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Growth Analysis and Age Estimation of Human Fetus from Human Fetal Humerus

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Abstract:
Metric study on human fetal bones does not limit itself in understanding the prenatal growth pattern alone. Rather it holds its application in the areas of anatomical, clinical and forensic sciences. Few studies are available on fetal diaphyseal length of humerus correlates with crown-rump length /crown-heel length (CRL/CHL). However the earlier studies show variations in the objectives, methodology adopted to prepare the bones, measurements selected and sample size which all depict an unreliable data base. The present study has been carried out on 912 fetal humeri from 456 human fetuses (244 males, 212 females) the largest sample ever reported, of varying periods of prenatal growth from 11 weeks to 40 weeks. Another significant aspect of the present study is that the humeri have been prepared through simple maceration process to preserve the originality of the bones. The maximum length of the diaphysis of humerus (h-ml) has been taken. It has been observed that there exists an overall prenatal growth pattern of fetal humerus based on the h-ml. There is a highly significant correlation observed between the h-ml and the CRL and CHL. Bisexual and bilateral differences have been analyzed. On the basis of the h-ml, regression equations have been calculated to estimate the fetal age, a crucial factor in medico-legal cases.

1. Introduction

Study on biological aspects on mankind is a very vast field of science. Human growth and developmental aspects have wide range of applications and scope. Very extensive studies have been carried out, in respect of growth on infants and children in many parts of the world (Tanner et al.1956; Hauspie et al. 1980; Corlett 1986; Tsuzaki et al.1990). However, very less attention, has been given in the case of growth studies on human in his intrauterine life. The reason for this lacuna may be lying on difficulties in making the availability of the required human specimens in their prenatal stages and also lacking of necessary sophisticated techniques and equipment to study such human material.

Attempts are being made from different angles to acquire a better understanding in dealing with the prenatal growth pattern of man. Although human embryologists and anatomists have achieved some break-through in understanding the pattern of growth and development of human, the studies still remain as an unfinished task. The study on human fetuses does not limit itself on just to understand growth and development only. It has got a wide range of ramifications of a vast network of its applications. Anatomists are routinely requested by legal authorities to provide an expert opinion about the age and sex of deceased fetuses under investigation.

The earlier fetometric studies carried out so far, lack the required coherent approach on this topic, because of the variations in the objectives, methodology adopted to prepare the fetal specimens on too less sample size. Gray and Gardner (1969) measured and sectioned only on 40 pairs of humeri from formalin fixed human fetuses. Oliver and Pineau (1960) reported linear correlation between the diaphyseal length of fetal bones and the height of fetus. Mehta and Singh (1972) measured the fetal diaphyseal lengths only on 50 fetuses. Kosa (1997) observed that the linear regression did not reveal any correlation between the weight of the limb bones and the body length of fetuses studied. Methodology adopted and techniques involved in the earlier studies to prepare the fetal long bones were not uniform. In various studies, observations and measurements were taken from photographs (Burdi 1969), radiographs (Scheuer et al.1980) and ultra sonographs (Falkner and Roche 1987; Mankeekar 1993). Moss et al. (1955) studied fetal bones prepared with alizarin staining. In some other studies (Ford 1956; Mehta and Singh 1972) fetal bones were dissected from preserved fetuses and measured.

Looking through the procedures followed in the earlier studies, it strikes in our minds, whether the measurements taken on the fetal bones, from these techniques are accurate ones as there is every chance of bones being oblique in their presentations in the photographs, radiographs, ultra-sonographs etc. Moreover the bone measurements taken on these graphs are only one-dimensional

approach, while the bone presents multi-dimensions in its form. In the case of dissected bones also, not only there is every chance of the tender fetal long bones getting damaged, but also the removal of soft tissues from the bones may not be that perfect which may result in distorted measurements. Measurements on long bones, which are obtained from chemically preserved fetuses, definitely differ from that of original long bones.

With different aims, variations in methodology adopted to prepare and also to measure the fetal specimens on too less sample size, the whole scenario depicts an incomplete picture. Therefore a systematic study is necessary to be undertaken considering the pitfalls highlighted above and with a view to clear the outstanding knots which are still intriguing in understanding the growth and development of human fetus.

The specific objectives of the present study are the following:

i. To analyze the fetal growth pattern and the rate of fetal growth based on the maximum length of fetal humerus (h-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 h-ml;
iii. To correlate the h-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 osteometry 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 investigation. 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 humeral shafts are tiny and delicate, wherever found necessary, Magnifying Reading Glass and even the Stereoscopic microscope were also used to take the osteomtry. 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 h-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 h-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

On the basis of the analysis on osteometry, the present study reveals that there is a positive growth trend, which has been observed in the h-ml from all the four age groups considered in the present study. Growth of fetal long bones has been studied by Smith (1939); Streeter (1949); Feltz (1954); O'Rahilly and Meyer (1956). Dupreux and Fontaine (1951) recorded a slow growth in both the bones of fetuses of four to six months only. The length of humerus and femur 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. Saettle (1951) plotted growth curves of shafts against fetal height on the basis of the growth curve which was more accurate. Oliver and Pineau (1960) have observed a linear correlation between diaphyseal length and fetal height. Gray and Gardner (1969) measured the shaft of the humeri in a total sample of only 40 fetuses that also from preserved ones. Moss et al. (1955) measured 106 fetuses from the total sample of 119 fetuses, which were cleared and stained with alizarin, ranging from 30 mm to 169 mm in CRL. Whereas in the present study, in the same age group (I and II) there were 333 fetuses measured. The present osteometric study has been carried out on dried bones from 456 fetuses. Slight variations observed in the earlier results might be attributed to the very less sample size and the mode of preparation of the bone material for the osteometric study.

Group	Gray and Gardner (1969)	Moss et al. (1955)	Present study
I	9.8	9.76	13.74
II	26.5	26.32	28.77
III	45.5	Nil	42.65
IV	57.75	Nil	57.37

Table 2: Mean of the maximum length of humerus: 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
h-ml	11.9819	27.9653	41.8954	57.3208

Table 3: Mean of the maximum length of humerus (h-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
h-ml	15.5125	29.5743	43.3988	57.4268

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

Studies on bone growth, based on quantitative analyses, help better understanding the growth pattern with its clinical applications. Moss et al. (1955) stated that the several combinations of osseous shaft lengths revealed a constant ratio between the specific growth rates of all the bones. Mehta and Singh (1972) measured humerus and femur. For every one 1 mm increase in CRL the length of humerus increases by 0.18 mm. Vare and Bansal (1977) observed a linear correlation between the diaphyseal length of upper and lower limbs and the CRL from 185 fetuses with CRL ranging from 185 - 415 mm. The bones were dissected from the body and got them measured. Although the muscles and connective tissue were removed from the bones, periosteum was left intact. For every one 1 mm increase in CRL the length of humerus increases by 0.17 mm.

HUMERUS		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
h-ml	CRL	-8.004	.237	-5.998	.220	10.699	.138	-1.179	.189
Females		11-16wks (04)		17-24wks (152)		25-32wks (42)		33-40wks (14)	
h-ml	CRL	-4.700	.204	-5.434	.220	5.846	.164	-4.271	.203

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

HUMERUS		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
h-ml	CHL	-5.968	.149	-5.528	.146	3.968	.112	-3.334	.134
Females		11-16wks (04)		17-24wks (152)		25-32wks (42)		33-40wks (14)	
h-ml	CHL	-9.840	.174	-4.930	.144	1.211	.123	-6.673	.142

Table 6: Regression values (b0, b1) for growth rate in Humerus with CHL in four age groups in males & females.
b1 shows increase in the osteometric measurements for every one mm increase in CHL
Number in parentheses indicates sample size

In the group II (17 to 24 weeks) and the group III (25 to 32 weeks) the h-ml shows almost equal rate of growth both in males and females. In the group IV (33 to 40 weeks), the h-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 during intrauterine life of human fetus may be due to smaller sample size.

Inter-age groups

As the fetuses of the present study are divided into four age-range categories viz. I, II, III, IV, the absolute 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 humerus (h-ml), between the three inter-age groups. Each bar diagram shows the growth rate of two variables i.e. the h-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 h-ml but also shows male-female differences.

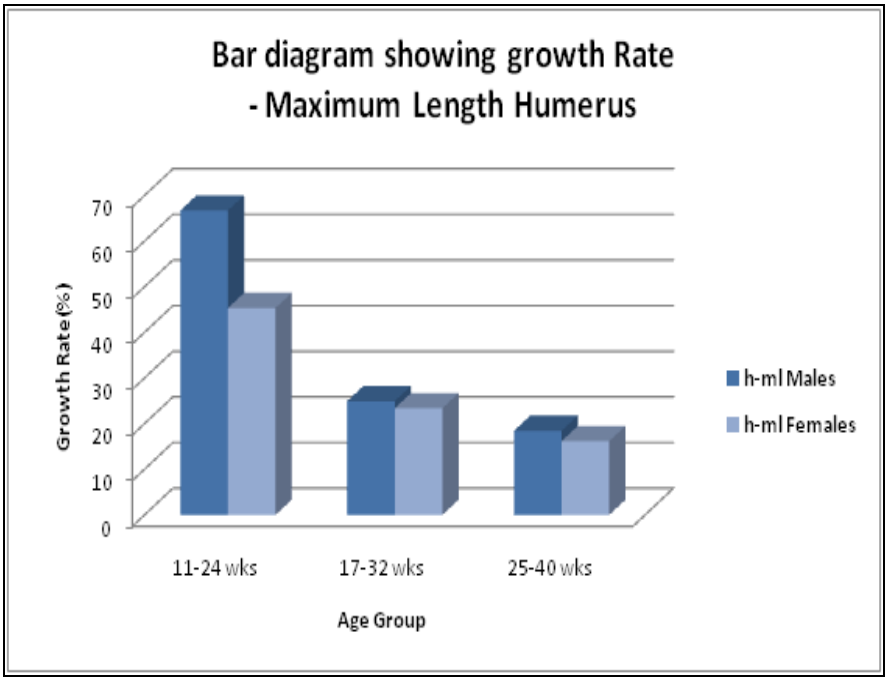

Figure 1

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

i. Absolute growth rate for the h-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.

3.4. Age Estimation

Studies pertaining to growth must consider the related variables and as such, the relationships among those variables can be brought out for a combined effect and thus revealing the dynamic relationship. The obtained correlation matrices for all the four age groups and among both the sexes have produced very high significant values for h-ml when related to the CRL and CHL. Accordingly, necessary regression equations were calculated to estimate the CRL and the CHL. 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 h-ml. The formula to calculate CRL/CHL from the h-ml is as follows:

CRL/CHL = (b1 x measurement) + b0

Regression values(b0,b1) for estimating CRL, from Humerus	Group –All	
	b0	b1
h-ml	18.824	4.862

Table 7: Regression values (b0,b1) for estimating CRL, from h-ml from the total sample of 456 fetuses

Regression values(b0,b1) for estimating CHL, from Humerus	Group –All	
	b0	b1
h-ml	27.475	7.264

Table 8: Regression values (b0,b1) for estimating CHL, from h-ml from the total sample of 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 include244 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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