Forensic Science International 231 (2013) 397.e1–397.e5

Radiographic fetal osteometry: Approach on age estimation for the portuguese population

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A B S T R A C T
The estimation of gestational age (GA) on fetal remains can be an important forensic issue. Forensic specialists usually use reference tables and regression equations derived from reference collections, which are quite rare in what fetuses are concerned. Since these tools are mostly grounded on ultrasonographic measurements, which are known to differ from real bones measurements or are based on ancient literature, this study aimed the construction of tables and regression equations for the Portuguese population on the basis of diaphyseal bone length measurements (femur, tibia and humerus) of 100 fetuses of known GA, using post-mortem radiographs. There is a strong correlation between the longitudinal length of studied bones and GA; the femur exhibits the strongest correlation ($r = 0.969$; $p = 0.000$), followed by the tibia ($r = 0.966$; $p = 0.000$) and the humerus ($r = 0.963$; $p = 0.000$). Therefore it was possible to obtain regression equations and to build tables with reference values for each of the diaphysis analyzed.

1. Introduction

Fetal age estimation is still a difficult task, especially since this kind of remains is unusually found, making it hard for the anthropologist to become comfortable when studying it [1,2]. However, it can be of important forensic value, particularly when it is necessary to determine the fetus viability, or in other words, if the fetus could have been born alive [3–7], even knowing that the skeleton alone will not convey the information about whether the fetus was born alive or dead, unless it is regarding neonate's remains [1–3].

The two main criteria used for fetal age estimation are dental mineralization and skeletal data, such as long bone diaphyseal length [1,3,6], which is highly correlated with gestational age [8– 13] and quite resistant to decomposition when comparing with other fetal structures [2,3,5,8]. Although dental age is recognized as more reliable than skeletal age, in many forensic instances the human remains do not include dentition. It is thus of upper-most importance to test the reliability of diaphyseal length for the purpose of age evaluation. Furthermore, recent studies advocate that the derived regression equations used for this estimation are, in some way, specific for each population and should be based on recent well-documented samples. As shown by various studies, size at birth is affected by secular trends which affect fetal length and depends on factors such as environmental improvements and socio-economical status [14–16]. Considering the size of fetal bones, even small differences may cause a big impact in age determination and, therefore, affect the outcome of forensic cases. When estimating skeletal age, forensic specialists typically use reference tables and specific regression equations that are derived from reference samples such as osteological collections. Yet, the lack of large identified skeletal collections including fetuses precludes the existence of appropriate formulae [8]. Consequently, these tables are mostly based on ultrasonographic measurements [9–12] that might differ from actual measurements on dry bone [17,18]. Moreover, formulae applicable on dry bones have twosteps and are mostly supported by outdated literature [8,13]. Until now, the most used reference to estimate fetuses' age at death has been Fazekas e Ko´ sa's data, which dates back to the 1970s and was derived from historical and not identified samples [8]. The need to develop new standards and to evaluate their specificity in relation to the population on the basis of which they were developed has become obvious. Recent studies, as is the case of Adalian et al. [4,17], who validated a methodology using measurements made on radiographies, which are more reliable than ultrasound measurements, used an identified sample, obtained from a hospital database.

As such, the main goal of the present study was to update fetal radiographic data for the Portuguese population, using a validated method [17]. The authors also sought to identify which of the three studied bones (femur, tibia and humerus) was more accurate to estimate gestational age. Also, it was intended to assemble reference tables for each bone to simplify gestational age comparisons. The final purpose of this study was to compare the obtained equation for the femur with the formulas developed by other authors with the same aim, namely those from Fazekas and Kosa, Adalian et al. and Scheuer and Black [8,17,19].

2. Materials and methods

The present study was based on a validated method, using plain radiographs (XR) from fetuses of known gestational age. The decision of employing XR measurements was due to the fact that they are more reliable than ultrasound measurements and can easily be used when the subject of study (in forensic context, for instance) retains soft tissues [4,17,18]. This was a retrospective cohort study. Anonymous fetopathological autopsy records from spontaneous and therapeutic abortions (meaning that none of the fetuses was born alive), performed at Hospital Garcia de Orta, E.P.E. (Almada, Portugal) were collected. All abortions occurred between 2000 and 2011. There were no ethical issues involved since there was authorization to perform plain radiographs and autopsies following the hospital's protocol, as well as to use these data in further investigations; on the other hand, there was no additional manipulation of the fetuses. The sample used in the present study consists of 100 fetuses (55 males; 45 females) with an age range between 13 and 40 weeks of gestational age (GA). The mean age at death is 26.11 weeks (SD = 7.74). The selection of the fetuses was made according to the following criteria:

_ GA between 13 and 40 weeks;
_ Absence of external limb malformation;
_ Absence of pathological alterations which could compromise normal skeletal
growth (e.g. Intra Uterine Growth Restriction);
_ Lack of maternal pathology;
_ Time elapsed between intrauterine death and fetal expulsion inferior to a week;

_ Twin pregnancies were included only when there were no signs of discordant
growth.

Diaphyseal bone length measurements of the femur, the tibia and the humerus were performed using post-mortem radiography (XR), taken with Siemens Mobilett II equipment (Global Siemens Healthcare Headquarters – Siemens AG, Healthcare Sector, Henkestrasse 127, D-91052 Erlangen, Germany); XR were then stored in a software application called Centricity1 Radiology, developed by General Electric Company_ (GE Healthcare Global Headquarters, Pollards Wood, Nightingales Lane, Chalfont St. Giles HP8 4SP, United Kingdom). Considering that XR records are collected form a hospital background, the fetuses belong to an identified sample, which is of great empirical value to develop formulas for each population [4,17].

Measurements of the larger dimension of the three long bones chosen for this study were taken with a 0.5 mm graduated metal ruler. Whenever it was necessary, the obtained value was converted to scale (included in the XR). As a rule, the measurements were performed on the left side, with the fetus placed anteroposteriorly (Fig. 1), otherwise the measurements were taken with the fetus placed laterally (Fig. 2). The calculation of GA was made in weeks, following the standard terminology used in obstetrics [9–11] and forensic sciences [7]. Classical formulae calculate GA in lunar months [8,13].

Statistical analysis was performed with SPSS1 (Statistical Package for the Social Sciences) 17.0. Gestational age and the longitudinal dimensions of the long bones were treated as continuous variables. The normal distribution of the variables was assessed through the skewness and kurtosis of the distributions [20] and the Q–Q plots. The equality of variances was evaluated with a Levene's test. All the variables are modeled by a normal distribution. The reliability of the method was evaluated with the relative Technical Error of Measurement (rTEM) [21]. A Student's t-test for independent sampled was used to evaluate if gender affected the length of the long bones. A linear inverse calibration model was used to predict gestational age at death, with gestational age as the response variable. 95% CI formulae were also included to encompass a range within which the parameter ''gestational age'' is estimated to be located. In order to construct easy access reference tables, the sample was divided in six groups comprising five gestational weeks. The authors also compared the formulae obtained in this study with similar ones, namely those calculated by Fazekas and Ko´ sa [8], Adalian et al. [17] and Scheuer and Black [19], in a validation sample, meaning that these fetuses were not used to obtain the new equations.

3. Results and discussion

To control the accuracy and precision of the measurements, the relative Technical Error of Measurement (rTEM) [21] was calculated. The lower the value, the better is the result. In this case, a value under 1% is considered acceptable for skillful anthropometrists, which means that these results are good, only the humerus is slightly above this limit. The intra-observer error (repeatability) results are presented in Table 1. The same is seen when evaluating the inter-observer error (reproducibility) where a value under 1,5% is considered acceptable for skillful anthropometrists. The results obtained in the present study are shown in Table 1. In this case, we can assert that the method used in this study provides reproducible results.

In the studied sample, longitudinal dimensions of the diaphysis of long bones are not significantly different between males and females (Femur: Student's t = 1.533; df = 98; p = 0.129; Tibia: Student's t = 1.698; df = 97; p = 0.093; Humerus: Student's t = 1.565; df = 98; p = 0.121). As expected [8–13], there is a very strong positive correlation between longitudinal length in the studied bones and documented GA. The correlation between the femur length and GA was the strongest (Pearson's r = 0.969; p = 0.000, see Fig. 3), followed by the tibia (Pearson's r = 0.966; p = 0.000) and the humerus (Pearson's r = 0.963; p = 0.000).

The obtained regression equations appear to be very useful for making predictions about GA, with each model explaining a very high percentage of total variance (Table 2). The use of conventional regression analysis to relate the indicator variable (in this study: the longitudinal length in long bones) with age (in this study: gestational age) involves a systematic bias in age estimation: there is a tendency to overestimate age in younger individuals, and to underestimate it in older individuals [22,23]. Claude Masset [24] suggested that this trend is related to the age distribution of the reference sample but it has been also proposed that the use of age as the dependent variable in the regression analysis weakens the estimates [25]. Notwithstanding, this bias is highly determined by the coefficient of correlation (r) and the coefficient of determination (r^2): a poorer correlation ($r < 0.7$) entails a greater bias. There are some circumstances when the inverse calibration method performs very well, one of them being when the age indicator is almost perfectly correlated with age [26]. That is the case of longitudinal length of the long bones in fetuses and gestational age. Besides obsolescent, formulae typically used by forensic anthropologists [8,13] are actually two-steps formulae: first they transform the bone length in body size and then they convert the body size into GA. On the other hand, one-step formulae are quicker in the estimation of GA and less prone to errors.

It was possible to build quick reference tables with the values of each bone for six age groups (in weeks of gestation), as follow (Tables 3–5). These tables are easy to use. The mean length of each type of bone, the standard error with a 95% confidence interval and the number of cases studied is given. From the tables observation, it can also be seen that there are some groups with few individuals; on the other hand, the groups with both special and legal interest, as is the limit of fetal viability (capability of surviving outside the uterus) which happens around the 24th gestational week, and the term of pregnancy (after the 37th week) have, respectively, 21 and 15 elements. Anyway, it is necessary to enlarge the sample in order to validate the results, which is being done.

The new regression equation for the femur was compared with two similar formulae, namely those obtained by Adalian et al. [17] and Scheuer and Black [19]. Adalian's sample is very similar to the one used in the present study (recent fetuses of known GA, from a European [French] hospital context) [17]. Scheuer and Black's study [19] is considered a reference; they also used fetuses of known GA but their sample comprises only individuals between 24 weeks of GA and six post-natal weeks. Both studies employed XR to obtain data. The results of the application of Fazekas and Ko´ sa's equation [8] were also compared with the new formula, since it is recurrently used in forensic context. The major problem with this procedure is that it first calculates body length, and only then GA is obtained in an age range of lunar months. The GA had to be converted to weeks, in order to compare the results obtained with the other three equations. Only the femur was compared since this bone is more closely related to fetal length and GA [3,9,13,18,19]. In order to avoid methodological bias, fifteen fetuses of known gestation age not included in the original regression analysis were used to evaluate which formula is the most reliable to estimate GA in Portuguese fetal remains. The results are collected in Table 6 and include the calculated GA (rounded to the closest whole number) and their difference to the real GA (residual).

The formula obtained in this study presents excellent results, comparable to the one by Adalian et al. [17], since the mean value of the residuals is slightly above 2 weeks in both cases (2.1 with the new formula and 2.3 with Adalian's formula). The application of Scheuer and Black's formula [19] tends to overestimate GA (the mean of the residuals is 4.7 weeks). The results obtained when applying Fazekas and Ko´ sa's formula [8] are similar to those calculated with the new and with Adalian's formula (the mean of the residuals is 2.1 weeks). But, as said before, the use of a single regression equation (as the one calculated in this study) is a much easier and quicker approach. The regression equation presented in the present study has the added advantage of being based on a Portuguese population, meeting the conditions to be tested on a larger sample.

4. Conclusions

In the present work, it was possible to obtain a useful regression equation for each studied bone: femur, tibia and humerus. Furthermore the goal of building quick reference tables for six age groups for each bone measured was achieved. In order to solidify the equations, the authors are currently enlarging the sample and plan to present the results in a near future, as well as to add the measurements of other long bones. Furthermore, in order to assure the use of the obtained results in every case, it is intended to apply the new equations on dry bones.

References

[1] E. Cunha, E. Baccino, L. Martrille, F. Ramsthaler, J. Prieto, Y. Schuliar, N. Lynnerup, C. Cattaneo, The problem of aging human remains and living individuals: a review, Forensic Sci. Int. 193 (2009) 1–13.

[2] L. Scheuer, Application of osteology to forensic medicine, Clin. Anat. 15 (4) (2002) 297–312.

[3] L. Scheuer, S. Black, The Juvenile Skeleton, Elsevier, London, 2004.

[4] M.-D. Piercecchi-Marti, P. Adalian, B. Bourliere-Najean, J. Gouvernet, M. Maczel, O. Dutour, G. Leonetti, Validation of a radiographic method to establish new fetal growth standards: radio–anatomical correlation, J. Forensic Sci. 47 (2002) 328–331.

[5] L.L. Klepinger, Fundamentals of Forensic Anthropology, Wiley-Liss, NJ, 2006.

[6] A. Huxley, R. Froede, W. Birkby, Strangulation of pregnant woman leads to one first-degree murder indictment for the death of the mother, Am. J. Forensic Med. Pathol. 22 (2001) 51–54.

[7] A. Huxley, J. Angevine Jr., Determination of gestational age from lunar age assessments in human fetal remains, Forensic Sci. 43 (6) (1998) 1254–1256.

[8] I.G. Fazekas, F. Ko´ sa, Forensic Fetal Osteology, Akade´miai Kiado´ , Budapeste, 1978.

[9] F.P. Hadlock, R.B. Harrist, R.L. Deter, S.K. Park, Fetal femur length as a predictor of menstrual age: sonographically measured, Am. J. Roentgenol. 138 (1982) 875–878.

[10] F.P. Hadlock, Ultrasound determination of menstrual age, in: P.W. Callen (Ed.), Ultrasonography in Obstetics and Gynecology, Saunders, Philadelphia, 1994, pp. 86–101.

[11] P.M. Doubilet, C.B. Benson, P.W. Callen, Ultrasound evaluation of fetal growth, in: P.W. Callen (Ed.), Ultrasonography in Obstetrics and Gynecology, 4th ed., Saunders, Philadelphia, 2000.

[12] P. Jeanty, C. Kirkpatrick, M. Dramaix-Wilmet, J. Struyven, Ultrasonic evaluation of fetal limb growth, Radiology 140 (1981) 165–168.

[13] G. Olivier, H. Pinneau, Nouvelle de´ termination de la taille foetale d'apre` s les longueurs diaphysaires des os longs, Ann. Med. Leg. 40 (1960) 141–144.

[14] L. Schack-Nielsen, C. Mølgaard, T.I. Sørensen, G. Greisen, K.F. Michaelsen, Secular change in size at birth from 1973 to 2003: national data from Denmark, Obesity 14 (2006) 1257–1263.

[15] G. Olivier, The secular change in birth height (from 1910 to 1972 in Paris), J. Hum. Evol. 6 (3) (1977) 293–296.

[16] L.T. Hop, Secular trends in size at birth of Vietnamese newborns during the last 2 decades (1980–2000), Asia Pacific J. Clin. Nutr. 12 (3) (2003) 266–270.

[17] P. Adalian, M.-D. Piercecchi-Marti, B. Bourliere-Najean, M. Panuel, C. Fredouille, O. Dutour, G. Leonetti, Postmortem assessment of fetal diaphyseal femoral length: validation of a radiographic methodology, J. Forensic Sci. 46 (2) (2001) 215–219.

[18] R. Sherwood, R. Meindl, H. Robinson, R. May, Fetal age: methods of estimation and effects of pathology, Am. J. Phys. Anthropol. 113 (2000) 305–315.

[19] L. Scheuer, S. Black, Developmental Juvenile Osteology, Elsevier, London, 2000.

[20] Kline, R.B. Principles, Practice of Structural Equation Modeling, The Guildford Press, New York, 2010.

[21] T.A. Perini, G.L. Oliveira, J.S. Ornellas, F.P. Oliveira, Technical Error of measurement in anthropometry, Rev. Bras Med. Esporte 11 (1) (2005) 86–90.

[22] A. Meinl, C.D. Huber, S. Tangl, G.M. Gruber, M. Teschler-Nicola, G. Watzek, Comparison of the validity of three dental methods for the estimation of age at death, Forensic Sci. Int. 178 (2008) 96–105.

[23] S.E. Calce, T.L. Rogers, Evaluation of age estimation technique: testing traits of the acetabulum to estimate age at death in adultmales, J. Forensic Sci.56(2011) 302–311.

[24] C. Masset, Age estimation based on cranial structures, in: M.Y. I˙s¸ can (Ed.), Age markers in the Human Skeleton, C. C. Thomas, Springfield, IL, 1989, pp. 71–103.

[25] D. Lucy, R.G. Aykroyd, A.M. Pollard, Nonparametric calibration for age estimation, Appl. Statist. 51 (2002) 183–196.

[26] R.G. Aykroyd, D. Lucy, A.M. Pollard, C.A. Roberts, Nasty, brutish, but not necessarily short: a reconsideration of the statistical methods used to calculate age at death from adult human skeletal and dental indicators, Am. Antiquity 64 (1999) 55–70.