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Maximum length of deciduous dentition as an indicator of age during the first year of life: Methodological validation in a contemporary osteological collection

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Maximum length of deciduous dentition as an indicator of age during the first year of life: Methodological validation in a contemporary osteological collection

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Abstract

Out of all the available methods for estimating age at death from immature human skeletal remains, those based on odontometric variables of deciduous dentition have proved to be one of the most accurate. The development of odontometric methods has been improved through the creation of documented human osteological collections, allowing their validation in different populations. The present study aims to test the regression equations for age estimation proposed by Liversidge *et al.* 1993, Irurita Olivares *et al.* 2014, and Cardoso *et al*. 2019, on the basis of the maximum length of deciduous teeth in an Argentinian sample of 35 infants of known age at death. The results showed that the absolute mean difference between estimated and chronological age was 5.76 ± 6.33

weeks for Liversidge's method, 5.71 ± 6.41 weeks for Irurita Olivares's method, and 6.79 ± 5.80 for Cardoso's method. It was also found that, for Liversidge's method, the canines provided the most accurate and the least biased estimations. For Irurita Olivares's method, mandibular anterior teeth were the most accurate, while the first mandibular molars offered the least biased estimations. For Cardoso's method, the canines presented the most accurate estimations, while the lateral incisors the least biased ones. Finally, 95% confidence intervals of estimated ages were calculated for each method, finding that Irurita Olivares's method provided the most reliable age estimations when using mandibular central incisors and mandibular first molars.

Keywords: Forensic anthropology population data, age estimation, deciduous dentition, maximum length.

Introduction

Age estimation from skeletal remains of subadults constitutes one of the most relevant forensic and bioanthropological issues, and requires the understanding of the biological processes that occur during ontogeny, associating the changes in hard tissues to a timescale (Scheuer and Black 2004; Cunha *et al.* 2009; Franklin 2010; Christensen *et al.* 2014; Spake and Cardoso 2018). Several age estimation methods based on different elements of the skeleton have been proposed, and teeth have proved to be the most accurate for estimating age in subadults. Teeth constitute very important elements for age estimation due to several reasons: they are the organs least affected by stress events during ontogeny and, therefore, the ones that present the greatest correlation between biological and chronological age. They are also the elements that show the best preservation in different environments (Saunders *et al.* 1993; Cardoso 2007; Luna 2008; Smith 2010; Elamin and Liversidge 2013).

There are different methods for age estimation of infants from dental remains that take into account eruption and degree of mineralization of dental tissues (Smith 1991; Halcrow *et al.* 2007). Methods based on eruption imply the identification of tooth emergence in relation to alveolar bone. This event is unlikely to be observed in skeletal remains of foetuses and newborns because alveolar tooth eruption is a process that begins around the fourth month of postnatal life, so until this moment there are no teeth erupting that could be used as a reliable indicator of age, and secondly, because during early stages of tooth formation, teeth are generally found loose in the tooth sockets or isolated (Lewis 2007; Saunders 2008; AlQahtani *et al.* 2010). Another problem associated with dental eruption is that it is more influenced by environmental factors than mineralization (Rösing *et al.* 2007; Saunders 2008).

Other methods that utilize teeth for age estimation are those that rely on stages of tooth development (Moorrees *et al.* 1963b; Demirjian *et al.* 1973; Irurita Olivares *et al.* 2014). Also, several studies have proposed methods that combined both tooth formation and alveolar eruption (Schour and Massler 1941; Ubelaker 1978; AlQahtani *et al.* 2010). Finally, some works rely on quantification of microstructural growth markers (Bromage and Dean 1985; Huda and Bowman 1995; Mahoney 2011; Birch and Dean 2014; Nava *et al.* 2017), and on odontometric variables (Deutsch 1985; Liversidge *et al.* 1993; Aka *et al.* 2009; Dagalp *et al.* 2014; Irurita Olivares *et al.* 2014; Minier *et al.* 2014; Viciano Badal *et al.* 2018; Cardoso *et al.* 2019), each method presenting different technical difficulties and degrees of precision. Particularly, odontometric methods are based on correlating age with certain measures of teeth, and from there, regression equations are developed for estimating age at death of non-adult individuals. Deutsch *et al.* 1985, Liversidge *et al.* 1993, Irurita Olivares *et al.* 2014, and Cardoso *et al.* 2019 presented equations for age estimation based on deciduous tooth length. However, the method put forward by Deutsch *et al.* 1985 is the least used due to its limited age range of application, and because it only presents equations for anterior teeth.

Studies on the development of the deciduous dentition from the fetal period to the first year of life have been limited to samples that include this age range, most of them from autopsy cases (Lunt and Law 1974; Sunderland *et al.* 1987; Aka *et al.* 2009; Dagalp *et al.* 2014) or from historical cemeteries (Liversidge *et al.* 1993; Irurita Olivares *et al.* 2014; Cardoso *et al.* 2019). In this context, subadult osteological collections become an important source of information, since they enable the validation of methods proposed for different populations, and an approach to inter- and intra-population variability in the process of tooth development, eventually generating new population-specific reference standards (Cardoso 2007; Halcrow *et al.* 2007; Iscan and Solla Olivera 2000; Hillson 2014).

The aim of this study is to evaluate the accuracy of the age-estimation equations proposed by Liversidge *et al.*1993 (from the English collection of Christ Church Spitalfields), by Irurita Olivares *et al.* 2014 (from the Spanish collection of San Jose’s Cemetery, Granada), and by Cardoso *et al.* 2019 (from the English collection of Christ Church Spitalfields and

the Lisbon collection) on a sample of infants (from birth to the first year of life) from the Lambre collection, Argentina.
This study represents a validation of different age-at-death estimation methods from the maximum length of the deciduous dentition in a contemporary Argentinian skeletal collection with associated personal information. The comparison of estimated age with the chronological age of individuals provides valuable information about the variability in the process of dental formation during the first year of life, and informs about the reliability of odontometric methods for its future application in bioarchaeological contexts and local forensic cases.

Materials and methods

This study was conducted on a sample from the Lambre collection hosted in the Faculty of Medical Sciences (National University of La Plata, Argentina). The collection is composed of 420 skeletons with associated personal information, of individuals who died in the second half of the twentieth century, obtained from the local cemetery of La Plata. One hundred and fifty-seven skeletal remains are from subadult individuals with ages ranging from the fetal period to three postnatal years (Salceda *et al.* 2012).
The study, conservation, and management of human remains in this research was in agreement with current national and international ethic codes (Aranda *et al.* 2014), and 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 2012). Information of age, sex, date and cause of death was obtained from the cemetery death records located in the cemetery archive. Chronological age of death is recorded in hours, days, months, or years after birth depending on the individual. An important fact to be considered is that for fetal individuals gestational age of death is not informed, and for postnatal individuals, if a premature birth has occurred, it is not specified either.
The sample for this study included individuals with developing well-preserved deciduous teeth without pathologies that could modify their morphometric features. Also, in order to avoid the inclusion of preterm individuals, only those individuals whose dental and skeletal ages (estimated by tooth development and length of long bones in a previous work) (García Mancuso 2014) resulted in more than 40 weeks of gestation were included.
The final sample was composed of 295 teeth of 35 infants, 20 males and 15 females, with ages ranging from birth to the first year of life (Fig. 1). Both sexes were considered

together, and estimated age (EA) and chronological age (CA) were standardized in weeks from conception to enable comparison between them. To standardize age in weeks after conception (including pre- and postnatal terms), two basic criteria were taken into consideration: a calendar year has 52 weeks, and births occur at 40 weeks of gestation, meaning that the first postnatal year equals 92 weeks (considering 40 weeks of gestation plus 52 weeks of 1 calendar year) (García Mancuso 2014). The decision to express EA and CA in weeks responds to the fact that the individuals of the sample cover an age range that does not exceed the first year of life (with the majority of individuals being between 40 and 50 weeks of gestational age), and the differences between EA and CA expressed in years are too small to be properly displayed in the results.

Maximum tooth length is defined as the maximum distance between the highest point of the crown and the lowest point of the developing edge, parallel to the longitudinal axis of the tooth. According to tooth type and its formation stage, this measure is taken from the highest point of the central lobe (incisors) or from the cusp tip (canines and molars) to the ridge of the crown or root of the developing teeth (Liversidge *et al.* 1993). Isolated teeth were measured using a digital calliper Schwyz (0.01 mm precision).

Intra- and interobserver error analyses of 36 pairs of measures corresponding to randomly selected teeth (20 single-rooted teeth and 16 molars) were made, and the measurements were taken with an interval of two weeks by one of the authors (SP) for intraobserver error, and by two of the authors (SP, GG) for interobserver error analysis. This subsample did not follow a normal distribution, so a Wilcoxon's test for related samples was used to compare pairs of measures. All statistical analyses were made using SPSS 23.1 considering a level of significance of 0.05.

When the left and right counterparts of the same tooth type were present in an individual, both maximum lengths were measured and compared in order to evaluate bilateral symmetry. Also, a comparison between maxillary and mandibular counterparts by tooth type was made in order to determine if there were significant differences between them. The Kolmogorov-Smirnov test determined that the sample did not follow a normal distribution ($Z_{k\text{-}s}$=0.11; p<0.05), for this reason, non-parametric tests were used for the comparisons.

Age at death was estimated from maximum length of each tooth in the sample using the regression equations proposed by Liversidge *et al.* 1993, Irurita Olivares *et al.* 2014, and

Cardoso *et al.* 2019, and the results obtained in years were transformed into weeks-after-conception and grouped by tooth type (Table 1).

Differences between EA and CA were calculated by tooth type. In order to assess bias in the estimations, differences between estimated age and chronological age were used to determine whether EA underestimated or overestimated CA (a positive value indicated an overestimation and a negative value an underestimation of the CA). To evaluate the accuracy of the methods, absolute differences between EA and CA were calculated allowing quantification of the magnitude of the differences (Lovejoy 1985; Santana *et al.* 2017). A Wilcoxon's test for related samples was applied in order to establish if significant differences existed between EA and CA for each method.

Individual age was calculated as the average of the EA for each tooth present. The variation of the individual EA was evaluated using the within-individual coefficient of variation ($CV= \sigma/\bar{x} * 100$), which expresses the standard deviation of the data as a percentage of the mean (Zar 2010). It was calculated for all individuals, whose ages were estimated using more than one tooth, on the basis of the contribution of different teeth to the overall mean age (Saunders *et al*. 1993).

Additionally, the differences between EA and CA of each individual of the sample were plotted against CA, in order to evaluate if the error in estimations increased with the age of the individuals, as reported in previous studies (Saunders *et al.* 1993; Liversidge 1994; Nawrocki 2010; Cardoso *et al. 2019*). Also, a Spearman's test was used to establish if a significant correlation existed between the variables.

Finally, in order to test the reliability of the methods, as a measure of their effectiveness (Walrath *et al.* 2004), the number of individuals whose true CA fell within the 95% confidence interval (CI) of the EA was quantified for Irurita Olivares's and Cardoso's methods, but not for Liversidge *et al.* (1993), because it did not provide standard deviations or standard errors of the model. For Irurita Olivares's method, 95% CI was calculated using the constants to estimate upper and lower limits for each function (information provided in Table 3, Irurita Olivares's *et al.* 2014). For Cardoso's method, 95% prediction intervals were calculated as detailed in the study, after checking that no recorded tooth measure in the present sample fell out of the length ranges provided by the authors (Cardoso *et al.* 2019).

Results

Intra- and interobserver error analyses did not indicate significant differences in maximum length (intraobserver error Z=-0.48 *p*=0.629; interobserver error Z=0.50 *p*=0.615). Likewise, the comparison between right and left counterparts by tooth type was not statistically significant (Table 2). Consequently, in those cases where teeth from both sides were present an average was calculated.

In turn, the comparison between maxillary and mandibular counterparts by tooth type was only significant for the central incisors. However, the lateral incisors and the first molars presented values that were not statistically significant, only marginal (Table 3).

With the purpose of assessing bias in age estimation, mean differences between EA and CA by tooth type were calculated for each method (Table 4). In the case of the estimations made from the equations proposed by Liversidge *et al*. 1993, the overall mean difference between EA and CA indicated an overestimation of 0.11 weeks. On the other hand, the overall mean values obtained by Irurita Olivares's and Cardoso's methods showed an underestimation of -3.29 weeks in the first case and of -2.56 in the second case.

As a measure of the accuracy of the methods, absolute differences between EA and CA were calculated by tooth type (Table 5). The canines provided the most accurate estimations for Liversidge's (4.13 ± 2.96 weeks for maxillary canines and 3.87 ±3.06 weeks for mandibular canines) and Cardoso's (5.27 ± 4.38 weeks for mandibular canines) methods. While for Irurita Olivares's method, the anterior mandibular teeth proved to be the most accurate, with a mean difference for all anterior mandibular teeth of 4.73 ± 6.33 weeks. On the other hand, for all methods, the second molars presented the greatest differences between estimated and chronological ages.

Wilcoxon's test showed that no significant differences existed between EA and CA for any tooth type using Liversidge *et al.* 1993 method. However, using the method developed by Irurita Olivares *et al.* 2014, significant differences were found in central maxillary incisors. When considering Cardoso's method, the difference between EA and CA was significant for three of the five mandibular tooth types analyzed (Table 6).

Variation in individual's overall mean EA was evaluated by the CV considering the contribution of different teeth to the overall mean age. The CV calculated by individual presented a minimum of 2% and a maximum of 13.52% for the EA by Liversidge *et al.* 1993; a minimum of 0.63% and a maximum of 13.78% in the EA by Irurita Olivares *et al.* 2014, and a minimum of 3.11% and a maximum of 19.24% for age estimations made by Cardoso *et al*. 2019.

Additionally, to assess whether there is a tendency for error estimations to increase with the age of the individuals, the mean difference between EA and CA was plotted against the CA of individuals in the sample for the three methods (Fig. 2). For Liversidge's and Cardoso's methods, no bias tendency related to age was observed (Fig. 2 a, c). On the other hand, Irurita Olivares's method showed a tendency to increase the error in estimations along with age, particularly to underestimate age in older individuals (Fig. 2 b). The Spearman's correlation test showed that for Liversidge's (r^2= -0.01; p= 0.92) and Cardoso's (r^2= -0.14; p= 0.45) methods there was not a significant correlation between the variables, while for Irurita Olivares's method, the correlation was significant (r^2= -0.34; p= 0.04).

To test the reliability of the methods, the 95% confidence interval (CI) of the EA was calculated for Irurita Olivares's and Cardoso's methods. The number of teeth and individuals whose true CA fell inside the 95% CI were quantified as correct assignations, and those that fell outside the range were quantified as incorrect (Tables 7, 8). The mean range of the 95% CI for Irurita Olivares's method was of 13.59 weeks, while Cardoso's method exhibited a 95% CI mean range of 50.33 weeks.

For Irurita Olivares's method, the teeth that provided the highest percentages of correct assignations were the mandibular central incisors and the first mandibular molars. On the other hand, maxillary canines provided the lowest percentages of correct assignations. For Cardoso's method, all tooth types exhibited percentages of correct estimations of 100%, except for the first molars, which showed 93.75% of correct assignations.

On the other hand, when quantifying the number of individuals whose true CA fell inside the 95% CI, it was found that in 72.42% of the cases CA was correctly predicted by Irurita Olivares's method, while by Cardoso's method, CA could be correctly predicted in 100% of the cases.

Discussion

Osteological collections with reliable age-at-death information are a useful source for the development and validation of methods that use odontometric variables for age estimation of human skeletal remains. As the validity of age estimation methods of forensic and bioarchaeological application should be assessed in the population in which they are intended to be used (Staaf *et al.* 1991; Nawrocki 2010; Stull *et al.* 2014; Henderson and Alves Cardoso 2018), in the present study, equations developed in Spitalfields, Granada, and Lisbon collections were tested in the Lambre Collection, evaluating the accuracy and bias of these equations on an Argentinian documented collection.

When considering the bias of the estimations for the three methods, it could be established that Irurita Olivares's and Cardoso's methods tended to underestimate CA, while the method developed by Liversidge *et al.* 1993 showed a slight tendency to overestimation of CA. In contrast to the results obtained by Liversidge's method, Cardoso's method presented a trend towards underestimating CA, which is especially striking taking into account that Cardoso *et al.* 2019 in their sample included individuals from Spitalfields original sample, analyzed by Liversidge *et al.* 1993. However, these results could be reflecting the fact that Cardoso's sample included a greater number of individuals older than one year than Liversidge's sample, producing an increase variation for age, and a tendency to increase bias when using this method to estimate age in a sample of individuals younger than one year.

The underestimation of the CA of the individuals of the Lambre collection when applying Irurita Olivares's method could be attributed to the fact that the sample used in the present study might include individuals of preterm birth, not detected by the exclusion/inclusion criteria that, as a consequence, might show a discrepancy between dental and chronological age (Smith 1991). Moreover, considering that the Granada subadult sample has a similar age distribution to the Lambre collection, and that despite this, the method developed by Irurita Olivares's is the one that provided the greatest underestimations, this could be reflecting the presence in the Lambre collection of individuals with smaller teeth than those from Granada for the same age.

The accuracy of the methods, represented by the absolute mean difference between EA and CA, was of 5.76 ± 6.33 weeks for Liversidge's method, 5.71 ± 6.41 weeks for Irurita Olivares's method, and 6.79 ± 5.80 for Cardoso's method. However, not all tooth types presented the same accuracy. The equations proposed by Liversidge *et al.* 1993 and by Cardoso *et al*. 2019 made it possible to obtain the highest degrees of accuracy when using the canines, while the equations proposed by Irurita Olivares *et al.* 2014 provided the most accurate estimations from mandibular incisors and canines. It should be pointed out that the equations proposed by Liversidge *et al.* 1993 combined maxillary and mandibular teeth, a methodology that was indicated by Cardoso 2007 and Irurita Olivares *et al*. 2014 as a factor that could produce inaccuracy in age estimation of their samples (Cardoso 2007; Irurita Olivares *et al.* 2014). The comparison of maxillary and mandibular teeth in the Lambre collection showed that, at least for incisors and first molars, the maximum length differed between superior and inferior tooth types. However, no differential accuracy was identified between maxillary and mandibular teeth, when considering Liversidge's method. The second molars represent the teeth with the greatest error in age estimation assessments by the three methods. At this point, it must be taken into account that second molars are the last deciduous teeth to be formed and, therefore, the ones that appeared underrepresented in this sample of infants that do not exceed the first year of life. For this reason, it is not clear if this trend for second molars to provide the greatest errors in the estimations is a true tendency, or an artifact resulting from the small number of teeth in the analyzed sample.

The CV has been used in previous studies as a measure of the variation contributed by different teeth to the overall mean age estimate (Smith 1991a; Saunders 1993; Cardoso 2007). In the present study, the calculated CV did not exceed 20% for any of the estimated ages by the three methods, results that agreed with those obtained by Cardoso 2007. Likewise, the CV range of variation reported by Saunders *et al.* 1993 for the permanent dentition (1-52%) was broader than the one obtained here for the deciduous dentition (Saunders *et al.* 1993). In the case of Cardoso 2007, the individuals of the sample presented five or less deciduous teeth for the estimations; in the case of Saunders *et al.* 1993 the majority of individuals presented one to six teeth for the estimations, and in the present study, the number of teeth present by individual ranged from one to ten, with an average of five. However, this information is difficult to analyze given that, to compare the CV results between samples, the same tooth types should be used in the estimations, especially taking into account that there is a variation in the results yielded by different types of teeth (Smith 1991a).

Several studies have proposed that, as the age of an individual increases, the sources of variation in the growth process also increase, and this could lead, as a consequence, to a decrease in the correlation between chronological and biological age (Saunders *et al.* 1993; Lewis and Flavel 2006; Lewis 2007; Saunders 2008; Nawrocki 2010). Even though dentition has proved to be a more accurate age estimator than other skeletal variables, it might be expected that age estimations from dental lengths will present greater errors when the age of the individuals increases (Smith 1991; García Mancuso 2014). This might be reflected in the significant correlation found between the difference EA - CA and chronological age when using Irurita Olivares's method, where a tendency to underestimate age in the older individuals of the sample can be seen. This correlation was not observed when using Liversidge's and Cardoso's methods, which did not present any bias tendency by age. However, two factors could be hindering the observation of a tendency to an increment in the error along with age: in the first place, the diminished number of individuals older than 60 weeks in the Lambre sample, and in the second place, the fact that the most represented age range in this study is 40-50 weeks, which, as said before, could include preterm individuals with small teeth for the age, producing an increment in the variation of the errors in the beginning of the age distribution of the sample.

Size and age distribution of the samples, as well as the statistical models used to derive equations, are important factors to be considered when validating age estimation methods, especially, to avoid confusing sample issues with population's growth differences (Franklin 2010; Nawrocki 2010; Cardoso *et al.* 2019). In this sense, it should be highlighted that the results of this study are clearly influenced by the age distribution of the sample, with the greatest number of individuals in the age range of 40-50 weeks, so it becomes particularly relevant for this age range.

It is relevant to distinguish that Cardoso *et al.* 2019 used a classical calibration for the development of age estimation equations, a factor that could produce a variation in the bias and accuracy of the method in comparison with the inverse calibration model used in the other two methods analyzed. Using inverse calibration implies that age is modeled as dependent on tooth length, resulting in a systematic bias, as noted by different authors (Aykroyd *et al.*1997; Cardoso *et al*. 2014; Cardoso *et al.* 2019). Classical calibration, on the other hand, which models age as the independent variable, presents a reduction in bias but also in the efficiency of estimates, with a larger variability than the methods derived from inverse calibration (Cardoso *et al.* 2014). However, it is not clear at what extent the differences in bias and accuracy results obtained by the three methods in this study respond to the statistical models from which the equations were derived, or to the variation ranges produced by the age distribution of the different samples.

To assess the reliability of the methods, the number of correct age predictions by tooth type and individual was quantified after establishing if CA fell inside the prediction intervals of the estimation. Liversidge *et al.* 1993 did not provide data to obtain these values, for this reason the method was excluded of this analysis. This fact highlights the importance of providing values to calculate the confidence limits when developing an age estimation method (Cardoso *et al.* 2019).

For Irurita Olivares's method, the most reliable teeth for age estimation were the mandibular central incisors and the mandibular first molars. Even though the second molars showed the greatest percentages of correct estimation, the small number of these teeth in the sample needs to be considered. On the other hand, the maxillary canines were found to be the less reliable teeth. For Cardoso's method, all teeth proved to be highly reliable with almost 100% of correct estimations.

When quantifying the number of individuals whose true chronological age fell within the prediction interval, Irurita Olivares's method correctly predicted chronological age in a 71.42% of the individuals, while Cardoso's method achieved 100% of correct individual age predictions. The high percentages of correct predictions made by Cardoso's method reflect the wide range of prediction intervals offered by this method. Even though the accuracy and bias of Cardoso's method proved acceptable for the age range of the Lambre sample, the wide prediction intervals make it less reliable than Irurita Olivares's method (White *et al*. 2012). This is clearly observed when calculating the mean range of the CI, which was 50.33 weeks for Cardoso's method, covering almost the total age range of the sample. On the other hand, the mean range of the CI calculated for Irurita Olivares's method was 13.59 weeks. It should be pointed out that Irurita Olivares's 95% narrow CI is not a prediction interval rather the 95% CI for the regression parameters and, as noted by Cardoso *et al.* 2019, this provides little information related to the variation about the regression line.

In addition, when comparing the results of the number of correct age predictions by individual and by tooth type for Irurita Olivares's method, the performance of the different teeth is highlighted. Even though this method has a lower percentage of correct individual age prediction than Cardoso's method, when utilizing central mandibular incisors and first mandibular molars, age was correctly predicted in 82.75% and 87.50% respectively, thus allowing to assert the reliability of these equations to estimate the age of infants from birth to one postnatal year.

Conclusions

The aim of this study was to validate three age-at-death estimation methods based on maximum length of deciduous dentition in an infant sample from the Lambre collection (Argentina). The methods proved to be accurate, although the results differed according to tooth type, being the canines the ones that provided the most precise estimations, and the second molars the ones exhibiting the greatest errors. Likewise, Liversidge's method tended to a slight overestimation of CA, while Irurita Olivares's and Cardoso's methods tended to underestimate CA. Also, Irurita Olivares's method tended towards an increase in the error in older individuals. However, the application of Irurita Olivares's method was found to be more precise than Cardoso's, since the latter had prediction intervals too wide for the age range of the sample.

Finally, the importance of validating the methods developed in other populations is highlighted, for which purpose documented osteological collections of subadults are essential, as they allow comparison of biological estimated age with chronological age, and therefore establish the accuracy of a method for forensic application.

CRediT Author Statement

Selene Petrone: Writing- Original draft preparation, Investigation and Conceptualization

Gonzalo Garizoain: Investigation, validation and writing.

Rocío García-Mancuso: Conceptualization and investigation.

Ana María Inda: Supervision and Project administration.

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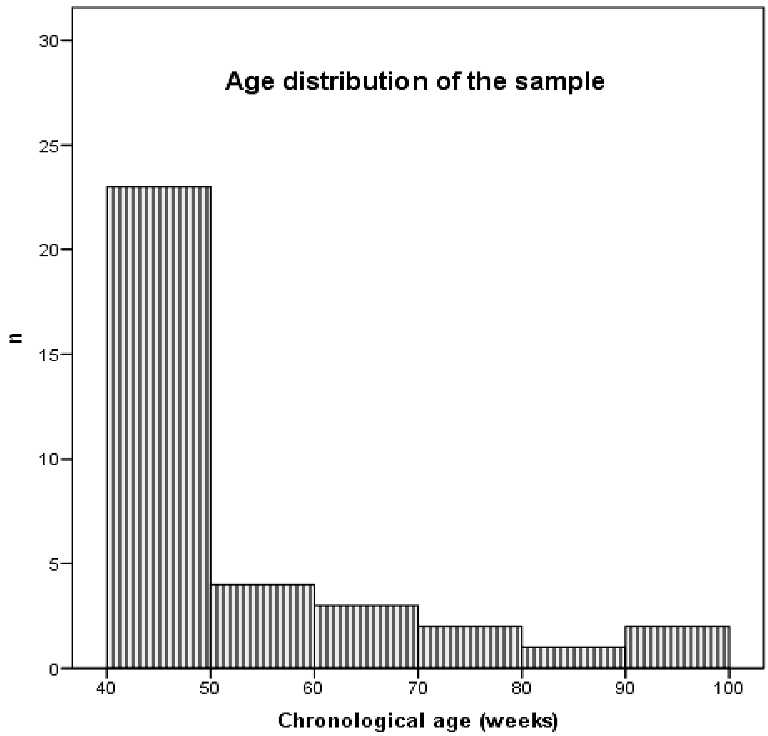

Fig. 1. Age distribution of the sample.Chronological age is expressed in weeks and 40 weeks coincide with the moment of birth

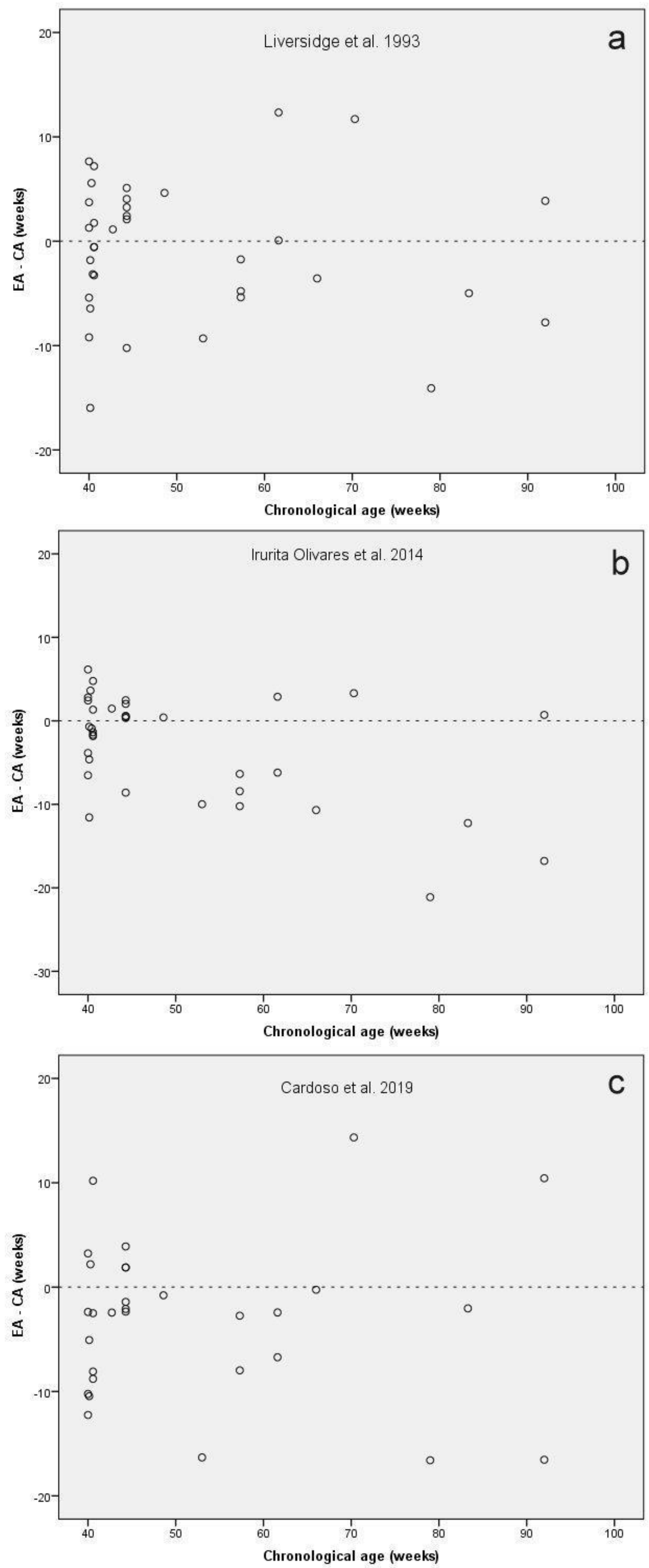

Figure 2. Scatter plot graph of the association between EA-CA and CA for Liversidge's (a), Irurita Olivares's (b), and Cardoso's (c) methods.

Table 1. Regression equations developed by Liversidge *et al.* 1993 (combining maxillary and mandibular teeth), Irurita Olivares *et al.* 2014 (specific equations for each tooth type), and Cardoso *et al.* 2019 (only for mandibular dentition), for age estimation from maximum length of deciduous teeth. x=tooth length, y= estimated age. (i^1: central maxillary incisor; i_1: central mandibular incisor; i^2: lateral maxillary incisor; i_2: lateral mandibular incisor: c': maxillary canine; c,: mandibular canine; m^1: first maxillary molar; m_1: first mandibular molar; m^2: second maxillary molar; m_2: second mandibular molar). (1) Estimated age in postnatal years; (2) Estimated age in years (includes gestational period); (3) Estimated age in postnatal years.

Tooth	Liversidge *et al.* 1993 (1)	Irurita Olivares *et al.* 2014 (2)	Cardoso *et al.* 2019 (3)
i^1	$y=-0.653+0.144*x$	$y=0.411*2.718^{0.120*x}$	
i_1		$y=0.457*2.718^{0.122*x}$	$y=(x-4.84)/5.53$
i^2	$y=-0.581+0.153*x$	$y=0.462*2.718^{0.123*x}$	
i_2		$y=0.492*2.718^{0.118*x}$	$y=(x-4.19)/5.51$
c'	$y=-0.656+0.210*x$	$y=0.550*2.718^{0.124*x}$	
c,		$y=0.544*2.718^{0.131*x}$	$y=(x-3.54)/4.26$
m^1	$y=-0.814+0.222*x$	$y=0.446*2.718^{0.154*x}$	
m_1		$y= 0.505*2.718^{0.139*x}$	$y=(x-4.06)/4.06$
m^2	$y= -0.904+0.292*x$	$y= 0.474*2.718^{0.179*x}$	
m_2		$y= 0.505*2.718^{0.168*x}$	$y=(x-3.27)/3.38$

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Table 2. Comparison between right and left counterparts. Mean, standard deviation, Wilcoxon (Z), and significance (*p*) of the difference in maximum length.

Tooth	n	Mean	SD	Z	*p*
i^1	11	-0.01	0.20	0.40	0.68
i^2	15	-0.11	0.18	1.84	0.06
c’	7	-0.08	0.31	0.93	0.35
m^1	11	0.07	0.14	-1.04	0.15
m^2	6	0.12	0.11	-1.90	0.05
i_1	16	0.00	0.19	0.18	0.85
i_2	13	-0.02	0.15	0.66	0.50
c,	13	-0.01	0.13	0.07	0.94
m_1	10	0.41	1.64	0.10	0.91
m_2	4	0.02	0.10	-0.50	0.58

Table 3. Comparison between maxillary and mandibular counterparts. Mean, standard deviation, Wilcoxon (Z), and significance (*p*) of the difference in maximum length (*p<0.05).

Tooth	n	Mean	SD	Z	*p*
i^1 - i_1	24	0.65	0.39	-4.20	**0.000***
i^2 - i_2	22	0.21	0.47	-1.91	0.055
c'- $c_,$	14	0.08	0.23	-1.18	0.235
m^1 - m_1	15	0.15	0.27	-1.72	0.084
m^2 - m_2	7	0.13	0.40	-0.84	0.398

Table 4*.* Mean, minimum, maximum, and standard deviation of the differences between EA and CA (in weeks) for the three methods.

		Liversidge *et al.* 1993				Irurita Olivares *et al*. 2014				Cardoso *et al*. 2019			
Tooth	n	Mean	Min.	Max.	SD	Mean	Min.	Max.	SD	Mean	Min.	Max.	SD
i^1	24	1.73	-15.98	19.10	7.94	-4.40	-22.18	9.09	6.83	-	-	-	-
i^2	23	0.59	-13.30	13.59	7.47	-3.02	-16.91	7.56	6.50	-	-	-	-
c'	14	0.33	-6.72	10.07	5.20	-4.21	-21.56	5.30	7.56	-	-	-	-
m^1	18	-1.30	-18.76	7.63	7.31	-4.78	-25.57	3.92	8.55	-	-	-	-
m^2	9	0.85	-17.07	17.33	10.73	-5.26	-23.45	6.28	9.08	-	-	-	-
i_1	29	-2.38	-15.97	8.75	6.53	-1.66	-18.87	15.46	7.06	-2.97	-17.84	18.81	8.27
i_2	26	1.25	-12.57	10.27	6.36	-1.85	-19.73	6.46	6.29	0.04	-15.25	11.98	7.16
c,	19	-0.08	-10.42	7.41	5.02	-2.40	-17.81	4.58	6.11	-3.58	-15.20	7.57	5.91
m_1	16	-0.53	-23.95	18.57	9.95	-0.25	-26.61	8.43	9.29	-4.59	-27.34	18.88	10.55
m_2	8	0.65	-18.59	16.42	10.95	-5.07	-23.64	5.67	10.66	-1.70	-21.00	14.25	11.02

Table 5. Mean, minimum, maximum, and standard deviation of the absolute differences between EA and CA (in weeks) for the three methods.

		Liversidge et al. 1993				Irurita Olivares et al. 2014				*Cardoso et al. 2019*			
Tooth	n	Mean	Min.	Max.	SD	Mean	Min.	Max.	SD	Mean	Min.	Max.	SD
i^1	24	7.04	1.30	19.10	4.40	6.08	0.73	22.18	5.31	-	-	-	-
i^2	23	5.97	0.15	16.59	4.35	5.24	0.53	16.91	4.81	-	-	-	-
c’	14	4.13	0.90	10.07	2.96	5.67	0.03	21.56	6.45	-	-	-	-
m^1	18	5.91	0.98	18.76	4.27	6.83	0.22	25.57	6.92	-	-	-	-
m^2	9	8.35	0.07	17.33	6.12	8.04	1.32	23.45	3.39	-	-	-	-
i_1	29	5.23	0.14	15.97	4.49	4.97	0.13	18.87	5.21	6.65	0.12	18.81	5.64
i_2	26	5.37	0.17	12.57	3.48	4.53	0.64	19.73	4.67	5.80	0.61	15.25	4.04
c,	19	3.87	0.18	10.42	3.06	4.71	0.47	17.81	4.47	5.27	0.52	15.20	4.38
m_1	16	6.92	0.11	23.95	6.95	6.75	0.44	26.61	6.14	7.89	0.79	27.34	8.21
m_2	8	7.79	0.33	18.59	7.15	7.88	0.50	23.64	8.49	8.35	1.70	21.00	6.72

Table 6. Wilcoxon (Z) and significance (*p*) of the comparison between EA and CA by tooth type.**p*<0.05.

		Liversidge *et al.* 1993		Irurita Olivares *et al.* 2014		Cardoso *et al.* 2019	
Tooth	n	Z	*p*	Z	*P*	Z	*p*
i^1	24	1.11	0.26	-2.61	**0.01***	-	-
i^2	23	0.48	0.62	-1.39	0.16	-	-
c’	14	-0.15	0.87	-0.35	0.72	-	-
m^1	18	-0.54	0.58	-1.26	0.20	-	-
m^2	9	0.41	0.67	-1.27	0.20	-	-
i_1	29	-1.41	0.15	-1.09	0.27	-2.1	**0.03***
i_2	26	1.07	0.28	-0.73	0.46	0.29	0.77
c,	19	0.36	0.71	-0.19	0.84	-2.45	**0.01***
m_1	16	0.46	0.64	1.12	0.26	-2.01	**0.04***
m_2	8	0.00	1.00	-0.41	0.67	-0.56	0.57

Table 7. Percentages (%) of correct/incorrect age estimations of the sample by tooth type for Irurita Olivares's and Cardoso's methods.

	Irurita Olivares *et al*. 2014				Cardoso *et al*. 2019			
	Correct		Incorrect		Correct		Incorrect	
Tooth	n	%	N	%	n	%	n	%
i^1	15	62.50	9	37.50	-	-	-	-
i^2	15	65.20	8	34.80	-	-	-	-
c'	7	50.00	7	50.00	-	-	-	-
m^1	12	66.66	6	33.34	-	-	-	-
m^2	8	88.88	1	11.12	-	-	-	-
i_1	24	82.75	5	17.25	29	100.0	0	0.00
i_2	17	65.38	9	34.62	26	100.0	0	0.00
c,	13	68.42	6	31.58	19	100.0	0	0.00
m_1	14	87.50	2	12.50	15	93.75	1	6.25
m_2	6	75.00	2	25.00	8	100.0	0	0.00

Table 8. Percentages (%) of individuals whose true chronological age fell inside the 95% CI for both methods.

	Irurita Olivares *et al.* 2014				Cardoso *et al.* 2019			
	Correct		Incorrect		Correct		Incorrect	
	n	%	N	%	n	%	n	%
Individual	25	71.42	10	28.58	30	100.0	0	0