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Age estimation by pulp/tooth ratio in lower premolars by orthopantomography

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ABSTRACT

Accurate age estimation has always been a problem for forensic scientists, and apposition of secondary dentine is often used as an indicator of age. Since 2004, in order to examine patterns of secondary dentine apposition, Cameriere et al. [1–6] have been extensively studying the pulp/tooth area ratio of the canines by panoramic and peri-apical X-ray images. The main aim of this paper is to examine the relationship between age and age-related changes in the pulp/tooth area ratio in monoradicular teeth, with the exception of canines, by orthopantomography. A total of 606 orthopantomograms of Spanish white Caucasian patients (289 women and 317 men), aged between 18 and 75 years and coming from Bilbao and Granada (Spain), was analysed. Regression analysis of age of monoradicular teeth indicated that the lower premolars were the most closely correlated with age. An ANCOVA did not show significant differences between men and women. Multiple regression analysis, with age as dependent variable and pulp/tooth area ratio as predictor, yielded several formulae. R^2 ranged from 0.69 to 0.75 for a single lower premolar tooth and from 0.79 to 0.86 for multiple lower premolar teeth. Depending on the available number of premolar teeth, the mean of the absolute values of residual standard error, at 95% confidence interval, ranged between 4.34 and 6.02 years, showing that the pulp/tooth area ratio is a useful variable for assessing age with reasonable accuracy.

1. Introduction

Although estimation of adult skeletal age at death is one of the most important identifying features in unknown individuals, it is also one of the most difficult to achieve [7–9]. This is due to the several differences (genetic, cultural and environmental factors, economic status) existing in the development and deterioration of the skeletal system, among individuals as well as across populations and between sexes [10,11].

Despite these problems, in the last few years the literature has been provided with several skeletal and dental methods for assessing age. Most of them apply many age indicators related to degenerative changes in the skeleton, such as modifications in the pubic symphysis [12,13], sternal rib ends [14], auricular surface of the ilium [15], endo- and ecto-cranial sutures [16,17], and radiology of the proximal femur and clavicle [18,19]. However, these specific skeletal structures are commonly subjected to taphonomic processes and are often not recovered or are too damaged to contribute to satisfactory osteological analysis. So it becomes necessary to check the validity of more accurate methods, less biased by other factors, such as the degree of age-related information contained within specific skeletal traits, as well as sampling strategies and statistical methods used to develop age estimation methods [20–25]. As Cunha et al. [8] noted, part of the age estimation error is the inevitable consequence of the statistical procedures used to extract an estimate of age from age indicators, and the magnitude of the error is inversely related to how well an age indicator is correlated with age.

Thanks to recent and substantial advances in physical anthropology, old methods have been improved and new techniques have recently been proposed [22,26–28]. However, there are still several problems about the standardization of techniques: the large age ranges of age phasing methods; observer subjectivity; bias and age mimicry when appropriate reference samples are not used; improper procedures and statistical parameters used to derive age-at-death estimates [8,29–35].

In the last few years, estimation of age at death has also received much attention in the forensic context. As a result of the global increase in unidentified corpses and skeletons, successfully determining the identity of a dead person is of great importance from ethical, legal and criminal perspectives. In addition, cases of age estimation in living persons have increased [8]. For these individuals, the main aim is to solve judicial or civil problems concerning the true age of minors with regard to questions of

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adoption, imputability, pedopornography and, for adults, civil issues on pensionable age and similar matters for individuals without valid documents of identification [6–8]. Due to additional ethical and medico-legal aspects, great precision, accuracy and a non-destructive approach are therefore required. Destructive methods, although more accurate, are not acceptable in many forensic cases, due to the loss of evidence, or in living subjects in whom the tooth must be sacrificed [36–38].

Several problems associated with age-at-death estimates can be minimized simply by applying the most appropriate methods. Due to the difficulties described above, and with the current move toward improving existing techniques of age-at-death estimation, more accurate age-related changes in teeth have recently been re-examined, as an alternative to skeletally based techniques [33,38–41]. Dental structures are becoming more and more useful, because of their resistance to physical and chemical agents [42,43]. In addition, the use of dental metric features eliminates subjective categorical classification and also helps to reduce the large age ranges which are usually associated with skeletal age-at-death estimates in adults [33].

1.1. Apposition of secondary dentine

One of the best-known features of aging is a reduction in size of the pulp chamber, caused by the continual secretion of dentinal matrix by odontoblasts (physiological secondary dentinogenesis). Dentine is a living tissue containing odontoblasts which form the tooth and which, during a person's lifetime, for both physiological and pathological reasons, deposit layers of secondary dentine, which gradually obliterates the pulp chamber [44–46]. The mean rate of increasing dentinal thickness has been found to be 6.5 μm/year for the crown and 10 μm/year for the root. The effect of continuous dentine deposition is a progressive increase in dentinal thickness, of 0.45 mm (17.1%) and 0.60 mm (24.3%) in the crown and root areas, respectively [46]. However, as Murray et al. [46] noted, age-related differences are observed between different tooth types: little increasing dentinal thickness is detected in the crown aspect of canine teeth (+3.4%), whereas the crown dentine of incisors and premolars may increase by 15.5% and 34.1% respectively, in older patients. Irrespective of age, mean dentinal thickness is observed to vary significantly across tooth types, with differences between all tooth types, except between canine and premolar teeth.

Secondary dentine has been studied by several methods: examples are sectioning [36] and X-rays [46,47] or X-rays alone [1–6,46,48,49]. Both methods reduce possible errors caused by magnification and distortion of X-rays and provide better image quality. Since 1925, when Bodecker [44] ascertained that the apposition of secondary dentine was correlated with chronological age, new detailed studies of the pattern and rate of secondary dentine apposition in maxillary and mandibular anterior teeth have been performed. Secondary dentine deposition was included in the method pioneered by Gustafson [36], in which dentine transparency and secondary dentine values showed the highest correlation with age. Philippas [50] was one of the first to use the radiographic method to verify the influence of age on the formation of dentine. In 1995, Kvaal and Solheim [47] developed a new method for estimating age in adults, based on the relationship between age and pulp size on peri-apical dental radiographs. Paewinsky et al. [49] also tested the method of Kvaal and Solheim [47] on digital panoramic radiographs, but specific regression formulae were developed by these authors for their sample. Currently, thanks to newly developed techniques, such as standard X-rays and micro-focus X-ray computed tomography [41,50–52], apposition of secondary dentine is a useful tool for age estimation in adults [8,53].

Cameriere et al. [3,4] studied the relationship between age at death and the ratio of the pulp/tooth area in digitalized peri-apical X-rays of upper and lower canines of several individuals in an identified Italian osteological collection. A computer-aided auto-drafting program was used to determine the outline of the pulp cavity and tooth. The researchers reported extremely high regression coefficients ($R^2 = 0.94$) when using labio-lingual X-ray images of upper and lower canines. The reliability of this method has recently been tested in both archaeological and other identified osteological collections [5,39,54], and clearly shows that this technique has high repeatability and is easy for others to learn and replicate. Last but not least, the dental features examined are robust enough to withstand long-term taphonomic effects [39,55–58].

Taking into account several previous studies in which the relationship between the ratio of pulp/tooth in the canine and age was investigated [1–6,39,55], the main aim of this study is to verify, by applying the age parameters of Cameriere et al. [4,5] in a Spanish sample of digital orthopantomographs, the amount of correlation between age and lower premolars.

2. Materials and methods

2.1. Samples

Panoramic dental X-rays of 637 Spanish white Caucasian patients aged between 18 and 75 were analysed. All subjects were chosen from the collections stored at the private radiology department in Bilbao (Spain) and the Faculty of Odontology of the University of Granada (Spain). The samples from Bilbao (Spain) were obtained directly by digital radiological technology and collected during the year 2006. The orthopantomograms from Granada (Spain) were digitized with a scanner, and images were recorded on computer files. The patient's identification number, sex, date of birth and date of X-rays were recorded. Protocols to collect orthopantomograms for human subjects were approved by the Ethics Committee for Research Involving Human Subjects of the University of Granada (Spain), and the study was conducted in accordance with the ethical standards of the Declaration of Helsinki. Patient's medical history was not taken into account when selecting the X-rays. The World Medical Association (WMA) has developed the Declaration of Helsinki as a statement of ethical principles for medical research involving human subjects, including research on identifiable human material and data.

Patients with lost or extracted single-rooted teeth, as well as those with root fillings, crown restorations, severe caries or other abnormal dental anatomy which might cause difficulty with measurement were excluded from this analysis. Orthopantomograms showing badly rotated teeth or teeth with large areas of enamel overlap between neighboring teeth were also excluded. The study samples have also minimal attrition. A total of 31 orthopantomograms was thus discarded, because all lower single-rooted teeth were absent or with pathologies. In fact, most of the older individuals were toothless, which made it difficult to find these teeth for the higher age groups. A total of 606 orthopantomograms (289 women and 317 men) was finally examined. The selected nomenclature to classify the teeth is that proposed by the Fédération Dentaire Internationale (FDI).

2.2. Measurements

Following Cameriere et al. [1], save the radiographic images as high-resolution JPEG files on a desktop computer and import to Adobe Photoshop CS4® image-editing software program (Adobe Systems Incorporated, San Jose, CA, USA). Next, open the image file, enlarge the working area, and zoom in. Adjust brightness/contrast and sharpness if needed. Some X-ray images are sometimes over- or under-exposed or a little blurred. With the aid of some Photoshop tools, it is possible to correct some image problems or enhance some edges not clearly visible (Fig. 1a).

Select the polygonal lasso tool from the tool bar. In order to select the entire premolar area, click in the premolar image to set the starting point of the premolar shape; move the cursor to a close point of the tooth profile and click again. A straight line from the first point selected will be drawn. Continue clicking to set endpoints for subsequent segments along the premolar profile. A minimum of 20 points from each tooth outline has now been identified and connected with the line tool (Fig. 1b). Now copy and paste the selected area on a new layer, which will be added to the active working area superimposed on the premolar image. This new layer, renamed "PREMOLAR", will be added to the layer palette. In order to select the pulp chamber area, proceed as previously for the entire premolar, following the pulp chamber profile with the polygonal lasso. A minimum of 10 points will also have been marked on the pulp outline, although up to 15 points are marked in some cases (Fig. 1c). Copy and paste the pulp chamber selection to a new layer and rename it "PULP CHAMBER". The new "PULP CHAMBER" layer contains only the premolar pulp chamber area pixels, as the "PREMOLAR" layer contains the entire premolar area pixels. To know how many pixels there are in each layer, activate the histogram

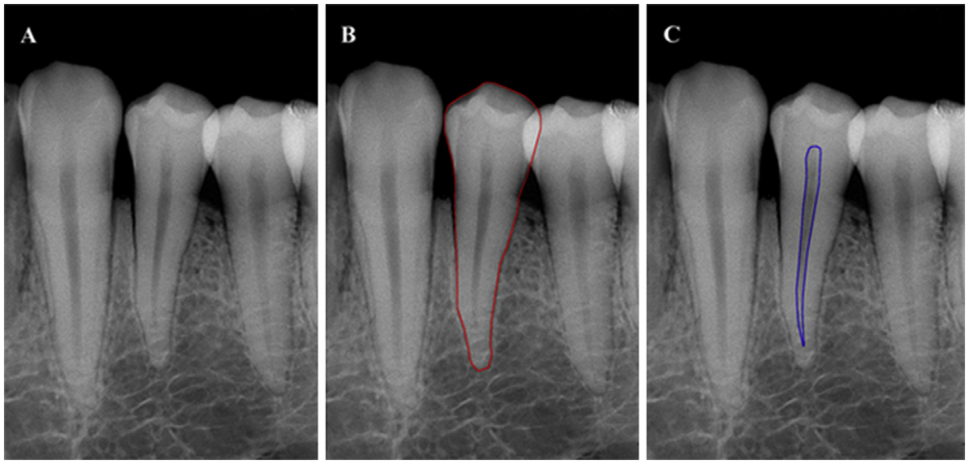

Fig. 1. Radiographic image of left lower premolar (34), after processing and measuring pulp and tooth areas with line tool: red line, tooth area; blue line, pulp area.

palette (windows > histogram) and the ''PULP CHAMBER'' layer by clicking on the layer name in the layer palette. In the histogram palette from the source menu, now choose SELECTED LAYER and double-click on the histogram image. Read the number of pixels contained in the ''PULP CHAMBER'' layer in the histogram palette. This value represents the first needed variable (pulp chamber area). Next, select the ''PREMOLAR'' layer, double-click on the histogram image and read the number of pixels contained in the entire premolar. This value represents the second needed variable [1].

2.3. Intra- and inter-observer agreement

Each file was numbered consecutively from 1 to 606, being part of a blind set-up. When analysing the radiographs, the observer had no idea of the chronological age of the individuals examined. All measurements were carried out by the same observer (RC) with ample experience of this technique. Since being able to replicate measurements reliably is an essential component of any metric study, tests for intra-observer error were performed. To test intra-observer reproducibility, a random sample of 50 panoramic radiographs was re-examined after an interval of at least 2 weeks and was studied with the concordance correlation coefficient.

2.4. Statistical analysis

We used R_{ij} to indicate the pulp/tooth area ratio of the tooth in position ij (i = 1, 2, 3, 4; j = 1, 2, 5) in the FDI notation. For example, we indicated by R_{34} the pulp/tooth area ratio of the lower left first premolar, R_{11} the pulp/tooth area ratio of the upper right central incisor, and R_{42} the pulp/tooth area ratio of the lower right second incisor.

For each orthopantomograph of the sample, dental maturity was evaluated by measuring the pulp/tooth area ratio of monoradicular teeth, except for incisors. The morphological variables, R_{34} (i = 1, 2, 3, 4; j = 1, 2, 4, 5) and the individual's sex were entered in an EXCEL file, for use as predictive variables for dental age estimation in subsequent statistical analysis. Chronological age was also recorded, and was calculated by subtracting date of birth from the date of the radiograph. Analysis of covariance (ANCOVA) was then applied, to study possible interactions between age, lower premolars and sex.

As regards model validity, the Variance Inflation Factor (VIF) was evaluated for the estimated parameters of each regression model. The VIF is a statistic which can be used to identify multicollinearity in a matrix of predictor variables, and shows how much the variance of the coefficient estimate is being inflated by multicollinearity [59,60]. Multicollinearity is said to be a problem when the variance inflation factor of one or more predictors becomes large. How large it may be, appears to be a subjective judgment. According to Haan [59], some researchers use a VIF of 5 and others 10 as critical thresholds. Some compute the average VIF for all predictors and state that an average ''considerably'' larger than one indicates multicollinearity [59,60]. At any rate, it is important to keep in mind that multicollinearity requires strong correlation of predictors, not just non-zero correlation.

In addition, to evaluate the accuracy of a regression model, the age of each subject (Age_i, $i = 1, \ldots, n$) was compared with estimated ages ($\mathrm{Age}_{\mathrm{est},i}$, $i = 1, \ldots, n$) by means of the mean prediction error (ME), which is the mean of the absolute values of the differences between chronological and estimated ages (residuals):

$$\mathrm{ME} = \frac{1}{n}\sum_{i=1}^{n} E_i = \frac{1}{n}\sum_{i=1}^{n} |\mathrm{Age}_i - \mathrm{Age}_{\mathrm{est},i}|,$$

where n is the number of subjects in the sample, and E_i ($i = 1, \ldots, n$) is the absolute value of the ith residual, i.e., the difference between the chronological and dental ages of the ith individual:

$$\delta_i = \mathrm{Age}_i - \mathrm{Age}_{\mathrm{est},i}, \quad i = 1, \ldots, n.$$

A positive value of δ_i indicates underestimation and a negative value overestimation.

The models were validated by estimating their predictive accuracy, tested bootstrapping the model fitting process. We used the bootstrap for validation purposes by estimating the *optimism* of R^2 as an index of predictive accuracy. *Optimism* is a positive bias in predictive accuracy, which usually occurs when we assess predictive accuracy on the same data set used to fit the model [62]. Lastly, subtracting the estimated expected optimism from the model R^2, we obtain bootstrap R^2, R^2_{boot}, which estimates the predictive accuracy of the model [60].

All statistical analyses were performed using the SPSS 15.0 software program (SPSS Inc., Chicago, IL) [62] and Microsoft Excel®. Significance level was set at 5%.

3. Results

There were no statistically significant intra-observer differences between the paired sets of measurements carried out on the re-examined orthopantomographs. Morphological changes in the pulp cavity in different age groups were observed. Regression analysis of age with central incisor, lateral incisor, and lower premolar gave the following R^2 values: 0.35, 0.45 and 0.86, respectively. These results demonstrated that the lower premolar has an R^2 which is significantly greater than that of the other teeth, which suggested that this tooth type should be used in order to continue data analysis. For each premolar, patients' sex and age distribution are listed in Table 1. Table 2 lists the frequency distribution, for male and female groups, of premolars present on each of the 606 evaluated orthopantomographs. This study was based on the evaluation of 1674 premolars. The pulp/tooth area ratio of lower premolars regularly decreased with age and ranged

Table 1
Age and sex distribution for each lower premolar tooth.

Tooth	Sex	Age cohort							Total
		–20	21–30	31–40	41–50	51–60	61–70	71–	
34	W	7	52	48	35	44	11	3	200
	M	19	78	50	53	37	24	4	265
35	W	9	60	38	32	35	8	2	184
	M	18	78	42	45	30	25	3	241
44	W	6	41	37	36	44	8	4	176
	M	11	63	35	46	28	23	2	208
45	W	13	66	39	31	35	10	4	198
	M	20	82	36	41	24	26	3	232

Table 2
Frequency distribution, by male and female groups, of the number of premolars (N) present in all the 606 orthopantomograms examined. The numbers 1, 2, 3 and 4 correspond to the number of premolars present in each sex or age group.

Number of premolars	Men	Women
1	26	59
2	74	69
3	96	83
4	121	78
Total	317	289

from 0.018 to 0.20. Fig. 2 shows the pulp/tooth area ratios, by age groups, of all lower first and second premolars.

An ANCOVA was performed to study the possible effect of sex on the linear regression model. With the measurements of the pulp/tooth area ratio of the lower premolars, this analysis of the F-values (Table 3) did not reveal any significant differences in either intercept or slope between men and women, and consequently sex was not included in the statistical models for any of the premolars.

Multiple regression analysis, describing age as a linear function of the pulp/tooth area ratio of the lower premolars, yielded several formulae. These regression formulae were subdivided according to the number and location of premolars, and only significant interactions were included in the models (Tables 4–6). Table 7 lists the regression model with all lower premolars. Depending on the available number of premolar teeth in an individual, R^2 ranged from 0.69 to 0.75 for a single lower premolar tooth and from 0.79 to 0.86 for multiple lower premolar teeth. When predicted age was obtained only with tooth 45 in Table 4, the residual standard error was 7.42 years and the mean of the residuals was ME = 6.02 years, with 95% confidence interval = (5.60, 6.44). When the pair of teeth 35 and 45 were used, the residual standard error was 5.75 years (Table 5) and the ME was 4.63 years, with 95% confidence interval = (4.25, 5.02). Lastly, when more than two lower premolars were used, the residual standard error decreased to 5.31 years (Table 6) and the ME was 4.34 years with 95% confidence interval = (3.92, 4.79). Although there was a positive association between the maturation of the different teeth (correlations ranged from 0.82 to 0.86), the values of the VIF for the linear models without interactions was always less than 6. In addition, the relative standard error of the parameter estimates

Table 3
Summary table of ANCOVA.

	df	SS	MSS	F	p
			R_{34}		
R_{34}	1	65,545	65,545	1029.42	<0.0001
g	1	138	138	2.17	0.14
Residuals	462	29416	64		
			R_{35}		
R_{35}	1	64,951	64,951	1133.96	<0.0001
g	1	211	211	3.68	0.056
Residuals	422	24171	57		
			R_{44}		
R_{44}	1	56,535	56,535	1046.16	<0.0001
g	1	56	56	1.04	0.031
Residuals	381	20590	54		
			R_{45}		
R_{45}	1	70,366	70,366	1284.31	<0.0001
g	1	186	186	3.39	0.066
Residuals	427	23395	55		

Table 4
Regression equations using single lower premolar teeth.

Tooth	N	Equation	R^2	SE
34	465	Age = 73.53 − 330.99·R_{34}	0.69	7.99
35	425	Age = 70.27 − 299.96·R_{35}	0.73	7.59
44	384	Age = 77.0 − 353.90·R_{44}	0.73	7.35
45	430	Age = 76.29 − 362.57·R_{45}	0.75	7.42

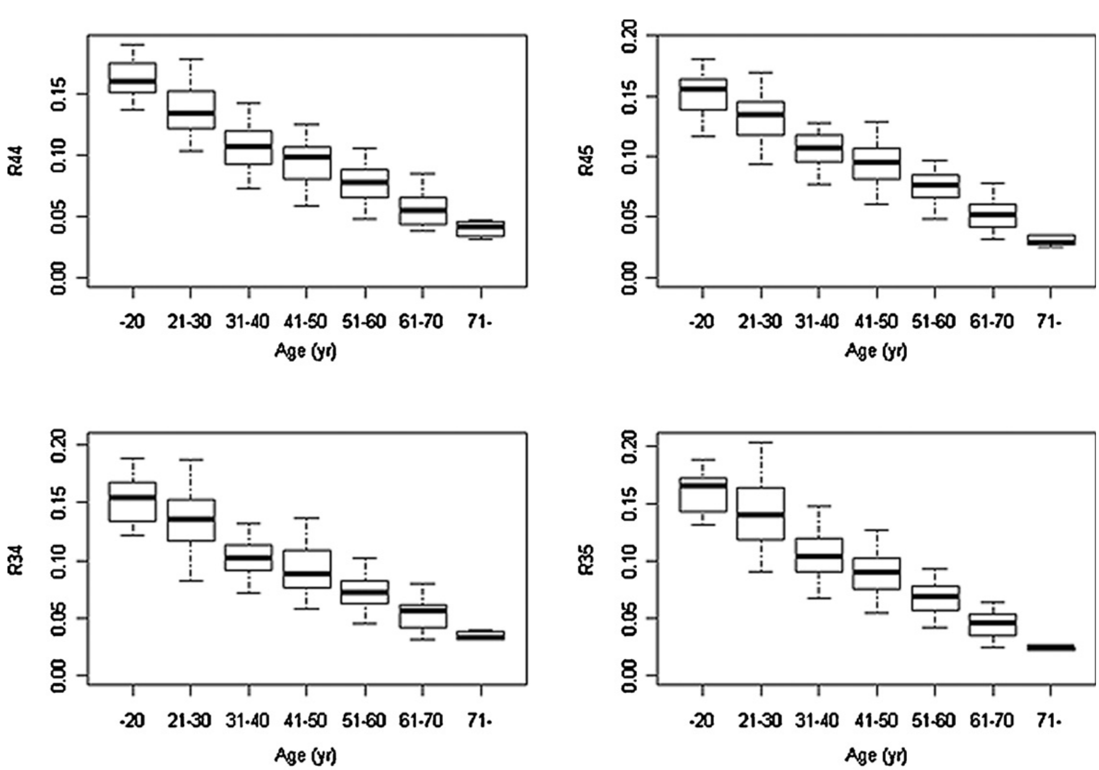

Fig. 2. Boxplots showing pulp/tooth area ratios, by age group, of lower first and second premolars. Pulp/tooth area ratio ranges from 0.018 to 0.20.

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Table 5
Regression equations using a pair of lower premolar teeth.

Tooth	N	Equation	R^2	SE
34 + 35	361	Age = 93.55 − 360.43·R_{34} − 380.69·R_{35} + 1855.3·R_{34}·R_{35}	0.81	6.29
35 + 44	274	Age = 94.48 − 357.17·R_{35} − 379.38·R_{44} + 1783.88·R_{35}·R_{44}	0.82	5.87
34 + 45	325	Age = 104.00 − 423.39·R_{34} − 481.12·R_{45} + 2428.31·R_{34}·R_{45}	0.81	6.24
44 + 45	287	Age = 99.26 − 401.23·R_{44} − 387.75·R_{45} + 1874.37·R_{44}·R_{45}	0.83	6.07
35 + 45	310	Age = 100.23 − 417.87·R_{35} − 437.67·R_{45} + 2274.03·R_{35}·R_{45}	0.84	5.75
34 + 44	317	Age = 94.77 − 337.10·R_{34} − 372.85·R_{44} + 1578.33·R_{34}·R_{44}	0.79	6.38

Table 6
Regression equations using more than two lower premolar teeth.

Tooth	N	Equation	R^2	SE
34 + 35 + 44	249	Age = 95.39 − 289.54·R_{34} − 300.52·R_{35} − 150.15·R_{44} + 1728.73·R_{34}·R_{35}	0.84	5.68
34 + 35 + 45	269	Age = 103.47 − 180.05·R_{34} − 392.02·R_{35} − 335.77·R_{45} + 815.16·R_{34}·R_{35} + 1646.64·R_{35}·R_{45}	0.85	5.55
34 + 44 + 45	240	Age = 99.68 − 287.39·R_{34} − 362.58·R_{44} − 138.82·R_{45} + 1831.66·R_{34}·R_{44}	0.83	5.96
35 + 44 + 45	219	Age = 99.6 − 334.8·R_{35} − 373.8·R_{44} − 105.5·R_{45} + 2006.5·R_{35}·R_{44}	0.86	5.39
34 + 35 + 44 + 45	199	Age = 101.56 − 151.05·R_{34} − 312.68·R_{35} − 126.83·R_{44} − 256.07·R_{45} + 901.15·R_{34}·R_{35} + 1226.10·R_{35}·R_{45}	0.86	5.31

Table 7
Regression analysis predicting chronological age from all lower premolars.

	Estimate	St. error	t value	p
Intercept	101.56	3.24	31.35	<0.001
R_{34}	−151.05	54.88	−2.75	0.0065
R_{35}	−312.68	37.27	−8.39	<0.001
R_{44}	−126.83	24.96	−5.08	<0.001
R_{45}	−256.07	62.24	−4.11	<0.001
$R_{34} \times R_{35}$	901.15	450.20	2.00	0.0467
$R_{35} \times R_{45}$	1226.10	488.60	2.51	0.0129

Table 8
Number of premolars (%) for age cohorts.

Age class	Number of premolars					Total
	0	1	2	3	4	
–20	0.00	11.43	17.14	37.14	34.29	100
21–30	0.00	12.57	11.98	26.95	48.50	100
31–40	3.20	12.80	28.00	32.80	23.20	100
41–50	4.92	12.29	25.41	31.15	26.23	100
51–60	8.33	10.19	28.70	22.22	30.56	100
61–70	9.84	19.67	26.23	27.87	16.39	100
–71	31.58	31.58	21.05	5.26	10.53	100

indicated the absence of multicollinearity among the examined teeth [59,60].

When all lower premolars were used, the residual plot (Fig. 3, right panel) showed no obvious pattern, and a few observations appeared to be outliers (with errors greater than ten years). The observed versus predicted plot (Fig. 3, left panel) shows that the regression model fits the trend of the data reasonably well. Hence, both diagnostic plots support the chosen model.

As expected, the percentage of people without teeth increased with age. However, less than 10% of those individuals aged between 61 and 70 years had lost their premolars, and about 32% of subjects over 70 had no premolars (Table 8).

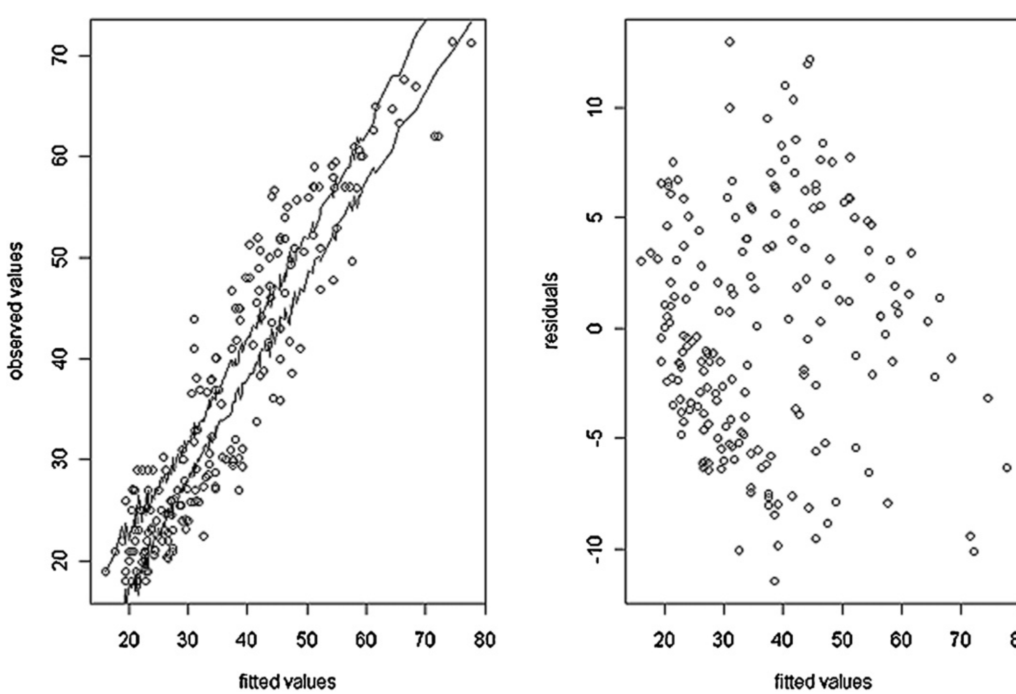

Fig. 3. Plot of observed against predicted values (left panel) and plot of residuals against fitted values (right panel) by regression model with all premolars (last equation in Table 7).

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4. Discussion

As Maples [63] stated, age estimation of skeletal remains is ultimately an art, not a precise science. Next to sex, age is an essential basic biological parameter which facilitates the identification of human remains in both forensic and archaeological contexts [64]. Due to the inherent challenges in aging and the need for better understanding of age estimates from adult skeletons, forensic anthropologists must constantly develop and test techniques for age estimation, mostly drawing on samples of known age at death. The method used and error range may be crucial in matches with missing persons.

In previous studies [1–6,39,55], analysis of the pulp/tooth area ratio in canines has been shown to provide reliable age-at-death estimates, and may help to narrow an estimate when used in combination with other morphological markers. The apposition of secondary dentine has also proven to be a robust age indicator and can be used as a univariate age indicator in forensic applications. This may assist in solving several problems associated with assessing age at death, such as observer subjectivity and taphonomic/preservation issues. In addition, these teeth are less readily damaged by direct heat or traumatic force, since they are covered by the soft tissue of the cheek; in addition, they are less likely than posterior teeth to suffer wear as a result of particular work, and are not as easily lost in dry skull material, as molars tend to be [51,52].

To examine the accuracy of this age indicator in other teeth, orthopantomographs were taken to make morphological measurements of the lower premolars. The major reason why it seemed so difficult to find suitable radiographs in the older age category was that at least one lower premolar should be present. A total of 31 orthopantomographs was thus discarded, because all lower premolars were absent or with pathologies. Further exclusion criteria, such as the presence of impacted teeth or teeth with vestibular radio-opaque fillings and crowns, pathological processes in the apical bone visible on the radiograph, or teeth with endodontic treatment, did not facilitate the selection procedure.

The mean values of the pulp/tooth area ratio by age group indicated a general inverse relationship between age and the ratio, the steepest reduction occurring in older subjects. This corroborated previous results with pulp and tooth measurements as age-related variables [47–49]. As in canines [1–6], ANCOVA analysis showed that sex did not affect the models in premolars. Murray et al. [46] investigated possible changes in pulp cell density, pulp area and dentinal thickness of all teeth with age. Analysis of the data according to patient's sex revealed no statistically significant differences between any of the measured variables (ANOVA, $P > 0.05$). Other anthropological indicators, such as the pubic symphysis [12,13], auricular surface of the ilium [15] and metamorphosis of sternal rib ends [14], depend on sex, which is not always easily diagnosed in archaeological material or in calcined remains and decomposed bodies which may include putrefied, mummified, saponified or burnt bodies, and all states of preservation, or lack of it, which severely alter physiognomy [8,39].

Interpreting the above tables and graphs, a different degree of accuracy can be observed. The accuracy of estimated ages from a single premolar turned out to be worse than that obtained from any two-premolar model which included that premolar. Although these results were slightly weaker than the coefficient of estimation when both lower premolars were included in the model, they still remained significant. Conversely, the differences were not significant when two-premolar models versus any model with more than two teeth were compared.

Obviously, there is a correlation between the pulp/tooth ratios of the lower premolars, but the values of standard errors of the parameter estimates are much lower than the estimated values (Table 7). The residuals do not indicate a trend in the distribution of the errors and the values of the VIFs showed the absence of collinearity among the examined measurements in teeth.

The mean prediction error (ME) of this technique was lower than 3.7 years in canines [1]. The accuracy achieved by analysing canines was better than that obtained with premolars, the values of which ranged between 4.34 and 6.02 years. The main source of error in OPG measurements seemed to be difficulties in identifying reference points on the radiographs when viewed on the monitor, and therefore in defining the line to be measured [65]. In 2000, Schulze et al. [66] investigated the precision and accuracy of measurements in digital panoramic radiographs, and reported that vertical measurements were less reproducible and less accurate than horizontal ones. Many key factors which may influence these results must be also taken into account, such as individual variability of tooth size, variations in patterns of secondary dentine apposition, differences in magnification of radiographs, and angles between X-ray beam and film. Several aspects, such as errors in exposure, projection angle, radiation dose, accuracy of patient positioning, and position of the tongue can distort the image quality of radiographs [66,67]. However, the results of this study showed a very high degree of intra-and inter-observer agreement, indicating high reproducibility of measurements.

Methods to assess internal validity vary in the amount of data used to estimate the model and the amount of data kept out to test the model. For example, with the split-half approach, the performance of the full model was underestimated, since only half the data was used to construct the model. The estimate was also very unstable, since half the sample was used for validation. This problem increased in cross-sectional studies. Hence, we validated our models using the bootstrap method, taking 2000 repetitions to estimate bootstrap R^2 (R^2_{boot}) as a measure of predictive accuracy of the model. In addition, a thorough theory has been developed to support bootstrapping as a universal validation technique [60,61].

Future research should aim at determining regression equations with higher accuracy by studying patterns of secondary dentine apposition in lower premolars and other teeth together, by means of peri-apical X-rays. The higher image quality of this technique will probably narrow the age estimation error and improve dental age estimation. Although OPGs are superior to smaller peri-apical films in their diagnostic examination, it is generally accepted that a radiographic image of a finite site found on an OPG is less clear than that shown by a peri-apical film [1,3,68]. The image quality of orthopantomograms has also been shown to depend to a great extent on the patient's age and sex. Gelbrich et al. [21] showed that image quality decreased with patients' increasing age. In the future, image analysis programs which can recognize pulp outlines in radiographic images will be very useful in minimizing human manual measurement of morphological parameters, and will probably reduce both inter- and intra-observer variability.

5. Conclusions

From the results of this study, it may be concluded that the use of Cameriere's age-related variables in lower premolars and the application of the new regression formulae on data obtained from orthopantomographs lead to accurate age estimates, if at least the selection criteria are respected and good quality orthopantomographs with clear radiological images are used. Future research modifying the presented technique, together with expected further improvements in peri-apical radiography by direct digital radiography (DDR), may provide an easy and optimized dental age estimation technique. The higher image quality of this technique will probably narrow age estimation error and improve dental age estimation. In addition, due to the fact that the rate of physiological secondary dentinal secretion is not constant throughout life [46], it would be of great interest to examine the patterns of secondary dentinal apposition according to age group, in order to check the accuracy of this age indicator in older individuals, in which the reduced size of the root canal has also been associated with calcification-related diseases such as arthritis, gout, kidney stones, gallstones, atherosclerosis and hypertension [69].

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