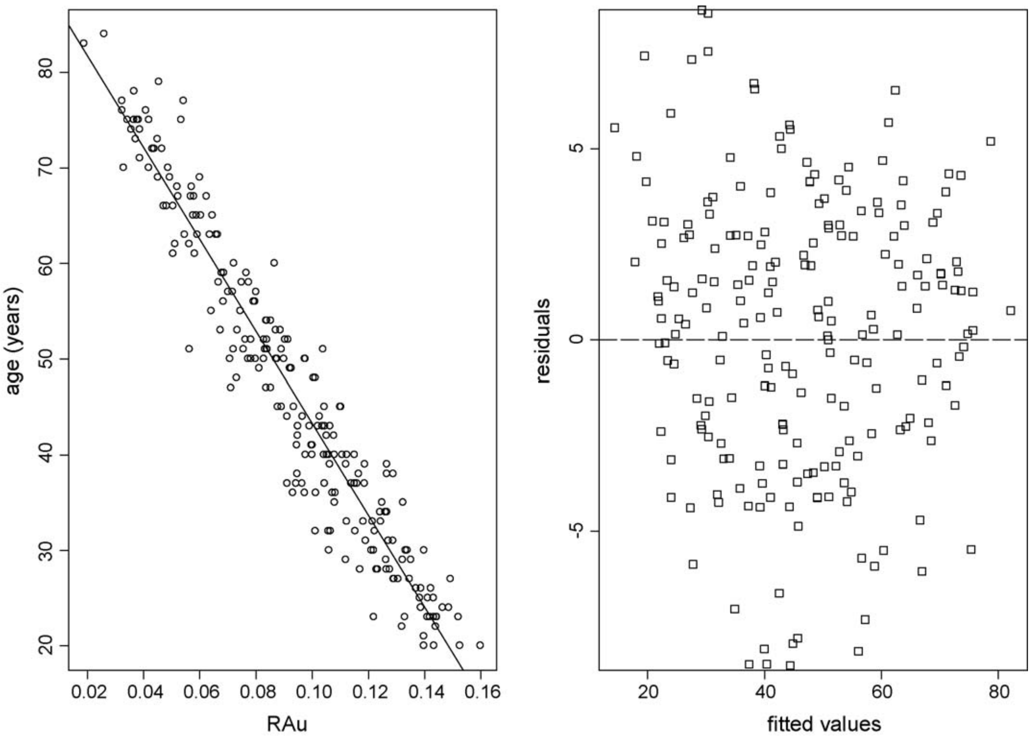

Fig. 4. Plots of data and regression line (left panel) and residuals against fitted values (right panel) using a simple linear regression model (Eq. (4)) to describe age as function of RA_l for both Italian and Portuguese individuals.

labio-lingual and mesial projections, were significantly better than those evaluated by measuring the pulp/tooth area ratio on upper and lower canines by labio-lingual X-rays only.

We therefore demonstrate here how dental age may be evaluated as a function of RA by means of a single formula for both Portuguese and Italian subjects (Eqs. (3) and (4), for upper and lower canines, respectively). In addition, according to previous results, sexual dimorphism does not significantly improve the formulas.

The proposed equations for age estimation are not only useful in assessing the chronological age at death in skeletal remains, but are also reliable tools in determining the age of living persons. This procedure could be easily employed on people without any identity document or in the increasingly frequent situations in which pensionable age must be evaluated in subjects without valid birth certificates or documents for pension purposes, or suffering loss of memory, dementia, etc.

With the RA method, the error of age estimation (about 2.5 years) is much lower than most that of anthropological methods, which give an error of more than 5 years.

These results suggest the use of the RA method also for paleodemographic studies [24–27]. Paleodemography examines the structure and dynamics of pre-modern populations. As its analyses are mainly based on expected longevity, infantile mortality and mortality rates, it can benefit from any method which is able to determine age at death with greater precision and accuracy.

Future research should aim at acquiring larger sample sizes, in order 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, in order to improve dental age estimation.

Disclosure

The authors wish to make clear that they receive no commercial benefits from, nor have interests Aribex (USA) and Anthos (Italy) Companies.

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