TECHNICAL NOTE

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Validation of a Radiographic Method to Establish New Fetal Growth Standards: Radio-Anatomical Correlation

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ABSTRACT: In forensic medicine, specialists might face difficulties when estimating age at death from fetal remains. Depending on the state of preservation, this age assessment is essentially based on the diaphyseal size of long bones. In a previous work, for the measurement of fetal femoral ossified shafts, we already established a simple and reliable method using a radiographic protocol. Since we previously stated that radiographic measurement values were closer to real anatomical size than ultrasonographic ones, in the present study we decided to check the importance of the difference between radiographic and anatomical measurements. Therefore, we dissected 30 pairs of fetal femurs and compared the difference between the two kinds of measurements (in percentages). This difference seemed to be slight (4.027%), but it was large enough to entail significant differences ($p < 0.001$). In order to provide a correction factor for radiographic measurements, we established a linear regression formula, which was tested on another sample of 30 pairs of dissected femurs. As a consequence of the good results, we improved the linear regression using a powerful statistical tool: the bootstrap. Finally, we obtained a simple equation that allowed us to figure out the real anatomical size with an R^2 of 99% and a mean relative difference of 0.153% (with a standard error of 0.252 mm, and therefore a 95% confidence interval with limits of −0.35 and 0.657 mm). This difference did not entail any significant differences ($p = 0.498$), and therefore, we concluded that with the proposed correction, radiographic measurements can easily be used by forensic specialists in their daily tasks or to establish new growth standards in order to best fit their population of interest.

KEYWORDS: forensic science, forensic anthropology, fetus, femur, diaphyseal length, radio-anatomical correlation, dissection, gestational age

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In forensic medicine, estimating gestational age is an important issue (independently of the cause of death) in several conditions: assessment of vitality, diagnosis of pathological conditions that could affect growth, and distinction between aborted fetuses and stillborns or between legal and illegal abortions.

Gestational age is determined by estimation of the fetus's developmental age concluded from the skeletal growth. With the development of prenatal ultrasonography during the last 30 years, several abacuses became available for complete fetuses or ossified parts of developing bones (1). Some authors, comparing these ultrasonographic measurements to the real anatomical ones, proved that they contain slight errors (2).

In forensic practice, abacuses can be useful in real anatomical conditions, however, radiographic methodology has to be applied when skeletal preparation is impossible or undesirable. Therefore, precise radiographic osseous criteria have been sought for many years, especially focusing on the appearance of ankles' and knees' ossification centers, but variation was observed up to several weeks (3,4).

In a previous work, we proposed a new radiographic methodology and validated a qualitative criterion allowing radiographic measurements: a net and clear-cut conjugal plate (5). In the present study, we compare femoral lengths obtained by the application of the previously cited radiographic protocol with the real anatomical length obtained after femoral dissection.

Materials and Methods

Sample

Anonymous fetopathological examination records were collected from spontaneous abortions, in utero deaths, and stillborns. Four hundred ninety eight fetuses were selected according to the following criteria: agreement between gestational age, morphological data (weight, height, foot length) (6–14), age-corresponding external appearance, absence of external malformation, or any fetal pathological alterations, normal caryotype, and lack of maternal pathology.

We randomly selected 30 fetuses: ten ranging from 18 to 20 weeks, ten from 26 to 28 weeks, and ten from 33 to 35 weeks, in order to preclude the influence of increasing calcification rate in anatomical condition.

Radiographic Protocol

The radiological examination was performed with a PHILIPS Diagnost 4 radiography table and a PHILIPS PCR/ACE treatment console using a focus-film distance of 1 m. The X-rays were taken on standard V phosphorus screen cassettes and printed on hard copy films.

For the lateral view, the inferior limbs were half bent in order to avoid any superposition. The applied radiographic parameters were 41 kV and 2 mAs.

Femoral Sections

The 30 pairs of femurs were dissected and cut in the sagittal plane in order to reproduce the radiographic lateral view. In case of fetuses older than 26 weeks, the bones were decalcified in a mixture of formic acid (50%) and sodium citrate (20%) during less than one day.

Measurement Technique

Measurements were taken with a .5 mm graduated plastic ruler both on radiographs and dissected bones. We noted the radiographic diaphyseal size, correlated with the scale plotted by the radiologist in order to figure out the real diaphyseal size, and we noted the real anatomical size obtained after femoral section. It was not necessary to use histological staining to distinguish cartilage and bone formation, since they were grossly identifiable (Figs. 1 and 2).

All the measurements were taken independently by two observers and repeated twice by one of them (with three-month intervals) (14).

Statistical Analysis

In order to take into account the importance of error compared to the value, the differences between the measurements performed with each method on the same ossified shafts were expressed in percentages. The Wilcoxon non parametric test for paired values was used in search of statistically significant differences.

FIG. 1—*Sagittal section of a fetal femoral bone.*

FIG. 2—*Determination of the ossified shaft's limits after sagittal section of the previously shown femoral bone.*

Results

The repeatability test, of which purpose is to check on eventual differences between two series of measurements taken by the same observer at two different times, did not show any significant differences to the threshold of 0.05 for either radiographic ($p = 0.857$) or anatomical measurements ($p = 0.734$).

The reproducibility test, whose purpose is to check on eventual differences between two different observers' measurements, did not show any significant differences to the threshold of 0.05 for either radiographic ($p = 0.582$) or anatomical measurements ($p = 0.682$).

The average relative difference between radiographic and anatomical measurements was 4.027% (with a standard error of 0.234 mm, and, therefore, a 95% confidence interval with limits of 3.62 and 4.55 mm), and the Wilcoxon non parametric test showed that this entailed a significant difference ($p < 0.001$). Therefore, we divided the sample into two age groups in order to check if the increasing calcification rate could influence the determination of the radiographic measurement landmarks. Sample A contained the 15 younger fetuses and Sample B contained the 15 older ones. However, this precaution did not reveal the determining influence of the calcification rate, since we obtained some significant differences, too (both P values were <0.0001) (Table 1).

Finally, in order to correct the observed radiological error and stay as close as possible to the real anatomical size, we established a linear regression using the least square method.

Linear Regression

The obtained regression formula was: *Anatomical size estimation = 0.958 * radiological size + 0.462*, which allowed us to determine the anatomical size with an R^2 (determination coefficient) of 97%.

To test the reliability of the preceding estimation formula, we decided to check its accuracy on a sample that was not included in its

TABLE 1—*Results of the Spearman correlation test and the Wilcoxon non parametric paired test in the two examined age groups (in gestational weeks).*

	Group A 18 to 28 GW	Group B 28 to 40 GW
How effective was the pairing?		
rs (Spearman, approximation)	0.9228	0.9856
P value (one tailed)	$P < 0.0001$	$P < 0.0001$
P value summary	…	…
Was the pairing significantly effective ?	Yes	Yes
Wilcoxon test for paired values		
P value	0.0003	0.0001
Exact or approximate *P* value?	Gaussian approx.	Gaussian approx.
P value summary	…	…
Are means significantly different? ($P < 0.05$)?	Yes	Yes
One or two tailed value?	Two tailed	Two tailed
Sum of positive, negative ranks	117; −3	105; 0
Sum of signed ranks (*W*)	114	105

formulation. Therefore, we randomly selected 30 other fetuses, their gestational age ranging from 18 to 36 weeks, and noted the radiological and anatomical measurements in the same way as before. We applied the established formula on this new sample, and observed a mean relative difference of 1.46%, which did not entail any significant differences ($p = 0.773$).

In order to refine this estimation, we decided to apply the bootstrap method on all of the 60 fetuses. The bootstrap is a powerful tool in assessing the accuracy of estimators and testing hypotheses for parameters in case of small data samples. Most techniques for computing variances of parameter estimators or setting confidence intervals for the true parameters assume that the size of the available set of values is sufficiently large, so that "asymptotic" results can be applied. However, in most of the conditions, this assumption cannot be made because of the sample constraints. The bootstrap provides the possibility of computing an important number of times the establishment of a regression equation on a randomly selected sample. In this study, we randomly selected 30 fetuses among the 60 composing the overall sample, and repeated 5000 times the establishment of the linear regression. The coefficients of each equation were saved, and the final equation is based on the mean values of these 5000 previously saved coefficients. This methodology provided the following estimation formula: *Bootstrap anatomical size estimation = 0.94 * radiological size + 0.811,* which allowed us to determine the anatomical size with an R^2 of 99% and a mean relative difference of 0.153% between the real anatomical size and the anatomical size estimation (with a standard error of 0.252 mm, and, therefore, a 95% confidence interval with limits of −0.35 and 0.657 mm) (Fig. 3). Moreover, this difference did not entail any significant differences ($p = 0.498$).

Discussion

Despite the considerable genetic differences in growth, genetic background has less to do with fetal growth than environment, and there is considerable evidence that the pronounced variations in birth length of different ethnic groups are largely attributable to socio-economic influences (15). These growth affecting elements imply an important variability all along the *in utero* development, and this makes age estimation even more difficult. Therefore, the possibility of miscalculating gestational age is a serious problem in developmental studies and for obstetricians, pediatricians, and forensic surgeons. With increasing interest in the precise evaluation of gestational age, it becomes necessary to revise previously published abacuses.

Skeletal length is considered a good marker of developmental age (16,17). Since in post mortem assessment the putrefactive process can interfere, long bone length seems to be a reliable marker.

Several results reported in the literature are flawed by the rather small-sized samples, important sources of bias, or lack of standardized procedures (18–20). Among them, the bias originating from inaccurately reported menstrual dates was excluded by the choice of our sample (correspondence between gestational age and morphological data limits the possibility of errors concerning the time of conception). Fetuses have to be well-identified; it is imprecise to make first an approximation in order to obtain a second one. For example, it is not conceivable to determine age on the basis of Haase's rule, and take this result as a reference for other measurements (17).

Until now only few studies have evaluated the correlation between values obtained by ultrasonographic or radiographic protocols and the real anatomical size (2,21). The ultrasonographically measured femur length was found to be significantly shorter compared to the anatomical length, depending on the different gestation periods, image resolution, and fetal movements. As a consequence, the proposed regression formulae were not useful in anatomical conditions.

Our predictive equation is pertinent for the second and third trimesters of gestation.

The measurements do not seem to be affected by the modification of the calcification rate. The fact that the cartilage and the ossified part of the developing bone are always well-defined, was emphasised by the lack of statistical difference in case of the repetition of the measurement as well as the change of observers. This measurement method is also applicable on dry bones, and age estimation can be calculated after the application of Huxley's correction for dry to flesh bones (22).

Moreover, the bootstrap is essentially a computer-based method that substitutes considerable amounts of computation for theoretical analysis. The bootstrap does with computer what the experimenter would do in practice, if it was possible. With the bootstrap, the observations are randomly reassigned, and the estimates are recomputed. These assignments and recomputations are carried out thousands of times and treated as repeated experiments.

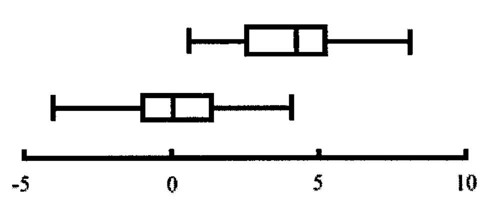

FIG. 3—*Graphic representation of the distribution of the differences (expressed in percentages) between: a) In situ (radiographic) and anatomical size values (upper boxplot); and b) Anatomical and estimated values (after the bootstrap correction) (lower boxplot).The extremities of the moustache represent respectively the 5th and 95th percentiles, the extremities of the box represent the 1st and 3rd quartiles, and the line inside the box represents the median of the distribution.*

The possibility of creating new standards with the application of such a methodology is very attractive, since—as Warren also stated—we have to establish comparative data for a variety of populations (23). Furthermore, the found differences have potentially important evolutionary and legal implications.

Conclusion

In a preceding study, we validated a qualitative criterion, which allowed us to take radiographic measurements of long bones' ossified shafts, and we proposed the use of this methodology to establish new fetal growth standards. In the present work, we demonstrated that these measurements can also be used in real anatomical conditions, after the application of a correction factor on radiographic values. This affirmation is enforced by the use of several statistical tests (repeatability and reproducibility tests) and a powerful statistical tool: the bootstrap.

This new methodological proposition is also based on a simple and easily applicable experimental protocol, which gives the opportunity to forensic specialists to realize extensive studies, and then determine the abacuses that best fit their population of interest.

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