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Estimation of age at death based on the analysis of third molar mineralization in individuals from Brazilian archaeological populations*

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Abstract

Estimating the age at death of archaeological individuals is critical for the reconstruction of the demographic profile of past populations. Teeth are very resistant to mechanical, chemical and physical damage. Thus, dental age estimation methods have been proven remarkably useful, especially when the other available bone remains are poorly preserved. In this study, we estimated the age of subadult individuals from pre-colonial archaeological sites in the state of São Paulo, Brazil, which are under the curation of the Museum of Archaeology and Ethnology at the University of São Paulo (MAE-USP). Age estimation was based on the analysis of dental mineralization stages originally proposed by Demirjian et al. (7) and applied to third molars by Soares et al. (11). Teeth (n = 18) were radiographed on a portable X-ray device and the images were analyzed in DICOM extension. The intraexaminer reliability test showed excellent agreement regarding the classification of mineralization stages (Kappa value = 0.94). This age estimation method showed good agreement with the previously cataloged age estimates, which were used for comparison. Of the 18 teeth examined, 15 had their age correctly estimated within the comparative range and only three did not match, of which two were very close to the cataloged estimates and one was underestimated by several years. Collectively, our findings suggest this method can be accurately applied to archaeological individuals.

Keywords: Determination of age by teeth; paleodontology; archeology

Introduction

Age estimation methods are commonly based on the analysis of human growth and development indicators. The approaches used for estimating the age of bone remains can be divided into two broad analytical categories, namely: skeletal development and dental development (1,2).

In the case of skeletons of immature individuals, the following anatomical structures are usually examined for age determination: tooth eruption and the degree of dental development, closure of the epiphyses and the length of the diaphysis of long bones, and the appearance and fusion of ossification centers. Nevertheless, in cases of archaeological remnants, the application of some of these methods may be limited due to poor skeletal preservation resulting from taphonomic processes (2).

Estimating the age at death of individuals from an archaeological collection is challenging, yet essential for reconstructing the demographics of that population; therefore, estimates very close to the chronological age are highly desirable. The study of archaeological individuals contributes to understanding the evolution of human populations, including their growth patterns, diseases, living conditions, health, well-being, and activities (2,3).

Dental age estimation is considered more accurate than other methods that evaluate the process of epiphyseal union and/or the length of the diaphysis, and it can be even more accurate if the available teeth are well preserved (3,4). However, from the age of 14 up to approximately 25 years, the third molar is the only immature tooth available to estimate the individual's age, which can render the identification process even more complex (5). The accuracy of age estimates determined by the analysis of third molars depends both on the correctness of the classification of the mineralization stage and the method of choice (6).

In 1973, Demirjian et al. (7) developed a method to estimate the dental age of living individuals based on the analysis of radiographs of seven index teeth (excluding the third molar) on the left side of the mandible. Eight stages were defined, from A to H, known as Demirjian's mineralization stages, which ranged from the first appearance of calcified spots until apical closure. A score was assigned to each tooth according to its mineralization stage; the scores are then summed up to indicate the dental age estimate based on preset sex-specific tables.

In 1993, Mincer et al. (8) carried out a study to assess radiographically third molar development according to the eight stages proposed by Demirjian et al. (7). Regression formulas were obtained to estimate whether an individual was under or over 18 years of age. Since then, several studies have been carried out to validate age estimates in different populations using Demirjian's mineralization method applied to third molars (9). While this method has shown satisfactory results in several samples, including Brazilians (10,11), its applicability to Brazilian archaeological populations is yet to be determined.

Thus, this study aimed to estimate the age at death of individuals from three Brazilian archaeological skeletal series (Moraes, Buracão, and Piaçaguera shell mounds) from the State of São Paulo, based on the analysis of third molar mineralization.

Materials and Methods

This study was previously approved by the Research Ethics Committee at the University of São Paulo School of Dentistry (FOUSP), under protocol number 2.943.91720/18.

Twenty-one third molars from 11 archaeological individuals from three sites in the state of São Paulo, Brazil, were radiographed. Of these, three teeth had their apex closed and were excluded from the analysis. Then, 18 developing teeth from 9 individuals were considered eligible (maxillary third molars, n = 4; mandibular third molars, n = 14).

The included teeth were from the following archaeological sites: (i) Piaçaguera (n = 2), a coastal shell mound dating back to approximately 7200-4200 before the present (BP), located in the municipality of Cubatão in the Baixada Santista region (12,13); (ii) Buracão (n = 2), a coastal shell mound close to Santo Amaro Island in the Baixada Santista region, dating back to 1700-1400 BP (12); and (iii) Moraes (n = 5), a riverine shell mound dating back to 6800-5000 BP,

located in the municipality of Miracatu in the Ribeira do Iguape Valley (14). These archaeological individuals are under the curation of the Museum of Archaeology and Ethnology at the University of São Paulo (MAE-USP). According to MAE-USP's records, three of them were females and eight had undetermined sex.
The teeth were radiographed on a portable Nomad direct current X-ray device (Aribex, Utah, USA) with an indirect digital sensor using photostimulable plates from the Digora Optime digital system (Soredex, Tuusula, Finland). After the sensor was exposed to the X-rays, laser scanning was performed on the Digora Optime equipment and the images were obtained in DICOM extension (Digital Imaging and Communication in Medicine) and stored in the Digora program for Windows (Soredex, Tuusula, Finland).
Before estimating the age of the archaeological individuals, the Kappa coefficient test was used to determine intraexaminer agreement regarding the classification of the mineralization stages. The entire sample was analyzed, with an interval of 21 days between the first and second sessions.
DICOM images were viewed using the free software StellarPACS DICOM Viewer® (Figure 1). All teeth were evaluated and classified according to their degree of development, as described by Demirjian (7) (Figure 2): (A) mineralization of cusp tips, without fusion; (B) union of the cusps, with an anatomical definition of the crown; (C) half of the crown formed, with a visible pulp chamber and beginning of dentin deposition; (D) complete crown, with the presence of the cementum-enamel junction; (E) beginning of interradicular bifurcation, root length shorter than that of the crown; (F) root length greater than or equal to that of the crown, tapered root end; (G) complete root, with parallel walls and open apex; (H) apical closure.
To estimate the individual's age, mineralization stages were converted into numbers, from 0 (stage A) to 7 (stage H), and applied to the regression formula developed by Soares et al. (11), as follows:

- Age = 9.8 + (1.2 x development stage)

The data regarding sex and identification, analyzed teeth, classification of the mineralization stages assigned to each tooth, and age estimates obtained through the formula developed by Soares et al. (11) were entered into 2020 Microsoft® Office Excel spreadsheets. Then, the age ranges previously estimated (cataloged) for each individual were added to the table and compared against those obtained through the method. Individuals were cataloged by the MAE-USP according to the site and number of their burial; for instance, "Burial #1 – Buracão site". In this study, the nomenclature was abbreviated as follows: initial of the archaeological site and number of burial (SB_1).
The individuals from the Piaçaguera and Moraes sites had their age estimated, whenever possible, by combining the analysis of tooth development and eruption with the appearance and fusion of the epiphyses (15). One individual from Buracão (SB_1) had their age estimated according to the stages of tooth eruption and fusion of ossification centers in the long bones (16). Another individual (SB_7) had their age estimated based on tooth development and closure of the epiphyses according to unpublished cataloged data of the MAE-USP.
Considering that age estimates based on tooth development and closure of the epiphyses are given in age ranges, only the mean age at death of each individual was used in subsequent analyses. As the data showed a non-normal distribution (Shapiro-Wilk W normality test = 0.76935, $P = < 0.001$), non-parametric statistical tests were used. The Wilcoxon test was applied to test whether discrepancies between the estimates obtained through the analysis of third molars and those based on tooth development and closure of the epiphyses were statistically significant. The correlation between the age estimates of both methods was determined by Spearman's correlation coefficient.

Results

The intraexaminer reliability test showed a nearly perfect agreement regarding the classification of the mineralization stages (Kappa = 0.94; 95% CI [0.81, 1.00]).
Table 1 describes the archaeological site, individual's identification number, teeth included for analysis, mineralization stage assigned to each third molar, and a comparative between age estimates obtained through Soares et al.'s formula (11) and the cataloged age ranges estimated by the MAE-USP.
The age of the nine individuals included in the analysis was estimated based on third molar development according to the stages proposed by Demirjian (7). Age estimates were calculated for each tooth; we note that some individuals

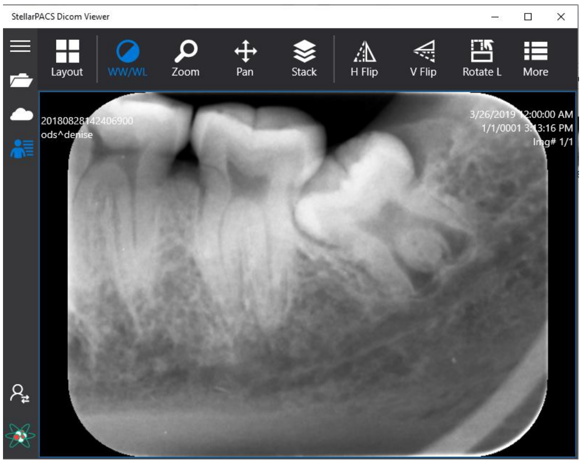

Figure 1. Radiographic image in DICOM format viewed in the StellarPACS DICOM Viewer® program.

might have had only one third molar analyzed whereas others two or four.
Overall, the method presented a good agreement with the cataloged age ranges used for confrontation, with no statistically significant differences between them (Wilcoxon test W = 127, P = 0.27). Furthermore, there was a positive correlation between estimates based on third molar development and those previously established based on tooth development and epiphyseal closure (Spearman S = 388.35, P ≤ 0.01) (Figure 3).
Nevertheless, in one case (SB_7), the age estimated by the analysis of third molar development was slightly below the minimum age of the range. In this individual, the two mandibular third molars were analyzed, with tooth #38 being classified into stage D and tooth #48 into C. Thus, this individual's age was estimated at 13.4 years based on tooth #38 and 12.2 years based on tooth #48, resulting in a difference of 1.6 years and 2.8 years from the minimum age of the range (15 years) used for comparison, respectively.
Another case with conflicting results was that of individual SM_XVA, whose age estimated by the method was 17.0 years as compared to 24-35 years of the comparative cataloged data.

Discussion

The method of choice and index teeth used to estimate dental age vary according to the age classification of the sample: fetus (prenatal and neonatal), children, adolescents, and adults (17). In a bioarchaeological context, tooth development is used to estimate the age at death of subadult individuals. Teeth are usually better preserved than bones and have a very important role in the reconstruction of the biological profile of damaged human remnants. Moreover, tooth development seems to be less affected by exogenous factors and pathological conditions, which renders dental age estimates more accurate than those based on bone development (4, 18-20).
Some limitations of the modified Demirjian's method should be considered, namely: (i) there are situations in which the third molar is the only tooth available for the analysis of development; (ii) the third molar is the tooth with the most variable formation and eruption time from one individual to another; and (iii) there may be a total absence of third molars or situations in which their disposition within the bone can make it

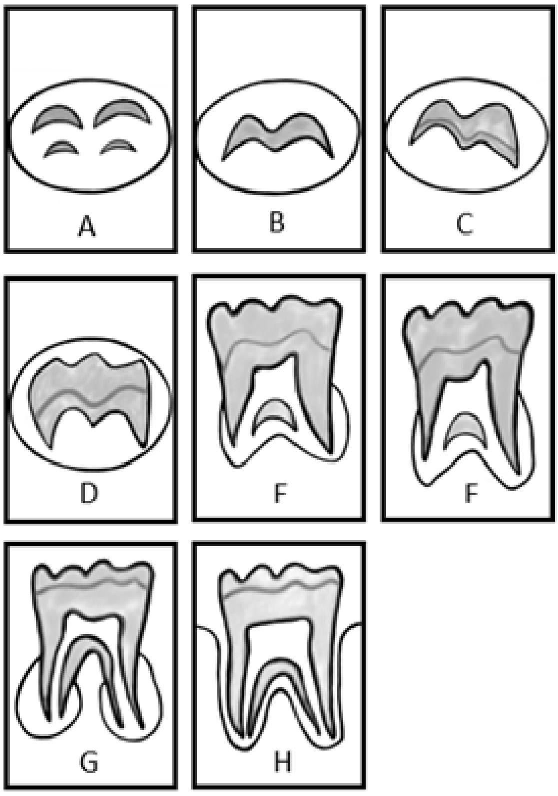

Figure 2. Tooth development stages proposed by Demirjian.

impossible to determine their development stage (8, 21, 22).
Third molar eruption can vary between 12 and 22 years of age and their development takes place from 9 to 23 years. The radiographic analysis of third molars can assist in the estimation of the individual's age range as it allows studying the development of the crown and root independently

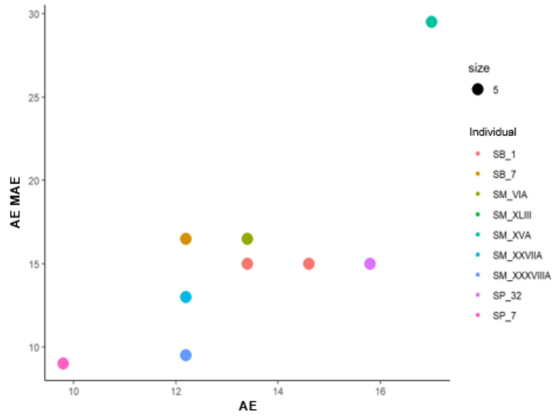

Figure 1. Correlation between age estimates by the MAE-USP versus those obtained through the analysis of Demirjian's mineralization stages applied to third molars.

of eruption. In skeletal remains of subadult individuals, the analysis of third molar development associated with bone development can result in more accurate age estimates (1).

In our study, the age of individuals from Brazilian archaeological collections was estimated by Dermijian et al.'s method (7) modified for third molars by Soares et al. (11). While the analysis of Demirjian's development stages has been successfully applied in contemporary populations, there are no records of its applicability in historical and/or archaeological collections.

This study showed good agreement between the estimates obtained through the radiographic analysis of third molars and those obtained previously through methods commonly used in archaeology, with no statistically significant differences between them (Wilcoxon test W = 127, P = 0.27). Importantly, there was a positive correlation between the estimates obtained herein and those previously established based on tooth development and closure of the epiphyses (Spearman S = 388.35, P ≤ 0.01). The results tend to be more consistent when there are enough bone and dental elements to estimate the age by usual methods. In contrast, when there are fewer elements available, the method's accuracy is likely to decrease.

Lopez et al. (10) applied Demirjian's method to living individuals from São Paulo, Brazil, aged between 15 and 23 years, and developed different formulas for females and males. Soares et al. (11) applied the method to individuals from northeastern Brazil and found no statistically significant differences between the sexes and mouth quadrants. For this reason, they suggested that a single regression equation should be applied to estimate the age, regardless of the individual's sex and third molar being analyzed. In an archaeological context, sex determination can be highly impaired depending on the skeleton's preservation conditions. Hence, methods that estimate the age independently of sex are advantageous to analyze skeletons of immature individuals since their sex determination is often challenging (23).

In our study, nine subadult individuals were analyzed, of which only two (SB_7 and SP_32) had their sex previously estimated. For this reason, we preferred to use a method that did not require sex-specific formulas. Moreover, the study by Soares et al. (11) was carried out with individuals from 6 to 22 years of age, which provided greater accuracy in the analysis of younger individuals. The mean age found for stage A was 9.8 years; as stage A is converted into number zero in the equation (11), when a third molar was classified at this stage, the age of 9.8 years was assigned to the individual. In our analysis, one individual (SP_7) had their age estimated at 9.8 years, which coincided with their previously estimated age range (9 years ± 24 months) (15).

As previously discussed, the third molar is a tooth with very variable development between individuals. According to Roselth et al. (9), it is possible to observe a difference as large as 4.7 to 6.8 years in the Demirjian's stages, suggestive of great individual variability in the mineralization process. The authors pointed out that the conflicting inter-study outcomes in different populations have been correlated with population-specific differences in tooth development. However, they suggest this may be a consequence of age mimicry when the sample is not evenly distributed across the age groups due to sample selection bias. The authors concluded that there is not enough scientific evidence to confirm these significant differences between different populations.

In our study sample, two individuals had their age underestimated by the method. In individual SB_7, the mandibular third molars analyzed (teeth #38 and #48) underestimated the age by a few years compared to the age range previously established based on tooth development and closure of the epiphyses, according to the MAE-USP's cataloged data. However, considering that third molars have a quite variable development, such differences seem not to dramatically affect the accuracy of the age estimates.

In individual SM_XVA, the radiographic analysis of the mandibular left third molar (#38) showed root apexes almost closed, yet with an incomplete apical end. The tooth was classified into stage G (complete root and open apex), given that stage H already indicates apical closure. Hence, the age of this individual estimated by Soares et al.'s method (11) was 17 years old, that is, 7 years below the minimum age of the age range previously cataloged (25 to 34 years) based on the general aspect of joint surfaces and tooth wear. The skeleton of this individual, despite presenting a regular bone preservation, was quite incomplete to estimate the age at death based on more elements.

As third molars are highly variable in terms of development, there is often a large gap between development stages. A study carried out in South Africa found a maximum age of 25.17 years for stage G in tooth #38 (24) as compared to 24 years in South Korea (25). Another study carried out with the Brazilian population (10) found a maximum age of 22.8 years for stage G among females and 22.7 years among males. Therefore, if only the third molar was considered, individual SM_XVA was likely to be over 20 years of age, but not older than 30. A combined analysis of the markers previously used could provide a more accurate estimate.

The method applied in our study is conservative (non-destructive) and easily reproducible if the examiner is properly trained, as confirmed by the intraexaminer reliability coefficient described herein. Another advantage is the fact that the method does not depend on the eruption of the third molar(s) because it is based on a radiographic analysis. Although tooth development does not seem to be directly affected by endocrine and metabolic diseases, the eruption of permanent teeth can be delayed in malnourished and sick children (21). Thus, an imaging examination can provide more accurate age estimates even in archaeological individuals who may not have a visible third molar in the mandible or maxilla. Moreover, the radiographic analysis allows detecting the entire process of tooth mineralization from the first stages up to apical closure (26).

Skeletons of immature archaeological individuals commonly present poorly preserved bones due to taphonomic processes (2). Thus, this method can usefully estimate the age of archaeological individuals who may not necessarily bear additional elements required for the application of other identification methods.

In our sample, age estimates were more accurate than those previously cataloged in individuals who had preserved bones and enough elements for the analysis. This demonstrates that the usual methods can effectively estimate the age of shell mound collections when there is enough material available, eliminating the need for more costly techniques. However, in the absence of sufficient elements for a direct inspection, the analysis of the Demirjian's stages applied to third molars proved to be relevant. It is important to consider that while it is not a costly approach, it does require the availability of a radiographic device, which is not always possible in an archaeological context.

In archaeological research, age estimates are usually given in ranges and the use of accurate methods can help narrow such intervals. Therefore, whenever possible, the modified method presented herein should be combined with other techniques for greater accuracy, particularly in the analysis of immature individuals from archaeological populations whose demographic profile needs to be established.

Conclusion

To conclude, our findings indicated that age estimation based on Demirjian's method applied to third molars showed good results in subadult individuals from a Brazilian archaeological collection. Further studies on different and larger archaeological samples are suggested. To improve the accuracy of the estimates, Demirjian's method should be combined with other approaches commonly used in archaeology.

Conflict of interest statement

The authors declare no conflict of interest.

Authors' Contributions

The authors contributed to this work, as follows: conceived and designed the experiments (DRM, VW, DF, MGHB), performed the experiments (CC, DRM, DF), analyzed the data (MGHB, EMC), contributed materials/analysis tools (CC, MGHB, EMC), wrote the paper (DRM, MGHB), edited the final version of the submitted paper (DRM, DF, VW, MGHB, EMC), read and approved the final revised version (DRM, DF, CC, EMC, VW, MGHB).

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Table 1. Age estimation based on third molar development in a sample of Brazilian archaeological individuals from three different shell mound sites.

Archaeological site	ID	Sex	Tooth	Stage	AE	AE by the MAE	Method(s) used by the MAE-USP	Bone preservation
Buracão	SB_1	Unclear	38	D	13.4	12-18	The stage of fusion of the epiphyses of long bones and vertebral body ring	Poorly preserved bones
			48	E	14.6	12-18		
	SB_7	Female	38	D	13.4	15-18*	The stage of fusion of the epiphyses of long bones and the stage of the eruption of mandibular third molars (fully impacted, with an open bone window for eruption)	Poorly preserved bones
			48	C	12.2	15-18*		
Moraes	SM_VIA	Unclear	18	D	13.4	13-14	Stage of fusion of the epiphyses of long bones and stages of tooth development and eruption	Preserved, complete bones
			28	D	13.4	13-14		
			38	D	13.4	13-14		
			48	D	13.4	13-14		
	SM_XLIII	Unclear	38	F	15.8	15 ± 36m	Stage of fusion of the few epiphyses of long bones available and stages of tooth development and eruption	Very incomplete bones
	SM_XVA	Unclear	38	G	17.0	25-34	Overall appearance of joint surfaces and tooth wear.	Preserved, very incomplete bones
	SM_XXVIIA	Unclear	48	C	12.2	13 ± 36m	Stage of fusion of the epiphyses of long bones and stages of tooth development and eruption	Preserved, very complete bones
	SM_XXXVIIIA	Unclear	18	C	12.2	9-10 ± 36m	Long bone epiphyseal ossification centers, stage of fusion of the epiphyses of long bones, stage of coxal bone formation and tooth development, and, in addition to some measurements of the length of long bones.	Preserved, very complete bones
			28	C	12.2	9-10 ± 36m		
			38	C	12.2	9-10 ± 36m		
			48	C	12.2	9-10 ± 36m		
Piaçaguera	SP_7	Unclear	38	A	9.8	9 ± 24m	Presence of long bone epiphyseal ossification centers, stage of coxal bone formation, and stage of tooth development and eruption	Preserved, very complete bones
	SP_32	Female	38	F	15.8	15 ± 36m	Stage of fusion of the epiphyses of the long bones and stage of coxal bone formation	Preserved, very complete bones
			48	F	15.8	15 ± 36m		

AE: age estimate (in years) based on the analysis of third molar mineralization; AE by the MAE-USP: age range previously estimated by the Museum of Archaeology and Ethnology at the University of São Paulo.

*Unpublished data cataloged by the MAE-USP.