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Growth Pattern and Fetal Age Estimation from the Diaphyseal Length of Femur

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Abstract:

Study on prenatal growth and development of human aspects has always been a neglected part. Expert opinion is routinely asked by the legal authorities to the anatomists regarding the age, sex and the cause of death of human from his skeletal remains. The anatomists could answer such queries, if the deceased under question belong to postnatal stage, childhood, adolescence or adult. But if such queries are asked on the prenatal stage of human, difficulties are faced to provide answers to such questions. The reason for this lacuna is precisely because of the unavailability of proper reference on such field. In the present study the maximum length of the diaphysis of femur (f-ml) was measured on 912 fetal femora, the largest ever reported, from 456 human fetuses (244 males and 212 females) of age range between 11 weeks to 40 weeks of intrauterine life. The fetuses were collected over the period of more than 25 years and the femora were prepared through simple maceration process. The f-ml has been correlated with CRL and CHL. Bisexual and bilateral differences have been analyzed. Growth pattern of the fetuses has been analyzed and the growth rate has been calculated on the basis of f-ml. Required regression equations were calculated to estimate fetal age, a crucial factor in medico-legal cases.

Keywords: fetus, femur, osteometry, growth, age estimation

1. Introduction

Fertilization, embryo, fetus, growth, adolescence, aging are but the process and a few steps through which all the animal forms transcend progressively before death. Man's search for understanding the dynamics of his own prenatal growth and development has not ended. A lot more is yet to be achieved to understand this mystic phenomenon. Study on human fetus has got a wide range of ramifications of a vast network of its applications. It extends its scope into the fields of anatomical, clinical, forensic disciplines, etc. Although there are few studies available to understand the fetal growth indirectly, a composite and anatomic metric approach on fetal growth dynamics is conspicuously missing.

Although studies on growth are available on infants and children very less attention has been given on fetal growth and development. (Watson and Lowrey 1967; Bhalla et al. 1986; Hazza 1990). Feltz (1954) studied only on 53 femora. Moss et al. (1955) measured the ossified shafts of long bones from a series of only on 119 embryos and fetuses. Gardner and Gray (1970) measured and sectioned only on 40 pairs of femora from formalin fixed human fetuses. Fazekas and Kosa (1966) calculated the mean lengths of all six limb bones at two week intervals. Musgrave and Harneja (1977) pointed out that the determination of the age of a fetus from the long bones was more difficult. Vare and Bansal (1977) estimated crown-rump length from the diaphyseal length of fetal long bones. However the earlier studies do not fulfill the necessary requirements to understand the aspects of fetal growth because of the variations in the objectives, methodology adopted, parameters selected and sample size. There is a growing demand to undertake the intrauterine growth studies from various quarters of scientific fields, and the present study aims to fulfill this demand.

Thus it is thought worthwhile to undertake an osteometric study on human fetuses of different sizes of their intrauterine period of life, so as to understand the growth pattern of human fetus in the best possible way on the basis of quantitative analysis, and also to highlight the significance of this study and its crucial applications. The present study has also taken necessary steps to overcome the limitations and difficulties observed in the earlier studies.

The specific objectives of the present study are the following:

- i. To analyze the pattern and the rate of human fetal growth based on the maximum length of the diaphysis of fetal femur (f-ml);
- ii. To assess the extent of bisexual differences and bilateral differences, if any, in the growth pattern of human fetuses on the basis of the f-ml;

- iii. To correlate the f-ml with crown-rump length (CRL) and crown-heel length (CHL) to estimate the fetal age;
- iv. To examine the applied significance of the selected measurements in terms of anatomical, clinical and medico-legal aspects.

2. Material and Methods

Undertaking any study on human fetus is not an ordinary task. The very basic problem is, in fact, to get this unique material, i.e. 'human fetus'. Making availability of this precious material that also in good number, methodology to be used to prepare the fetal skeletons the later being a herculean task, to measure the bones by using delicate techniques with sophisticated instruments, all needed to be taken care of.

Fresh human fetuses for the present study have been collected from the Sassoon General Hospitals, Pune, India, over the period of more than 25 years. Fetuses which appeared normal, only considered for the present study. In all there have been 912 humeri from 456 normal fetuses included for the present work. Among the 456 fetuses, 244 are males and 212 are females. All the fetuses have been categorized, by following the scales referred by Davies (1967); Okajima (1975); Williams and Warwick (1980).

Group	Weeks	CRL (mm)	CHL (mm)	Osteometry (456)		
				Male	Female	Total
I	11-16	51-100	Up to 150	18	4	22
II	17-24	101-200	151-300	159	152	311
III	25-32	-	301-400	49	42	91
IV	33-40	-	401-550	18	14	32
Total				244	212	456

Table 1: Distribution of human fetuses for the present metric study

After the collection of fetuses, the umbilical cord of the fetus was tied tightly with a thread near the umbilicus. The part of the umbilical cord along with the placenta was cut off and removed. The purpose of the tying up of the thread was to stop the oozing out of the fetal blood from the fetus. The fetuses were then washed with running water for about few minutes. Sex of the fetus was noted down. The CRL and the CHL were taken. Then the fetuses were kept in glass jars containing water for maceration. During maceration, plenty of maggots formed in the contents of the maceration jars and started eating the soft tissues of the fetus. At the end of the maceration process, fetal bones got separated from the fetal soft tissues, the later were eaten up by the maggots. The indication of the completion of the maceration process was that no more living maggots, found in the macerated contents. Only a few dead maggots were found floating on the liquid contents. Then the macerated contents were washed and the bones were collected. The bones then got dried in the normal room temperature. After drying, the bones were kept in suitable containers.

Thus in the present study the bones have been prepared through natural maceration process, without using any chemical, so as to retain the originality of the bones. And therefore in the present study, the osteometry was taken on the naturally prepared dried bones. More over on these macerated bones, multidimensional approach could be applied while taking the osteometry on the bones which are themselves multidimensional in their form.

Although the full sets of fetal skeletons were prepared and stored, only the diaphyses of humeri have been selected and measured. For the present study, the maximum length of the diaphysis of humerus (h-ml) has been measured. It is the maximum straight distance between the highest point on the proximal end and the lowest point on the distal end of the shaft of the fetal humerus. Dial Caliper with 0.5 mm accuracy was used to measure the bones. As the femoral shafts are tiny and delicate, wherever found necessary, Magnifying Reading Glass and even the Stereoscopic microscope were also used to take the osteometry. Appropriate statistical analyses have been done to find out the correlation coefficients and also calculated regression equations to find out the growth rate and estimate the fetal age.

3. Results and Discussion

3.1. Bilateral Differences

In the present study, the bilateral differences are found very negligible, on the basis of f-ml. Therefore, in the present study, the bilateral measurements have been clubbed together and the average of the bilateral measurements were used to calculate the mean and standard deviations, the correlation coefficients, the regression values and also to analyze on growth and age estimation.

3.2. Bisexual Differences

In the present study, the bisexual differences are also found very limited, on the basis of f-ml. However, in the present study, the mean and standard deviations, the correlation coefficients, the regression values, analyses on growth and age estimation have been presented on both the sexes separately.

3.3. Bone Growth

Dupreux and Fontaine (1951) recorded a slow growth in humerus and femur of fetuses of four to six months only. The length of both the bones increased regularly from the fifth month onwards. They also found that the growth of humerus during that period was slow

compared to femur, resulting in a marked difference in the length of both the bones at the time of birth. Moss et al. (1955) noted a characteristic interphase in the growth of the body shaft in the CRL interval of 80 - 89 mm. Before this interval, the body shafts of all the long bones grow relatively faster than the CRL and after that the growth rate was not as fast as it was before. They stated that the several combinations of osseous shaft lengths revealed a constant ratio between the specific growth rates of all the bones. Feltz (1954) measured 53 femora, mixed left and right, 8.4 mm to 110.6 mm in total shaft length, from 53 fetuses and prenatal infants ranging from 31 mm to 485 mm CRL (ninth fetal week to third postnatal week) and calculated that for every 1 mm increase in CRL, the length of femur increases by 0.21 mm. The later result has also been reported by Mehta and Singh (1972) and Vare and Bansal (1977). Gardner and Gray (1970) measured the shaft of the femora in a total sample of only 40 fetuses that also from preserved ones.

Falkner and Roche (1987) while measuring femoral lengths from full-term infants on radiographs stated that correlation is higher for femur with recumbent length (CHL) than for femur with CRL; presumably because the femur contributes to recumbent length (CHL) and not to CRL. In the present study also the f-ml correlates more with CHL rather than with CRL. Oliver and Pineau (1960) observed that a linear correlation between the diaphyseal length of fetal long bones with that of height of fetus.

In the present study, it has been found that there is a faster rate of growth in the femur (female) from the group I (11 to 16 weeks). For every 1 mm in the CRL, the f-ml is increased by .421 mm. In the females the f-ml shows two fold growth from group I (11 weeks to 16 weeks) to group II (17 weeks to 24 weeks); three fold growth from group II (17 weeks to 24 weeks) to group III (25 weeks to 32 weeks); four fold growth from group III (25 weeks to 32 weeks) to group IV (33 weeks to 40 weeks). In males the f-ml shows four fold growth from group I (11 weeks to 16 weeks) to group III (25 weeks to 32 weeks). In the group I (11 to 16 weeks) females show faster rate of growth in the f-ml, for every 1 mm increase in the CRL and CHL. In the group II (17 to 24 weeks) and the group III (25 to 32 weeks) the f-ml shows almost equal rate of growth both in males and females. In the group IV (33 to 40 weeks), the f-ml shows faster growth rate in females when compared to male, for every 1 mm increase in CRL. The differential growth trends in males and females in the groups I and IV may be due to smaller sample size.

Group	Gardner and Gray (1970)	Moss et al. (1955)	Present study
I	9.27	10.04	13.31
II	26.77	26.1	29.66
III	48.5	Nil	46.56
IV	63.75	Nil	65.05

Table 2: Mean of the maximum length of femur: Comparison

Males	Group -I	Group -II	Group -III	Group -IV
	11-16wks (18)	17-24wks (159)	25-32wks (49)	33-40wks (18)
	Mean	Mean	Mean	Mean
f-ml	11.5667	28.5593	45.2648	65.0083

Table 3: Mean of the maximum length of femur (f-ml) in four age groups in males
Number in parentheses indicates sample size

Females	Group -I	Group -II	Group -III	Group -IV
	11-16wks(04)	17-24wks(152)	25-32wks(42)	33-40wks(14)
	Mean	Mean	Mean	Mean
f-ml	15.0625	30.7737	47.8708	65.1679

Table 4: Mean of the maximum length femur (f-ml) in four age groups in females
Number in parentheses indicates sample size

FEMUR		Group -I		Group -II		Group -III		Group -IV	
Males		11-16wks (18)		17-24wks (159)		25-32wks (49)		33-40wks (18)	
Dependent Variable	Independent Variable	b0	b1	b0	b1	b0	b1	b0	b1
f-ml	CRL	-6.414	.213	-9.706	.248	3.244	.186	-4.198	.224
Females		11-16wks (04)		17-24wks (152)		25-32wks (42)		33-40wks (14)	
f-ml	CRL	-26.600	.421	-9.716	.254	-1.904	.217	-11.678	.253

Table 5: Regression values (b0, b1) for growth rate in Femur with CRL in four age groups in males and females.
b1 shows increase in the osteometric measurements for every one mm increase in CRL
Number in parentheses indicates sample size

FEMUR		Group -I		Group -II		Group -III		Group -IV	
Males		11-16wks (18)		17-24wks (159)		25-32wks (49)		33-40wks (18)	
Dependent Variable	Independent Variable	b0	b1	b0	b1	b0	b1	b0	b1
f-ml	CHL	-4.609	.135	-9.177	.164	-4.864	.148	-6.810	.158
Females		11-16wks (04)		17-24wks (152)		25-32wks (42)		33-40wks (14)	
f-ml	CHL	-25.722	.280	-9.044	.166	-8.638	.165	-15.709	.179

Table 6: Regression values (b0, b1) for growth rate in Femur with CHL in four age groups in males and females.

b1 shows increase in the osteometric measurements for every one mm increase in CHL

Number in parentheses indicates sample size

Table 3.T.85

3.4. Inter-age groups

As the fetuses of the present study are divided into four age-range categories viz. I, II, III, IV, the growth rate is calculated between these four groups. Thus, there are three inter-age groups are formed from the four basic groups as I-II (11 to 24 weeks); II-III (17 to 32 weeks); III-IV (25 to 40 weeks). Each intergroup has the total number of fetuses of both the groups concerned.

Bar diagram prepared for the present study reveals the absolute growth rate for the maximum length of the shaft of femur (f-ml), between the three inter-age groups. Each bar diagram shows the growth rate of two variables i.e. the f-ml of both the males and females. Thus the prepared diagram not only helps to understand the trend of the absolute growth rate of the f-ml but also shows male-female differences.

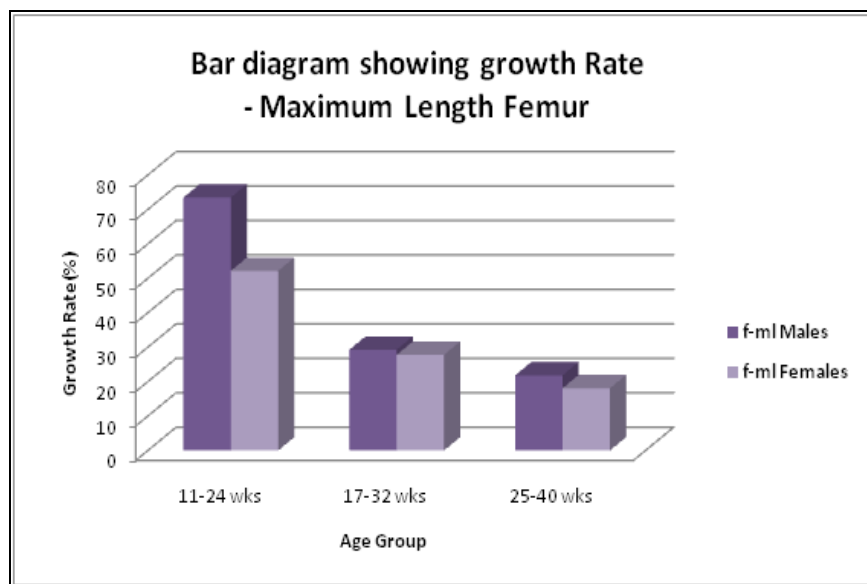


Figure 1

From the obtained bar diagram the following salient features have emerged:

- i. Absolute growth rate for the f-ml is higher in the inter-group I-II (12 weeks to 24 weeks) and gradually declining through the proceeding two inter-groups II-III (17 weeks to 32 weeks) and III-IV (25 weeks to 40 weeks).
- ii. In the overall growth pattern, males show faster rate of growth than females.

3.5. Age Estimation

As it has been revealed by the present study about the highly significant correlation obtained between the CRL/CHL with the f-ml, the next step has been taken to calculate the necessary regression equations to estimate fetal age. Once the CRL and CHL are calculated, age can be further estimated from the already established age estimation scale.

Necessary regression values (b0, b1) have been calculated for the f-ml. The formula to calculate CRL/CHL from the f-ml is as follows:

- $CRL/CHL = (b1 \times \text{measurement}) + b0$

Regression values(b0,b1) for estimating CRL, from Femur	Group –All	
	b0	b1
f-ml	36.749	4.078

Table 7: Regression values (b0, b1) for estimating CRL, from femur from the total 456 fetuses

Regression values(b0,b1) for estimating CHL, from Femur	Group –All	
	b0	b1
f-ml	54.513	6.085

Table 8: Regression values (b0, b1) for estimating CHL, from femur from the total 456 fetuses

The application of the present fetal study, in the clinical aspects will enable us to advance a growth standard, which would help us to comprehend the differential growth pattern between the normal and the abnormal fetuses. The anatomical aspects of this study on growth pattern in relation to age, sex variations would open new vistas of researches in the fetal growth and development. The analysis in estimating the fetal age from various maximum possible measurements on the largest possible parts of the human fetal specimens will definitely help us in solving problems facing the estimation of fetal age, a crucial factor in medico-legal cases.

4. Conclusion

- i. The largest ever sample size (456 which include 244 males and 212 females) considered by any other study on human fetus.
- ii. The CRL and CHL were taken directly on fresh fetuses immediately after their collection.
- iii. Though time consuming, natural process of maceration technique adopted for the first time. No chemicals used. The harvested and cleaned bones were dried in natural shade before storage and measurements.
- iv. The humeri belong from 11 weeks to 40 weeks of the intra-uterine fetal period, and the fetuses were collected over the period of more than 25 years.
- v. The fetal humeri have been categorized into four age-wise groups to study the bone growth.
- vi. Bone analysis has been done on the fetal humeri to deal with the growth pattern and accordingly growth rate has been calculated.
- vii. The application of this present study focuses on the anatomical, clinical and forensic sciences.

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