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Differences in Morphological Age-Related Dental Changes Depending on Postmortem Interval

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ABSTRACT: Morphological methods for age estimation from teeth have been developed and applied to samples without taking the postmortem interval into consideration. We studied differences in morphological age-related changes between fresh extracted teeth and teeth from human skeletal remains in order to develop appropriate dental age estimation methods according to the time after death. Forty-three permanent teeth from dental patients were compared to 37 teeth obtained from human skeletal remains with a postmortem interval from 21 to 37 years. Morphological age-related changes were investigated by measuring variables on intact and half-sectioned teeth. A new computer assisted image analysis procedure to avoid subjectivity was developed to measure variables in sectioned specimens. Dental color, translucency length, attrition, cementum apposition, and secondary dentin showed higher values in teeth from human skeletal remains than in fresh extracted teeth. Variables obtained by morphometric analysis of computer-generated images (tooth length, tooth width, root length, and root area) showed higher values in fresh extracted teeth than in teeth from skeletal remains. The postmortem interval affects age-related morphological changes, and therefore different methods should be used for teeth of unknown postmortem interval.

KEYWORDS: forensic science, aging, morphological dental changes, postmortem interval

The identification of human skeletal remains is a recurrent problem in both forensic and anthropological contexts. Mineralized tissues (bone and teeth) have been used as the human tissues most resistent to degradation and putrefaction. In some cases, identification, and especially age estimation, have been undertaken without regard for the period of time after death. As a result, identical age estimation methods have been used for both corpses and human skeletal remains.

Since Gustafson introduced the first statistical method for age assessment in 1950 (1), several similar morphological methods have been proposed, which to a varying extent have improved the accuracy of age estimates (2–6). Moreover, computer-based techniques and computer-assisted image analysis have been incorporated to the methods used to measure morphological changes in teeth (7,8) in order to avoid the bias inherent in observer subjectivity. Nevertheless, to our knowledge, morphological methods for age estimation in the study of teeth have been developed and applied to samples without regard for the postmortem interval. A new approach was suggested by Solheim (6), who proposed multiple regression analyses to estimate dental age for each type of tooth, and different formulas for dental age estimation that were assumed to be valid regardless of the individual's sex and the color of the tooth. Of particular interest are the differences in tooth color depending on the condition of the body, which Solheim was the first to notice and describe (6).

This raises interesting questions: does the postmortem interval influence the age-related morphological changes classically measured as the best markers for dental age estimation? If so, should different dental age estimation methods be developed and used according to the time after death? The aim of the present study was to investigate differences in morphological age-related changes between fresh extracted teeth and teeth from human skeletal remains. We developed a new computer-assisted image analysis method for studying morphological changes, and assessed the reproducibility and efficiency of this method.

Materials and Methods

Human Teeth Sampling

Two different samples were studied. Healthy erupted human permanent teeth (Group I), extracted for valid clinical reasons (periodontal disease, malocclusion, or orthodontic treatment), were obtained from the public Oral Health Service in Santa Fe (Granada, Spain) and from private dental clinics. Forty-three permanent teeth, molars excluded, were taken from patients (19 women and 24 men) from 25 to 79 years of age. The other population group (Group II) was composed of 37 healthy erupted permanent teeth obtained from human skeletal remains (19 women and 18 men; age 22 to 82 years). The corpses had been buried at the local cemetery in Granada during a postmortem interval ranging from 21 to 37 years. Protocols to collect samples from human subjects were approved by the corresponding Ethics Committee for Research Involving Human Subjects, and the study was conducted in accordance with the ethical standards laid down by the Declaration of Helsinki.

Morphological Analysis Procedure

After extraction or removal from skeletal remains, teeth were kept in saline solution and sent to the laboratory for analysis. Teeth were cleaned of blood with distilled water and any attached soft tis-

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sue was carefully removed with a scalpel. In each tooth, morphological age-related changes were studied as follows. First, parameters in intact teeth were measured, including dental color, total length of the tooth and root, length of translucent dentin, periodontal recession, attrition, and root surface roughness. After the study of intact teeth, samples were sectioned with a rotating grinding stone following the procedure described by Solheim (9). The parameters measured in sectioned teeth were dental attrition, amount of secondary dentin, cementum apposition, and root translucency. Finally, the same sectioned specimens were studied with a new computer-assisted image analysis procedure.

Measurement of Parameters in Intact Teeth—Root dentin color was estimated, after grinding approximately 0.5 mm into the dentin, by comparison with a dental shade guide (Kerascope, Ivoclar®), as proposed by Solheim (10). Total tooth and root length were measured with a pair of vernier callipers. Transparent root dentin was measured from the apex of the root to the borderline between transparent and opaque dentin in front of a standard light source (3,11). As an expression of the loss of periodontal attachment, the distance from the cementum-enamel junction to the most gingival periodontal fibers attached to the root surface was measured in fresh extracted teeth (12). Periodontal recession was also estimated in teeth samples from human skeletal remains by measuring the distance between the cementum-enamel junction and the alveolar bone. The mesial, vestibular, lingual, and distal surfaces were measured with vernier callipers, and the mean was calculated. The total surface area of attrition was measured directly in a stereomicroscope, using a millimeter grid on transparent film (13). After careful removal of the remaining periodontal fibers, the juxtaapical root surface was studied in a stereomicroscope under low magnification and scored according to Solheim and Kvaal (14).

Measurement of Parameters in Sectioned Teeth—Dental attrition, amount of secondary dentin, and cementum apposition were assessed in sectioned teeth using a stereomicroscope according to the scoring system of Johanson (4). Root translucency was scored according to the Dalitz system (2).

Morphometric Analysis by Computer-Assisted Image Generation—Sectioned teeth (half-tooth) preparations were inlaid into black plasticine to avoid light artifacts during the image productions. Under identical lighting conditions, images of sectioned teeth were captured by a TV camera and digitalized at 768 × 576 pixels, 256 gray level format, using Matrox Intellicam Interactive (version 2.0) software (Matrox Electronic System Ltd., Quebec, Canada). Digitalized images were coded and stored in monochrome in the hard disk of a PC-based work station. Morphometric analysis was performed with Visilog 5 software (Noesis S.A., Quebec, Canada). A program for the image analysis process was developed. Automatic analysis was done as follows: the first step was calibration to convert pixel units to metric units. After that, pulp (*p*), root (*r*) and translucency areas (*t*) were delimited manually to obtain initial binary images. The automatic process excludes any external participation except the manual delimitation of the initial binary images. Four automatic binary images were obtained: Image 1: total tooth; Image 2: root of the tooth, consisting of structures in common from the total area of the tooth (Image 1) and the root area delimitated manually (*r*); Image 3: crown of the tooth, obtained by subtracting root area (Image 2) from total tooth area (Image 1); Image 4: root translucency, comprising structures in common in the total area of the tooth (Image 1) and the translucency area delimitated manually (*t*). A sequence of the images obtained by the new method is shown in Fig. 1. From these images the program automatically obtained the length and width (mm), and area (mm^2) of the total tooth, root, crown, pulp, and root translucency of each sample.

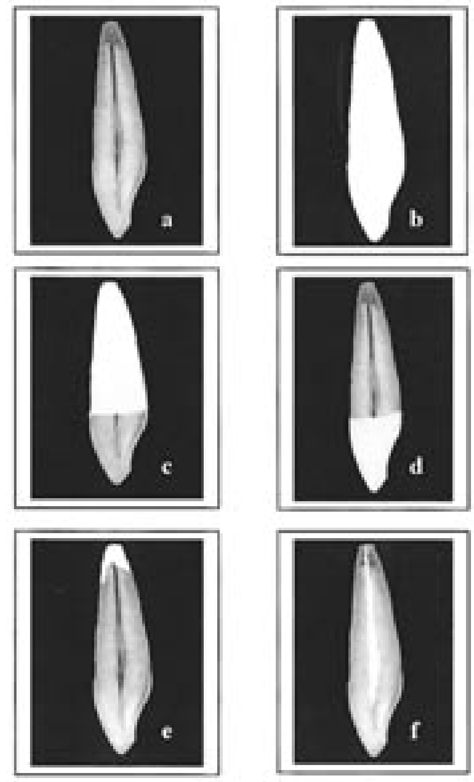

FIG. 1—*Procedure for image production by computer-assisted image analysis.*

Statistical Analysis

Data were exported to an Excel spread sheet and statistical analyses were done using the SPSS version 8.0 program for PCs. As an indicator of reproducibility, intraclass correlation coefficients (ICC) and their confidence intervals were calculated for duplicate analyses of the same sample done by the same observer (intraobserver assay) but at two different moments.

Differences and statistical comparisons were calculated to study the influence of sex and type of tooth for each variable by analysis of variance (ANOVA), as appropriate. Linear regression analysis was used to determine the presence of correlation between age and other variables. Differences between groups of samples were studied by one-way analysis of variance.

Results and Discussion

Current methods of estimating chronological age at death include the study of morphological changes in teeth (1–8, 10–14). It has been assumed that these changes are not affected by any external factors (such as putrefaction or mineralized tissue decay), therefore, these methods are applied to both fresh extracted teeth and teeth from human skeletal remains. There is evidence that some individual factors (such as sex or type of tooth) should be taken into account when the age of the corpse is diagnosed by estimating dental age (6). In addition, changes in tooth color have been observed when human populations of different postmortem intervals are studied (6,10). These previous studies suggest that a different scientific approach for dental age estimation is necessary when dental samples from human remains of unknown postmortem interval are evaluated.

To avoid any subjectivity in the procedure, we developed a new computer-assisted semi-automatic program to analyze the changes in teeth with aging. Intraobserver reproducibility of the measurements with the new computer-assisted image analysis method was evaluated using the ICC and confidence intervals, with excellent results (ICC close to 1) for all variables (results not shown). Once the system is set up and the conditions of the determinations are established, measurements of length, width, and area of dental structures are obtained automatically. This new computer-assisted image analysis method, presented for the first time in this paper, can be considered a reliable complement to classical methods used to study morphological dental age-related changes, thanks to the greater objectivity of the measurements. On the other hand, this method is easy to perform on any PC or Macintosh computer.

Table 1 presents the descriptive analyses, significant comparisons of variables, and noteworthy differences between types of sample (fresh extracted teeth versus teeth obtained from human remains). Significant differences in variables between the two sample groups were independent of the type of tooth and sex. Dental color, translucency length, attrition, cementum apposition, and secondary dentin show higher values in teeth from human skeletal remains than in fresh extracted teeth (Table 1). The causes of changes in tooth color after death have already been explained (6,10). Modification in translucency and secondary dentin could be the result of postmortem changes in chemical (mineral) composition that might happen after death. Nevertheless, more research should be done to account for the postmortem changes in attrition and cementum apposition. On the other hand, periodontal recession was greater in samples from extracted teeth than in human remains. This result may reflect the differences in the methods we used depending on the source of the sample (see Materials and Methods). In addition, the variables found to be significantly different with computer-assisted morphometric image analysis (tooth length, tooth width, root length, and root area) showed higher values in fresh extracted teeth than in teeth obtained from skeletal remains. These results may be explainable on the basis of postmortem biochemical changes such as the loss of tissue water and organic dentin matrix.

Once it is known that some variables are affected by the postmortem interval, it seems important to analyze the differences in these variables according to individual factors such as sex, type of tooth, and age. Table 2 summarizes the descriptive statistics for the variables affected by the postmortem interval in both sample groups according to sex. For the group of fresh extracted teeth, significant differences were found between men and women for all

TABLE 1—*Descriptive statistics and significant comparisons according to sample source.*

Variable	Group I*	Group II*	*F* exp	d.f.	*P*	Remarks
Color†	2.9 ± 0.6	4.6 ± 0.5	177.3	(1;74)	<0.001	Group II > Group I
Translucency length†	6.5 ± 2.7	9.1 ± 3.5	11.6	(1;74)	<0.001	Group II > Group I
Periodontal recession†	4.6 ± 1.6	3.5 ± 1.5	4.5	(1;74)	<0.04	Group I > Group II
Attrition†	10.9 ± 11.6	16.3 ± 18.4	3.9	(1;74)	<0.053	Group II > Group I
Cementum apposition‡	2.3 ± 0.9	3.5 ± 1.3	21.00	(1;74)	<0.001	Group II > Group I
Secondary dentin‡	3.3 ± 1.3	4.0 ± 1.3	6.75	(1;74)	<0.011	Group II > Group I
Tooth length§	23.6 ± 3.5	21.9 ± 2.0	11.3	(1;74)	<0.001	Group I > Group II
Tooth width§	8.9 ± 1.5	8.1 ± 1.0	16.9	(1;74)	<0.001	Group I > Group II
Root length§	16.6 ± 2.5	15.7 ± 1.6	10.2	(1;74)	<0.002	Group I > Group II
Root area§	90.3 ± 26.1	74.9 ± 13.6	30.6	(1;74)	<0.001	Group I > Group II

* Values represent mean ± standard deviation for Group I (fresh extracted teeth; $n = 43$) and Group II (teeth obtained from human skeletal remains; $n = 37$) for all variables except periodontal recession (Group I, $n = 26$; Group II, $n = 21$).
† Parameters in intact teeth.
‡ Parameters in sectioned teeth.
§ Parameters by computer-assisted image analysis.

TABLE 2—*Descriptive statistics and significant comparisons according to sample source and sex.*

	Group I*		Group II*	
Variable	Men ($n = 24$)	Women ($n = 19$)	Men ($n = 18$)	Women ($n = 19$)
Color	3.1 ± 0.5	2.4 ± 0.5†	4.7 ± 0.5	4.5 ± 0.6
Translucency length	7.2 ± 2.6	5.5 ± 2.6§	9.8 ± 3.0	8.4 ± 3.6
Periodontal recession	5.0 ± 1.6	3.6 ± 2.2§	4.3 ± 1.4	3.1 ± 1.4
Attrition	14.2 ± 13.1	6.5 ± 8.0	14.8 ± 8.1	17.7 ± 24.8
Cementum apposition	2.5 ± 0.8	2.1 ± 0.9	3.7 ± 1.4	3.4 ± 1.2
Secondary dentin	3.7 ± 1.4	2.7 ± 1.1‡	4.3 ± 1.4	3.8 ± 1.4
Tooth length	24.3 ± 3.5	22.8 ± 3.4	22.4 ± 1.9	21.3 ± 1.9
Tooth width	9.5 ± 1.5	8.1 ± 1.4†	8.6 ± 0.9	7.7 ± 0.9**
Root length	17.4 ± 2.6	15.6 ± 2.1§	16.2 ± 1.5	15.3 ± 1.6
Root area	102.1 ± 27.2	75.4 ± 15.1†	81.3 ± 12.5	68.8 ± 12.0**

* Values represent mean ± standard deviation for Group I (fresh extracted teeth) and Group II (teeth from human skeletal remains) of cases in parentheses for all variables except periodontal recession (Group I, men = 18, women = 8; and Group II, men = 7, women = 14). Variables affected by the postmortem interval were measured as described in the footnotes of Table 1 and the Material and Methods section.

Significant differences between men and women were † $p < 0.001$; ‡ $p < 0.01$; and § $p < 0.05$ for fresh extracted teeth and ** $p < 0.01$ for teeth from human skeletal remains.

variables affected by the postmortem interval except attrition, cementum apposition, and tooth length. Nevertheless, differences were also found for two variables (tooth width and root area) for teeth from human skeletal remains (Table 2), whereas the rest of variables affected by the postmortem interval did not differ between men and women in this sample group.

Differences in variables according to the type and position of the tooth from each sample group was another area of interest. As expected, differences in values according to the type of tooth were found for most variables (results not shown). Nevertheless, variables affected by the postmortem interval (Table 1) did not differ according to the type and position of the tooth for either fresh extracted teeth or teeth from human skeletal remains.

Correlations between variables affected by the postmortem interval and age of the individual at extraction or at death were calculated for each sample group. For fresh extracted teeth, correlation coefficients obtained from the linear regression models with age as the dependent variable were higher than 0.5 for color, attrition, secondary dentin, and cementum apposition. When teeth from human skeletal remains were analyzed, significant correlation coefficients were also obtained for secondary dentin and cementum apposition.

Our results suggest that postmortem interval affects age-related morphological changes, and that different methods of dental age estimation should be used depending on the time after death. Regression models for dental age estimation should be obtained for different postmortem intervals.

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