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TECHNICAL NOTE

ODONTOLOGY; ANTHROPOLOGY

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Age-at-Death Estimation by Pulp/Tooth Area Ratio in Canines: Study of a 20th-Century Mexican Sample of Prisoners to Test Cameriere's Method

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. Cameriere et al. studied the pulp/tooth area ratio by peri-apical X-ray images of the canines, to observe the apposition of secondary dentine. The present study examines the application of this technique in a Mexican identified sample coming from the Department of Physical Anthropology of the INAH, at Mexico City. The main aim of this work is to test the reliability of this method in a skeletal sample of a specific population, different from the samples used for its development. The obtained regression model explained 96.2% of total variance (R^2 = 0.962) with a standard error of estimate of 1.909 and a standard deviation of 1.947. These results demonstrate great reliability and that the age/secondary dentine relationship is not variable in this specific population.

KEYWORDS: forensic science, forensic odontology, age estimation, secondary dentine, pulp/tooth area, linear regression

Accurate age-at-death estimation is an important component of both forensic and archaeological studies (1). However, the aims and human material of these disciplines are different. In bioarchaeology and other population-focused studies (2,3), age estimation is essential for inferring derived demographic parameters such as growth rates, fertility schedules, and life expectancies (4–6). In forensic contexts, where both accuracy and reliability are required (7), this parameter is fundamental for identification of deceased victims and living persons, in connection with crimes, mass disasters, and migration movements (4,8).

Age-at-death assessment faces biological and methodological problems because of its great variation, both within and between populations. As skeletons delivered for examination are often incomplete, the possibility of identifying dead bodies with respect to parameters such as stature, age, sex, and individual traits by means of classical osteological methods alone (employed in both forensic medicine and anthropology) is usually limited. For this purpose, various parts of the body are examined and several techniques are employed (9–12). However, there is still much confusion about the standardization of methods, procedures, and statistical parameters (2,13). It is necessary to take into account many factors such as the context, costs, time, and equipment required, the examiner's qualifications and the various states of preservation of bodies. Choice also partly depends on how accurate the age diagnosis needs to be in the case in question (14).

With this move toward improving existing age-at-death estimation techniques, more accurate age-related changes in teeth have recently been re-examined as an alternative to skeletally based techniques (15–17). Age estimations from teeth are frequently used because teeth may be preserved long after all other tissues have disappeared (11,12,18). They are still preserved even when most of the bones have been destroyed, mutilated, or affected by other taphonomic agents (11).

Forensic odontologists have a number of such methods available for use in their discipline (19). The search for optimal age estimation procedures has continued over the years until the present day (2). Using the degree of aspartic acid racemization in dentine (20) or the determination of cementum annulation from tooth-root cross-sections (21), the error bounds for age estimates within 95% confidence intervals did not exceed 2.5 years (2,20). However, these dental age estimation methods most frequently referred to require extraction and, in some of them, preparation of microscopic sections of at least one tooth. These methods are time consuming and expensive, and a destructive approach may not be acceptable for ethical, religious, cultural, or scientific reasons (20,22–24).

Additional problems can be the reference samples on which the various methods have been developed, which are also of fundamental concern as the methodologies become too specific or dependent on the demographical and chronological profile of the series. Correct age estimation must consider this unavoidable limit to define with precision the limits of results provided (2,8,18).

For adults, the literature offers various possibilities, such as morphological, biochemical, and radiological techniques (2,25–27).

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Several very fine and relatively accurate dental methods are now available (19). One of them is apposition of secondary dentine which represents an ongoing, regular process, only modified by extensive caries or tooth wear (28,29). 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 (30,31). The mean rate of increasing dentinal thickness has been found to be 6.5 μm per year for the crown and 10 μm per year for the root. The effect of continuous dentine deposition is the progressive increase in dentinal thickness by 0.45 mm (17.1%) and 0.60 mm (24.3%) in the crown and root areas, respectively. The pattern of secondary dentine deposition varies with tooth type. As regards sex, no statistically significant differences are observed (32).

In 1925, Bodecker (31) ascertained that the apposition of secondary dentine was correlated to chronological age. Detailed studies of the pattern and rate of secondary dentine apposition in maxillary anterior teeth were performed by Philippas and Applebaum (28,29) but without the aim of estimating age at death. Secondary dentine deposition was included in the method pioneered by Gustafson (30), in which dentine transparency and secondary dentine values showed the highest correlation with age.

Secondary dentine has been studied by several methods: examples are sectioning and X-rays (33–35). However, radiographic analysis does not require grinding or sectioning of teeth, and therefore, it does not destroy any tooth substance.

Kvaal et al. (36) and Paewinsky et al. (34) obtained reproducible measurements of the dental pulp chamber and great correlation between the individual age and the dimensions of the pulp chambers.

Since 2004, Cameriere et al. (37) have published five 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, 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 examine. Currently, the apposition of secondary dentine by peri-apical X-ray may provide a useful tool in age estimation in adults (2,37).

Reliability is the degree to which a method produces the same results when it is used at different times, either by multiple observers or by the same observer. It can be tested by conducting inter-observer or intra-observer variation studies to determine error rates. Low inter-observer variation (or error) indicates high reliability (38).

The present study examines the reliability of Cameriere's method (39,40) in a sample of skeletal human remains from Mexico. This osteological collection belongs to the Department of Physical Anthropology of the INAH (Instituto Nacional de Antropología e Historia), in Mexico City. The importance of this collection lies in the characteristics of the individuals composing the sample: 85 Mexican male subjects of known age and cause of death who were detained in the National Penitentiary of Lecumberri (Mexico City) between 1901 and 1914 (Fig. 1). The most common cause of death was infections of various kinds (41).

The main aim of this work is to test the accuracy of the pulp/tooth area ratio by peri-apical X-ray images as an age indicator, using identified sample from a 20th-century Mexican skeletal collection. The method of Cameriere et al. (35) is the first to focus on analysis of this ratio to estimate age. Nevertheless, it is necessary to ascertain whether the original formulas predicted age accurately or if population-specific equation improved age assessment. As Cunha et al. noted (2), sometimes, the best method is that which has been tested by many on several and different populations, which is suitable for a specific forensic context, practical, quick, and not expensive.

FIG. 1—*Inaugurated by the president Porfirio Díaz and known in popular culture as the "Black Palace of Lecumberri," it was used as a penitentiary from 1900 to 1976. The building was decommissioned as a prison in 1976 and was turned over to the National Archive in 1980.*

Materials and Methods

Peri-apical X-rays of 103 canines from 85 male subjects aged between 18 and 60 years were analyzed (Table 1). Teeth were selected from the osteological collection, mentioned above, formed of the 122 skulls with mandibles of prisoners, aged between 18 and 75, who had died in the Lecumberri Penitentiary before completing their sentence. Owing to the parameters selected (presence of canine), it was possible to study only the 85 male subjects indicated before, excluding the individuals older than 60 years because of the absence of canines.

There was a clause in the prison's regulation, which stated that the bodies of all prisoners dying before the end of their sentence should be autopsied and their skulls retained. It was because of this rule that a collection of 130 male skulls was made (Fig. 2). Of the 130 skulls that originally formed the collection, eight were lost (41). It is currently composed of 122 complete skulls with mandibles, collected between 1901 and 1914. The prisoners' skulls were emaciated and cleaned after autopsy, so that the samples are generally exceptionally well preserved (Fig. 3). This condition facilitated radiographic analysis of teeth.

Of these 122 skulls, 119 had tags giving the personal details of each subject (Fig. 4): prisoner's name, date and place of birth, occupation, age on entering jail, marital status, religion, ethnicity, educational attainment, crime, sentence, date of admission to

TABLE 1—*Age distribution of Mexican sample of Lecumberri Penitentiary.*

Age	No.	%
18–20	1	1.18
21–35	59	69.4
36–55	21	24.7
56–60	4	4.7
Total	85	100

prison, date of completion of sentence, and date and cause of death. All the skulls belong to adult male subjects, divided into the following age ranges, as ranked by Hooton (1).

Subadult (18–20 years)	3 (2.4%)
Young Adults (21–35 years)	63 (50.0%)
Average-age Adults (36–55 years)	50 (39.7%)
Old Adults (56–75 years)	10 (7.9%)

FIG. 2—*Skull number 11 of the osteological collection of Lecumberri Penitentiary. Professor Nicolás León created this collection between 1901 and 1914. A successive numeration from 1 to 130 was assigned to the skulls in the Department of Physical Anthropology of the INAH (1983) (photo R. Enríquez, DAF-INAH).*

FIG. 3—*Skull number 91 has two reference numbers, one on the left parietal bone and the other on the mandible. To create an internal catalog of the skulls, a new label was assigned to them, from Da-2-530 to Da-2-653 and from Db-2-530 to Db-2-653. Da was the label selected to define normal skulls and Db to define pathological ones (photo R. Enríquez, DAF-INAH).*

Hooton (1) had taken his data before four of these 126 skulls had been lost (41). As the age appearing in the personal files is that at the moment of imprisonment, it was necessary to add the number of years they stayed in prison until they died. The highest number of deaths occurred between 1908 and 1911, peaking in 1909. Table 2 lists the numbers and dates of deaths.

Many of the detainees had been born in the central-southern parts of the Mexican Republic (Table 3), and many of them gave their occupations as day laborers (Table 4). A high percentage of them could not read, 30 could read and write, 18 could only read but not write, four had been to elementary school, and one had gone to high school. None of the others had any data regarding schooling. The crimes for which they had been sent to prison were mainly homicide, followed by assault and battery, or both (Table 5). Living conditions in the penitentiary were so poor that many prisoners died within a few months of entering. The average length of stay ranged from 5 months to 2 years.

Finally, causes of death were mainly infections. The most common causes were gastrointestinal diseases, acute or chronic, and tuberculosis, which killed at least one-fifth of the detainees every year. Cardiovascular diseases represented the main cause of death of the younger individuals (Table 6).

The method applied here to estimate age at death followed that previously published by Cameriere et al. (39,40). Only individuals with at least one canine were selected and, if both teeth were present, bilateral canines were studied. In addition, canines without

FIG. 4—*Personal tag of one prisoner. Doctor Nicolás León obtained the personal data of each detainee from the penitentiary authorities (photo R. Enríquez, DAF-INAH).*

TABLE 2—*Distribution of mortality in Lecumberri Penitentiary (modified from [41]).*

Year	No	%
1901	1	4.2
1902	6	5.1
1903	2	1.7
1904	3	2.5
1905	5	4.2
1906	11	8.4
1907	13	8.4
1908	20	16.8
1909	21	17.6
1910	17	14.3
1911	14	11.8
1912	5	4.2
1914	1	0.8
Total	119	100

TABLE 3—*Place of birth of the criminals detained in Lecumberri Penitentiary (modified from [41]).*

Place	No.	%
Mexico City	74	60.7
Guanajuato	20	16.4
Estado de Mexico	10	8.2
Querétaro	5	4.1
Puebla	4	3.3
Michoacan	3	2.5
Hidalgo	2	1.6
Chiapas	1	0.8
Jalisco	1	0.8
Moreos	1	0.8
Belice	1	0.8
Total	122	100

TABLE 4—*Main occupation of the detainees of the Lecumberri Penitentiary (modified from [41]).*

Occupation	No.	%
Various jobs	62	50.8
Day laborer	32	26.2
Craftsman	13	10.7
Domestic	3	2.5
Dealer	3	2.5
Peasant	1	0.8
Employee	1	0.8
Unknown	7	5.7
Total	122	100

TABLE 5—*Type of crime of the criminals of the Lecumberri Penitentiary (modified from [41]).*

Type of Crime	No.	%
Homicide	59	48.4
Robbery	45	36.9
Physical damage	6	4.9
Homicide and robbery	5	4.1
Rape	2	1.6
Homicide and damage	2	1.6
Failed homicide	1	0.8
Kidnapping	1	0.8
Robbery and damage	1	0.8
Total	122	100

endodontic treatment or any prosthetic fittings and canines without dental fillings and significant pathologies, such as wear, caries, and calculus, were chosen. The selected nomenclature to classify the canines is that proposed by the International Dental Federation (IDF).

Standard X-ray generating equipment (Toshiba® IRYOYO-HIN—Torex 20) for intraoral radiography was used, manufactured by Picker X-ray Corporation Waite Manufacturing Division, Inc. and International Association of Machinists and it was the property of the INAH (Instituto Nacional de Antropología e Historia). It is a mobile X-ray machine easy to use and simple to operate. The teeth were X-rayed in a designated area (controlled area) both isolated and *in situ*, depending on the state of conservation of each skull and on the possibility of extracting them without damage. Normal intraoral and peri-apical X-rays were taken directly by manual radiological techniques with an exposure of 40 mA at 70 kVp. Both paralleling and bisected angle techniques were utilized, depending on the situation of each canine inside the skull and mandible. Direct-action or nonscreen film (Kodak® INSIGHT dental film – 24 × 40 mm) was used as image receptor. This type of film is often used for intraoral radiography where the need for excellent image quality and fine anatomical detail is of great importance (31,42). Manual processing (developing and fixing) of radiographic images was carried out in a darkroom including a series of solution tanks, thermometer, timer, and film. X-ray was then digitized using a scanner (HP G4050 scanjet, at least 300 dpi resolution) (Fig. 5), and images were stored digitally. The images were saved in JPG format for further image analysis (Fig. 6).

Following Cameriere et al. (39,40), the radiographic 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 for each pulp outline were identified and connected with the line tool, also on the Draw Toolbox, and the area of both tooth and pulp was ascertained (37). All measurements were made without prior information about personal data of the subjects.

Age was estimated by applying the three equations proposed by Cameriere et al. (40) for upper canine (1), for lower canine (2), and for both canines (3):

Upper canine: Age = 99.937 – 532.775 (RAu);
lower canine: Age = 89.456 – 461.873 (RAl);
both canines: Age = 114.624 – 431.183 (RAu) – 456.692 (RAl) + 1798.377 (RAu)(RAl).

TABLE 6—*Main cause of death of the prisoners in Lecumberri Penitentiary (modified from [41]).*

Year	Gastrointestinal diseases	%	Tuberculosis	%	Epatic Disease	%	Cardiac Disease	%	Renal Disease	%	Syphilis	%	Rheumatic Disease	%	Lung Disease	%	Violence	%	Other	%	Total
1901	1	20			1	20											2	40	1	20	5
1902	1	16	3	50	1	16			1	16											6
1903	1	50																	1	50	2
1904									1	33							1	33	1	33	3
1905	2	40	2	40															1	20	5
1906	2	20	3	30			2	20			1	10			1	10			1	10	10
1907	3	30	1	10	1	10	4	40			1	10									10
1908	2	10	3	15	3	15	3	15			1	5	4	20	1	5	1	5	2	10	20
1909	5	23	4	19			7	33	1	5			2	10					2	10	21
1910	6	35	5	29	1	6	3	17											2	12	17
1911	5	35	6	42			2	14											1	7	14
1912	1	20	3	60													1	20			5
1914							1	10													1
1915							2	66					1								3
Total	29	24	30	25	7	6	24	20	3	2	3	2	7	6	2	2	5	4	12	10	122

FIG. 5—*The scanner HP G4050 scanjet is the property of the Laboratory of Anthropology of University of Granada (Spain). This equipment showed high pixel reproducibility and high image quality.*

FIG. 6—*Peri-apical X-rays of the lower left canine of skull Da-2-551. Observe the great contrast and the low image distortion that enable the analysis of the pulp chamber.*

RAu and RAl, respectively, represent the pulp/area ratio in upper and lower canines.

Statistical Analysis

For each skull in the Mexican sample, dental maturity was evaluated by measuring the pulp/tooth area ratio on upper (RAu) and lower canines (RAl). All measurements were carried out by the same observer with ample experience of this method. To test intra-observer reproducibility, all peri-apical X-rays were re-examined after an interval of 2 weeks. Intra-observer reproducibility of measurements was studied by Pearson's concordance correlation coefficient.

The accuracy of dental age estimation was defined as how closely chronological age could be predicted, measured as the difference between chronological age and dental age. To evaluate the accuracy of an age estimation method, the age of each subject (Age_i, $i = 1, \ldots, n$) was compared with estimated ages ($Age_{est,i}$, $i = 1, \ldots, n$) by means of the mean prediction error:

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

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

$$\delta_i = Age_i - Age_{est,i}, \quad i = 1, \ldots, n.$$

A positive value of δ_i indicates underestimation and a negative value overestimation. Data were analyzed by Statistical Package for the Social Sciences (SPSS) 15.0 software. The significance threshold was set at 5%.

Results

In the above sample, there were no statistically significant intra-observer differences between the paired sets of measurements carried out on the re-examined peri-apical X-rays ($p < 0.01$). Very good agreement was found between intra-observer measurements (Table 7).

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 (Table 8).

The regression model explained 96.2% of total variance ($R^2 = 0.962$) with a standard error of estimate of 1.909 (Table 9). The standard deviation is 1.947.

The residual plot (Fig. 7) shows no obvious pattern, and the data points did not plot outside the expected boundaries. The regression line (Fig. 8) shows that the regression model fits the data trends very well. Hence, both diagnostic plots support the chosen model.

Discussion

Age estimation is essential in both forensic and anthropological sciences. While sex assessment in adults generally does not present any difficulty, the estimation of age at death of adults remains

TABLE 7—*Pearson's correlation between measurements of the same observer (intra-observer error).*

		Dentalage 1	Dentalage 2
Dentalage 1	Pearson's correlation	1	0.998
	Sig. (bilateral)		0.000
	N	85	85
Dentalage 2	Pearson's correlation	0.998	1
	Sig. (bilateral)	0.000	
	N	85	85

TABLE 8—*Correlation coefficients between predictive morphological variables and real age.*

		Realage	Ptar43	Ptar23	Ptar13	Ptar33
Realage	Pearson's correlation	1	−0.947	−0.856	−0.964	−0.940
	Sig. (bilateral)		0.000	0.000	0.000	0.000
	N	85	19	25	31	22

Ptar, pulp/tooth area ratio.

TABLE 9—R^2 *of the linear regression.*

Model	*R*	R^2	Corrected R^2	Standard Error of the Estimate
1	0.981	0.962	0.962	1.909

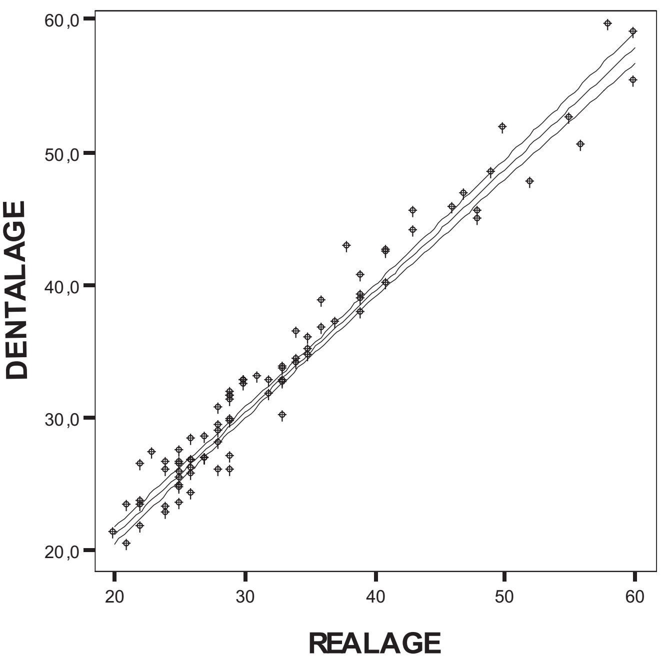

FIG.7—*Plots of data and regression line.*

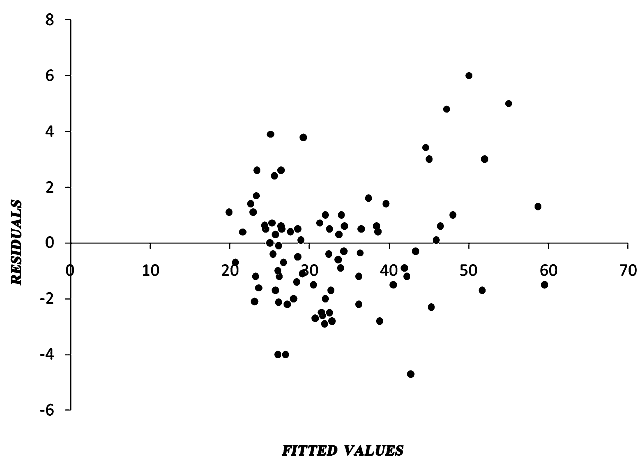

FIG. 8—*Residuals against fitted values using a simple linear regression model to describe age as function of pulp/tooth ratio.*

difficult and is subject to many types of error which can be prejudicial for forensic and bioarchaeological analysis (2).

Reliable aging techniques are available for many different anatomical regions of the human skeleton. However, elements commonly used for establishing age at death, such as the pubic symphysis, the auricular surface of the ilium, and the fourth rib, are sometimes missing, and body parts may be frequently damaged (7,43,44). 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 statistical methods used to develop age estimation methods (15–17,23,43,45). A large number of methods and variants have been developed, and this number is constantly increasing, reflecting the difficulty of diagnosis and dissatisfaction with available techniques. The key criterion for assessing the value of a method is the error of estimate (confidence interval, tolerance limits, etc.), that is, a measure of dispersion in the reference sample (24,46).

Study of dental characteristics is often the sole resource for age estimation, because of the relatively small error of estimation (22,26,46). However, several dental methods imply partial destruction of the tooth and seem to be very much complicated and expensive to be applied on a large scale (6,19,21,25,47).

The apposition of secondary dentine is a frequently used method of ascertaining age (2). Preliminary results by Cameriere et al. (48), applying orthopantomography, revealed a linear regression between age and pulp/tooth ratio of upper canines.

This new paper was organized following the above preliminary study but with peri-apical X-rays instead of orthopantomography. A more accurate radiographic image was produced by applying both paralleling and bisecting angle techniques. The paralleling technique provided low image distortion. The root and the crown of the canine were well shown enabling the study of tooth and pulp chamber. The resulting digitalized images showed greater contrast and had less chance of overlap. In addition, when extracted teeth were examined, peri-apical images of single canines made data analysis even easier.

Dental age was evaluated by measuring the pulp/tooth area ratio according to labio-lingual peri-apical X-rays on upper and lower canines. Measurements of pulp and tooth areas on digital peri-apical images of canines yielded more reliable and reproducible data than those achieved by orthopantomograms. The results did not reveal any statistically significant differences between Mexican and European samples and therefore suggested the possibility of applying the same regression equations to both populations.

The results of this study demonstrate the great accuracy of the pulp/tooth area ratio as an indicator of age at death. With this simple and accurate method, the error of age estimation (about 1.9 years) is much lower than that of most anthropological methods, which gives an error of more than 5 years. Also, it confirms the importance of studying anthropological frameworks for identification, which lead to reliable methods and allow for both quick and economic procedures.

Conclusions

In forensic contexts, accurate estimates of age at death are crucial in efforts positively to identify partially or completely decomposed individuals by narrowing down the missing persons list of potential victims. In this context, judicial requirements, time, and the accuracy of the age range are basic factors in developing identification procedures. In addition, as a result of the global increase in migration movements in recent years, there is a growing demand for age estimates of living persons. Furthermore, during U.S. federal legal proceedings, the reporting of statistic error in forensic science applications is necessary in cases of legal admissibility according to the Daubert standard (49). This means that forensic techniques must be accurate; they must lead to very precise results with ranges that will correctly classify an individual at least 95% of the time.

The origin of errors may frequently be found in variations both between and within studied populations, in observer variations and in the methods employed. Thanks to recent and relevant advances of physical anthropology, old methods have been improved, and new techniques have been recently proposed (25–27). However, because of genetic and nongenetic factors, when applied to independent populations of known age at death, many methods prove less reliable than the results obtained from the samples used to elaborate those methods (2,4,12).

The results of this study clearly indicate the following:

- Study of the apposition of secondary dentine by peri-apical X-rays (39,40) can be used to generate forensically significant age-at-death estimates, both in living and deceased persons.
- Owing to its relatively small error of estimation, the analysis of morphological parameters of teeth on peri-apical X-rays of adult humans is more reliable than most other methods for age estimation. Computer-assisted image analysis avoids the bias inherent in observer subjectivity and improves the reliability and statistical analysis of data.
- This application to a Mexican sample demonstrates that the same regression equations can be used without any statistically significant differences between individuals from a population of diverse origin.
- Study of the apposition of secondary dentine (39,40) is easy to apply, is not destructive, is easier to check scientifically, is less dependent on technical ability than other techniques, and does not require highly specialized equipment.
- The method (39,40) also can reveal its usefulness in both bio-archaeological and forensic anthropological settings, suggesting the use of the pulp/tooth ratio also for paleodemographic studies. Paleodemography examines the structure and dynamics of premodern populations (50). As its analyses are mainly based on expected longevity, infantile mortality, and mortality rates, it can benefit from any method that is able to determine age at death with greater precision and accuracy (3,5,14).
- This method (39,40) should be always used in conjunction with another to provide the most precise estimation of age possible.

Future research should aim at reducing standard errors of age estimation, at studying the effect of different races and cultures on model parameters, and at investigating the use of several teeth together, to improve dental age estimation.

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