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Assessment of Age at Death in Perinatal Individuals from Dental Histology Analysis. An Exploratory Study

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Abstract

Age estimation is one of the main data used in the study of child skeletal remains in forensic and bioarchaeological investigations.

Objective: Our aim was to assess the age at death attributed through methods based on bone development and by analysing the microstructure of tooth enamel.

Methods: Age at death was estimated using femur length and dental histology in 9 perinatal individuals of known age. Two methods were selected for the estimation of age at death from femur length. Previous work has reported that these two methods produce the best estimations of the age at death of individuals. For the dental histology analysis, longitudinal ground sections of 6 deciduous incisors from 5 individuals were examined. Incremental lines in the tooth enamel (striae of Retzius and the neonatal line) were used to estimate the age at death, assuming a daily secretion rate of 3.23 µm.

Results: The ages estimated by the two methods based on femur length differ, except for those concerning the last stage of gestation. It is interesting to note that the accuracy of the estimation depends on whether the method was established with prenatal or postnatal individuals. Estimated ages based on dental histology (N=3 individuals) are consistent with the recorded data.

Conclusion: The study of dental histology based on the analysis of enamel microanatomy has some limitations but allows the age at death in to be established very accurately. The quality and quantity of data that this technique provides is of prime importance for forensic studies and for analyses of historical populations and archaeological remains.

Introduction

Estimated age at death is one of the main data used in the study of child skeletal remains in forensic and bioarchaeological investigations [1,2]. Age data are fundamental in building up individual biological profiles, which contributes to the judicial system and has legal implications in forensic cases [3,4] as well as allowing reconstructions of mortality profiles and interpretations of the impact of changes in the lifestyle of past populations [5]. Furthermore, with accurate estimations of gestational and post-gestational age, this variable can be associated with factors that may have modified the normal growth trajectory [6].

The gradual changes that occur during growth and development are used as indicators to estimate age. Tooth formation, dental eruption and long bone development are the preferred anatomical references for establishing growth patterns in foetal, perinatal and infant individuals. These patterns are established from the analysis of individuals of known age and are used as a reference to estimate age in cases where it is unknown [7,3].

An enormous amount of data from long bone growth surveys has been used to develop regression equations for age estimation. It should be noted that some equations were developed to estimate gestational age in the prenatal period [8-10]. While others were proposed for the postnatal period [11-13]. There are many clinical studies on the gestational age of preterm infants and small babies that assess health, growth and development. However, these groups are impossible to identify when assessing the remains of long bones of foetuses and infants from archaeological sites [13]. Unfortunately, there is no method for estimating age using long bones that takes the pre-postnatal continuum into account [13]. This observation is important because the origin and characteristics of the samples from which the estimation methods are developed influence the results and interpretations when they are extrapolated to other samples or populations [14-17].

A method that allows age to be obtained directly, with inbuilt chronological identification, would avoid the problems of extrapolation. Dental histology studies can fulfil this purpose because they are based on the presence of growth lines in the enamel whose analysis in deciduous teeth can be used to identify premature births and estimate the age at death of infant individuals. These growth lines can therefore provide a precise chronology of the formation of the tooth [18-21] as well as the age at death if the tooth was not fully formed [22-25]. The enamel has two types of growth lines: cross-striations and striae of Retzius. The former, which are perpendicular to the axis of the prisms, result from the circadian activity of ameloblasts, so that each cross-striation corresponds to one day [26]. The striae of Retzius are arranged obliquely to the axis of the prisms and correspond to successive increments in the formation of the enamel matrix. Striae of Retzius have a regular systemic periodicity that can vary from tooth to tooth for about 6-9 days. Each stria corresponds to a specific moment of tooth formation and highlights the active ameloblasts at that moment. The etiology of the striae is unknown but they are the

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result of physiological, natural changes. Moments of stress produce accentuated striae [20], sometimes they are identifiable on the surface of the tooth as hypoplasias.

Birth is a time of stress, and is manifested in tooth enamel by a very marked stria known as the neonatal line (NNL) [27,28]. The link between NNL and birth can be observed in the difference between birth conditions. For instance, NNL virtually does not exist in stillbirth cases and NNL thickness is thicker after vaginal delivery than after caesarean section [29] which is used as an argument in favor of caesarean section being less stressful for the newborn [30]. Birth is easily identifiable in temporary teeth because their formation begins in utero and ends in early childhood. The NNL thus calibrates all temporary teeth and distinguishes between prenatal and postnatal enamel. In addition, given the stress sensitivity of striae, periods of childhood stress can be identified post-mortem through the presence of strongly marked lines formed after the NNL. Preliminary evidence suggested that deprivation or threat (physical and sexual abuse, neglect, trauma) may present themselves as stress lines in the enamel [31]. There are various potential causes of stress, such as infections, high fevers or periods of starvation. In addition, depending on the location of the NNL in the crown, it is possible to infer whether the birth occurred at term (location of the NNL similar to control individuals) or prematurely, in which case the NNL is closer to the dentin horn. Thus, the study of the micro-anatomy of the enamel in deciduous teeth not only provides a) the chronology of crown formation, but also b) calibrates it with birth and therefore c) suggests an age at death if crown formation was not complete, d) to identify whether the birth was at term, and e) to characterize the child's health.

The aim of this exploratory study based on a small sample size was to show the potential use of enamel microanatomy to suggest an age at death in immature individuals. To show the limitations of this analysis teeth whose crown development has ended are also included. A method based on bone development to suggest an age at death for the same individuals is presented for comparison.

Material

The individuals studied are from the Lambre collection, which is composed of skeletons donated by the municipal cemetery of the city of La Plata (Argentina) to the Faculty of Medical Sciences of the National University of La Plata for research and teaching purposes[32,33]. The sex, age, date and cause of death of the human remains in the collection are documented, but information on the gestational age of foetal individuals is lacking. The documented death dates for the children in the collection are between 1927 and 2015. The individual included in this study died between 1992 and 1997, so it can be said that the sample is representative of those who died in the last decade of the twentieth century in La Plata city.

Although there is no specific population characterization for the individuals of the Lambre collection, the most frequent nationality is Argentinian, with 58.46% for adults and all sub-adult individuals. The sample can be described as belonging to an urban and cosmopolitan population of the Metropolitan Area of Buenos Aires, thus reflecting a complex demographic history with multiple migration events and miscegenation events. Genetic studies indicate the presence of Amerindian, European and African components in proportions of approximately 14%, 82% and 4% respectively[34,35].

Methods of age estimation based on long bones were applied to a sample of 9 individuals from the Lambre collection. Of these, 6 individuals have a chronological age (CA) at death in the postnatal period; 2 individuals do not have documentary information and 1 individual died in the foetal period but no gestational age is available. Dental histology analysis was performed only for five individuals (268 [i1UR, i1LL], 286, 313, 318, 326). The individual 268 is represented by two teeth.

The study, conservation, and management of human remains in this research was in agreement with current national and international ethic codes. The research on the Lambre collection was approved by the Bioethics Committee of the Faculty of Medical Sciences of the National University of La Plata (COBIMED: 0800-013812/12-000).

Methods

Age at death was estimated using femur length (FL) and dental histology. Two equations were selected for the age estimation from femur length, one applied to foetal individuals and the other to postnatal ones. A selection was necessary because the individuals in the sample are pre- and postnatal in age and there are no age estimation equations covering both periods together. These two equations were chosen because they proved to give the most accurate results for estimating age in individuals up to one postnatal year in the Lambre collection [15]. [8] [FL * 0.3922) + 8.83] is the most appropriate equation for foetal individuals, and [13]. [(FL - 74.04)/42.01] for postnatal individuals. Age is given in weeks from conception and birth is assumed to have occurred at week 40 of gestation.

These equations produce a bias when used in individuals of other ages [8] (the Scheuer et alequation in postnatal cases and the Cardoso et al equation one in prenatal cases) (see Results). For this reason, in order to compare the age that was estimated according to the length of the femur with the age obtained from dental histology, the equation to be applied depended on whether the individual is pre- or postnatal, considering a femur length of 79 mm as the boundary between the pre- and post-natal periods [9,36]. In other words, the [8] equation was used for individuals with a shorter femur length, and [13] equation for those with longer femurs, so that the results could be compared with those from the dental histology analysis.

For the analysis based on dental histology, the teeth were sectioned using the methodology described in [37]. The age at death was estimated using the method described by [38,39]. The NNL was identified in each tooth section (Figure 1). The point of intersection of the NNL with the enamel-dentine junction (EDJ) was used as a starting point. In the prism placed at this intersection, a point was established at 100 µm from the EDJ. The stria of Retzius passing through this point was followed to its intersection with the EDJ. This triangle corresponds to one area of the enamel. The same procedure was performed up to the dentin horn and to the cervix to establish several areas of prenatal and postnatal enamel. [38] found an average of 3.23 µm for deciduous canines, established from 559 measurements of the daily secretion rate in the enamel 100 µm from the EDJ. They proposed that each area of enamel is therefore formed in 30 days. To obtain the age at death after birth, the number of areas of post-natal enamel in each section was multiplied by 30 and the length of prism formed after the last area of enamel was divided by 3.23 to add the last days of enamel formation.

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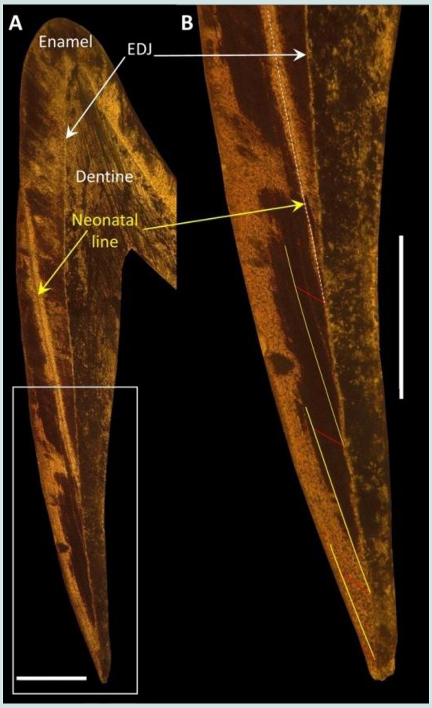

Figure 1: A. Thin section of the 268a tooth. The neonatal line (NNL) is clearly visible. B. Detail of the enamel close to the cervix showing the method usedto estimate age at death. Every 100 µm along the prism length (in red) corresponds to 30 days. Successive stages in enamel formation starting at the NNL are shown by striae of Retzius (yellow lines). In total, three successive 100 µm lengths and an additional prism length of 48 µm suggest anage at death of3.2 months. EDJ: enamel-dentine junction. The bar corresponds to 500 µm.

The sections were observed with a Leica M8 stereo microscope and a Zeiss Universal transmitted and polarized light optical microscope using x2.5, x6.3 and x16 lenses. The digital images were acquired using an adaptable IDEA® removable camera placed on the eyepiece of the optical microscope and captured on a computer using Spotbasic®software. The images were processed with Nikon ViewNX2® for contrast enhancement and adjustments, ImageJ® (Fiji) for line tracing and data acquisition, and Adobe Photoshop® for photo editing

Results

The chronological age and the age estimated from femur length are presented in (Table 1). The estimated ages of individuals 333 and 268 from femur length are very low relative to the chronological ages (Figure 2). This could indicate that these are premature individuals. According to their femur length, individuals 428 and 286 were in the last stage of gestation: both equations based on femur length yield similar results for these individuals. It is interesting to note that the ages estimated by the [8] method, based on foetal individuals, underestimates the ages of postnatal individuals (318, 455, 295, 313, 326) and, conversely, the ages estimated by [13] whose equation is based on postnatal individuals, underestimates the ages of foetal individuals (333, 268).

The estimated ages based on dental histology (EA H) are given in (Table 2). In individuals 268, 286 and 318, crown formation was not complete, while in the remaining two, 313 and 326, part of the root was already formed and thus the age at death cannot be provided. In the four teeth whose crown formation was not complete, the analysis of dental histology provides very accurate estimations. In 268a and 268b, the age at death is 15 weeks after birth (Figure 3). In tooth 286, the NNL was not observed and in 318, a thin layer of enamel is posterior to the NNL, suggesting death during the first month of extra-uterine life. These results are consistent with the cemetery records (Table 2), (Figure 3), which show that 268 died at 53 weeks (40 weeks gestation and 13 weeks postpartum), 286 died in the foetal stage (less than 40 weeks) and 318 died27 days (4 weeks) after birth

Table 1: Chronological ages and ages at death in weeks estimated from femur length

Individual	CA	FL (mm)	EA F1	EA F2
333	43.3	51.31	28.95	11.86
268	53	61.03	32.77	23.9
428	N/A	70.64	36.54	35.79
286	Foetal	73.60	37.7	39.46
318	43.8	81.83	40.92	49.64
455	44	82.43	41.16	50.39
295	44	85.91	42.52	54.69
313	N/A	98.89	47.61	70.76
326	57.3	106.96	50.78	80.75

CA: chronological age, Foetal: deceased during the foetal period, FL: femur length in mm, EA F1: estimated age at death using the Scheuer et al. (1980) method, EA F2: estimated age at death based on the Cardoso et al. (2014) method.

Table 2: Chronological ages and estimated ages at death in weeks

Individuals	Tooth	CA	EA F	EA H
268	i1U; i1L	53	32.77	55.00
286	i1U	N/A	37.7	<40.00
318	i1U	43.8	49.64	44
313	i1U	N/A	70.76	*53.00
326	i1L	57.3	80.75	*50.83

CA: chronological age, EA F: estimated age at death using femur length, EA H: estimated age at death based on dental histology, i: incisor, U: upper, L: lower, *: minimum age at death after completion of crown formation

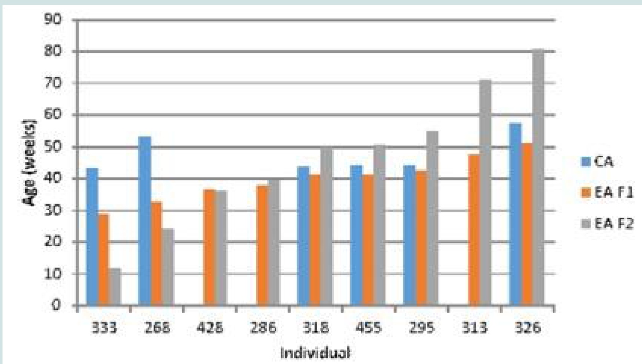

Figure 2: Comparison of documented chronological ages with estimated ages at death from femur length. CA: chronological age; EA F1: estimated age at death using the Scheuer et al. (1980) method; EA F2: estimated age at death using the Cardoso et al. (2014) equation.

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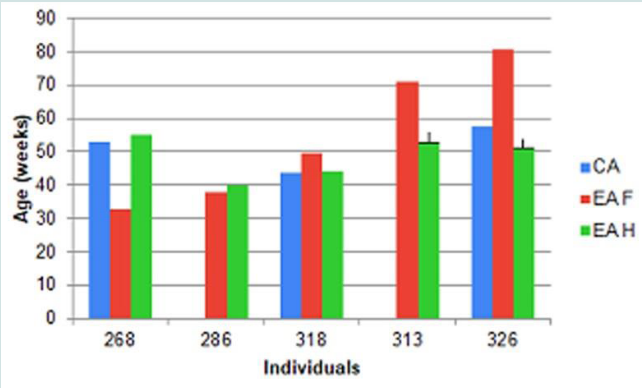

Figure 3: Comparison of documented chronological ages with estimated ages at death from femur length and dental histology. CA: chronological age, EA F: estimated age at death from femur length using the two methods combined for prenatal and postnatal individuals, EA H: estimated age at death from dental histology analysis. ⊥: Individuals 313 and 326 have full crown and root formation so that dental histology gives a minimum age.

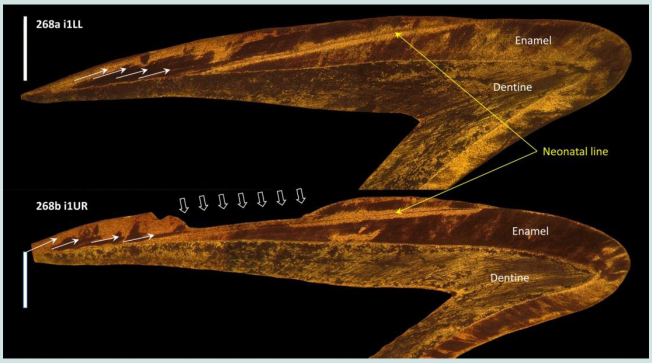

Figure 4: These two teeth from the same individual (268a, 268b) show accentuated striae (white arrows) in the postnatal enamel. These stress marks are more marked in the upper right incisor (268b) where ameloblasts precociously interrupted enamel secretion, producing a hypoplasia in the central area of the crown's buccal face (broad white arrows). i1LL: lower left first deciduous incisor, i1UR: upper right first deciduous incisor. The bar corresponds to 500 µm.

(44 weeks).

In tooth 268a (Figure 1), other marked striae of Retzius can be observed that formed after the NNL, thus indicating moments of postnatal stress. Tooth 268b (Figure 4) was even more strongly affected by stress, since the secretion of ameloblasts was interrupted at the level of the stress line following the NNL and is responsible for a significant hypoplasia in the middle crown zone.

The contribution of dental histology based on enamel analysis is limited in teeth where crown formation is complete. However, the time between birth and completed crown formation can be known. This is important because if the period is longer than usual, it can be inferred that the birth was premature. In 313 and 326, the time between birth and the end of crown formation is 3 and 2.5 months respectively (13 and 10.83 weeks), indicating that the birth was not premature. To obtain the age at death in these latter cases, the analysis should include dentine. Unfortunately, growth lines in this tissue are not easy to discern and observations can rarely be made. In cases where root formation has begun, an estimate based on dental metrics can be used [40,41] but this was outside the scope of our study.

Discussion

Several methods based on bone growth have been proposed to determine the age of foetal, perinatal and infant individuals. These methods have been established on the basis of surveys of populations that provide sound information through the biological profiles of individuals whose chronological age is known. They therefore become reference methods that are used to estimate ages in individuals whose biological profiles need to be reconstructed [42]. An extrapolation of the method was assessed to confirm its applicability to populations other than the one used to establish it [13,43]. However, the fact that a method works when extrapolated to a different population does not ensure that it can be applied to all populations.

In the case of our study, although the individuals analysed are close in origin to the populations used as a reference (Europeans), the method based on bone growth (length of the femur) suggests ages that do not agree with the records. According to this method, individual 268 died before birth, individual 286 near birth (between 38 and 40 weeks) and individual 318 a few months after birth (Table 1). The bias of these attributions does not follow a similar pattern either, since the age at death is underestimated for individual 268 and overestimated for individual 318. However, the results from both equations are consistent when the individuals died in the perinatal period, as is the case with individuals 286 and 318. This consistency is confirmed by the dental histology analysis (Table 2).

To estimate age by dental histology, we used a daily rate of enamel secretion (3.23 microns) determined from the study of an English population [38]. A close rate (3.26 microns) was obtained in a population of Baka pygmies [39]. The similarity of these results from two distantly related populations living in totally different habitats allowed us to extrapolate the same rate of enamel formation to the individuals we analysed in this study. The agreement between our estimates of age at death and the records indicates that the true rate of enamel formation is no different. If extrapolation has to be avoided, the daily enamel secretion rate can always be obtained in each individual under study by measuring the distance between adjacent cross-striations.

Comparison of chronological age with the two methods used (Table 2) shows that results from dental histology are closer to chronological ages than those from the femur length. Age estimations from femur length were obtained using two equations established from other population. Bone growth methods yield inconsistent results when applied to different populations, this limits the use of this method for comparison and may result in either overestimation of age or underestimation. Differently the methodology from dental histology allows to obtain the chronology directly through the presence of growth lines in the enamel.

However, the method based on the histology of tooth enamel has its limitations, as we have seen in cases when the formation of the crown is complete. Once formation of the root has begun, analysis of the enamel can only determine whether the individual was born at term but cannot provide the age at death. The growth lines can be analysed in the dentin, where the lines of Von Ebner and

Andersen correspond to the cross-striations and the striae of Retzius respectively [44]. However, observation of these lines is much more difficult except in very exceptional cases.

Another and much more important limitation of dental histology concerns the availability of the tooth and preparation of the thin section for analysis. In 4 out of 9 individuals, the dental histology analysis could not be performed. In the case of individuals 333 and 428 who died in the foetal stage, maturation of the enamel had not ended and the growth lines are not observable. In individuals 326 and 455, who died after birth, the observation of growth lines and prisms is of very poor quality, making it impossible to apply the methodology. This may be accounted for by different factors, such as biogenetic processes, conservation or preparation.

Analyses of infant mortality need to be addressed by age groups, since causes of death have different rates and interpretations in the neonatal and post-neonatal periods. When death is caused by endogenous factors, such as birth/genetic defects, they are fatal within a short period after birth. In contrast, exogenous causes such as infectious diseases and gastrointestinal disorders have a greater incidence in the post-neonatal period [45]. With improvements in treatment and care in neonatal care units, there is a higher survival of premature babies, which has changed the epidemiology of infant mortality. However, premature birth and low birth weight are still variables associated with a higher risk of death [46].

The study of dental histology cannot pinpoint the cause of death but can provide certain indications. In individual 268, the age according to dental histology coincides with the documented chronological age but differs from the age that long bone growth would indicate. This suggests that in this case there may have been a premature birth or severe restriction of longitudinal growth due to conditions of stress. In this same individual there are marked striations after the NNL (Figure 4). This indicates that there were several periods of stress after birth, one very pronounced which resulted in the formation of hypoplasia. Although the cause of death in this case is recorded as non-traumatic cardio-respiratory insufficiency, these signs of stress indicate that the death of this individual at four months of postnatal age was the result of a prolonged process and not of an abrupt event.

Conclusion

The study of dental histology based on the analysis of enamel microanatomy can establish the age at death of neonates. Although this type of analysis requires an invasive technique and has limitations that may prevent its application, the precision that can be obtained could justify its use in many cases. The quality and quantity of data that this technique provides on the growth and health status of individuals is of prime importance for forensic studies and for analyses of historical populations and archaeological remains.

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