Prenatal growth of human ulna and estimation of CRL and CHL based on osteometry on fetal ulna.

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ABSTRACT

In dealing with fetal bone growth and fetal age estimation from osteometry, the earlier osteometric studies were done from chemically preserved fetuses, photographs, radiographs and ultrasonographs which would not give perfect results. In the present study osteometry was taken directly on naturally prepared fetal ulna bones for the first time. There were six new osteometric measurements on human fetal ulna introduced. Possible osteometric measurements were taken on 912 fetal ulna bones (right and left) from 456 fetuses having age range between 11 weeks to 40 weeks of intrauterine life. Bilateral and bisexual differences were analyzed. It is found that there is positive growth trend found in fetal ulna on the basis all the osteometric measurements taken on fetal ulna. The fastest rate of growth in the length of fetal ulna was observed in females during 11 weeks to 16 weeks of the intrauterine life of human fetus. For every 1 mm in the CRL (crown- rump length), the Maximum Length of Ulna is increased by .306 mm. As there is no established scale available for fetal age estimation from fetal bones, even anatomists found difficulties to provide precise opinion regarding the age of

human fetus from fetal bones in suspected murder cases. In the present study regression values were calculated and found out a formula to estimate CRL and CHL (crown-heel length) from the osteometric measurements on fetal ulna. The age estimation is a crucial factor in dealing with medico-legal cases.

Key words: Fetus; ulna; osteometry; fetal growth; fetal age estimation.

Introduction

The phenomenon of intrauterine growth and development of human fetus involves both quantitative and qualitative perspectives. In the earlier studies comparatively, less attention was given on osteometric aspects than the developmental aspects. Studies were available in respect of growth on infants and children (Hazza 1990; Cardoso et al. 2014), children through adolescence (Kulkarni 1985; Padmanathan et al.1990), and also adults (Macho1986). However few studies were available on growth aspects of human in its intrauterine life (Vare and Bansal 1977; Simon et al. 1984). Although studies were available on fetal growth and development, the aims of the earlier studies were varied from one another. Most of the studies aimed to concentrate on developmental aspects (Okajima 1975; Hamilton and Mossman 1976; Kulkarni et al.1981). Few studies were aimed to estimate fetal age (Mehta and Singh 1972; Kosa 1997; Gosavi and Kulkarni 2013; Shirley 2009). Methodology adopted in the earlier studies dealing with prenatal growth and development was not uniform. Ford (1956) and Moore and Persaud (1993) measured human fetuses which were preserved in formalin. In various studies, observations and measurements were taken from photographs (Burdi 1969), radiographs (Carneiro et al. 2016; Carneiro et al. 2019) and ultrasonographs (Falkner and Roche 1987; Mahon et al. 2009). Mehta and Singh (1972) measured the crown-rump length of fetuses, after fixing them in 10% formalin for 4 to 6 months. As such that there was

no uniform method adopted in the earlier studies in preparing the fetal specimens. In dealing with fetal growth and fetal age estimation, the number of fetal specimens considered in the earlier studies varied from one another. It was noted that in many cases the number was found to be quite inadequate. Gray and Gardner (1969), Gardner and Gray (1970) studied a series of only 40 embryos and fetuses. Mehta and Singh (1972) measured the diaphyseal lengths of only 50 fetuses. Feltz (1954) studied only 53 femora. Because of the inadequacy as well as variability in the sample size, no proper comparison could be made between the studies. In the case of osteometry, from the earlier studies, which were aimed to estimate the age from fetal long bones, it was observed that not all the studies included all the long bones. (Feltz 1954; Moss et al.1955; Gray and Gardner 1969; Gardner and Gray 1970; Mehta and Singh 1972).With different aims, variations in methodology adopted to make the availability of the human specimens, and also with much variation in the methodology followed to measure the specimens, with less number of parameters on small sample size, the whole scenario depicts an incomplete picture to carry fetal growth studies on the basis of osteometry on fetal bones. Expert opinion is routinely requested from anatomists by legal authorities to know about age and sex of deceased from bone remains while dealing with suspected murder cases. While examining adult cases, as there are already established scales available for age estimation, it becomes easy to estimate the age of deceased adult from the available bones. On the contrary, when opinion about age of a deceased fetus is asked from fetal bones, as there was no established scale or standards available to estimate fetal age from fetal bones, even anatomists are not in a position, to provide precise information regarding the age of fetus, from the fetal bone remains. Thus, it is felt necessary that a systematic study must be undertaken considering the pitfalls highlighted above.

Objectives

The objectives of the present study are to assess bilateral differences, in the growth pattern on the basis of measurements taken on both side ulna bones of human fetuses; to find out the extent of bisexual differences, if any, in the growth pattern of human fetuses on the basis of metric analysis; to analyze the rate of fetal growth as exhibited through detailed osteometry on the basis of categorization of fetuses into four age groups; to correlate various osteometric measurements on ulna with CRL (crown-rump length) and CHL (crown-heel length) to estimate fetal age; to examine the applied significance of those selected measurements in terms of anatomical, clinical and medico-legal aspects.

Material and Methods

Source of Fetuses

Fetuses for the present study were collected from the Sassoon General Hospitals, Pune, India. The principal author of the present study was an Anatomy staff member in the Department of Anatomy, B.J. Medical College (BJMC) with its attached Sassoon General Hospitals, Pune, India since 1978 onwards for over the period of 32 years. The Deans of the BJMC and Sassoon General Hospitals, and the Heads of the Department of Anatomy, BJMC were kind enough to cooperate to collect human fetuses from the Sassoon General Hospitals. The Professors and Heads of the Departments of Obstetrics and Gynaecology and Forensic Medicine, BJMC were also cooperative to supply human fetuses from their respective sections. The fetuses were collected during the above period. The fetuses were from abortions of Medical Termination of Pregnancies (MTPs)/Still Births. The collections of fetuses were done by following official procedures. The study

was conducted in the Department of Anatomy, BJMC and continued in the Department of Anatomy, Dr. V. M. Government Medical College, Solapur, India. Required approval was obtained from the Ethical Committee of BJMC.

Population Base

Anatomical study on human is both on individual basis and population oriented. In the present study, name of the parents of the fetuses and their place of living indicated that all the parents located within the geographical area of Maharashtra, India. This broad population base of Maharashtra provided a vital significance, indicating that the fetuses belonged to the Maharashtra population of India.

Categorization of Fetuses

In all there were 912 fetal ulna bones (right and left side bones) included from456 normal fetuses for the present study. Among the 456 fetuses, 244 (53.51%) fetuses were males and 212 (46.49%) fetuses were females. The fetuses for the present study of varying sizes ranging from 51mm to 394mm in CRL with 70 mm to 577 mm in CHL having age range between 11 weeks to 40 weeks of intrauterine life. As there was earlier literature available to estimate fetal age from the CRL as well as CHL, the present study adopted the already established scales, (Davies 1967, Okajima 1975, Williams and Warwick 1980), to estimate fetal age from CRL and CHL. Osteometry was carried out on all the 456 fetuses. All the 456 fetuses were categorized into four age groups, each group having eight weeks duration of age range, except for the first group. The first group got only six weeks range, as because fetal long bones would be available for manual
measurements only after 11 weeks of the intrauterine period. (Table 1).

Table 1. Age wise, Group wise and Sex wise Distribution of Human Fetuses

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

Somatometry

When a fetus was brought to the Department of Anatomy, it was to be prepared for somatometric study. Only those fetuses, which appeared normal, were selected for the purpose of the present study. Firstly the umbilical cord of the fetus was tied tightly with a thread, near the umbilicus. The purpose of the tying up was to stop oozing out of the fetal blood from the fetus. The part of the umbilical cord along with the placenta was cut off and removed. The fetus was then cleaned with running water for about few minutes and kept ready for observation and taking somatometric measurements. Sex of the fetus was noted down. There were two somatometric measurements selected for the present study (Table 2).

Table 2. Somatometric Measurements

S.No.	Somatometric Measurement	Abbreviation
1	Crown-Rump Length	CRL
2	Crown-Heel Length	CHL

Earlier Methodology

The methodology adopted by the earlier workers to measure fetal bones from photographs, radiographs and ultra sonographs might not be accurate ones as there was every chance of bones being oblique in their exibits. Moreover the bone measurements taken on these graphs were only one-dimensional approach, while the bone itself exhibited in multi-dimensional form. In the case of measurements taken on the dissected bones not only there would be every chance of tender fetal long bones getting damaged, but also the removal of soft tissues from the bones might not be that perfect which might result in distorted measurements. Moss et al. (1955) studied fetal bones prepared with alizarin staining. In some studies (Ford 1956; Mehta and Singh 1972) fetal bones were dissected from preserved fetuses and measured. Measurements on long bones, which were obtained from chemically preserved fetuses, definitely differ from that of original long bones. Therefore it was necessary to prepare original bone and take measurements directly on the bone to get accurate results for the present study.

Maceration

The maceration involves the decomposition of soft tissues and the bones get separated from the decomposed soft tissues. During the process of maceration live maggots started consuming the soft parts of the fetuses. Once the somatometry was completed the fetuses were kept in glass jars labeled with identification number and date of collection of the fetuses. The jars contained sufficient amount of water so that the fetuses could immerse inside the water. Then the glass jar containing the fetus was kept in a maceration room on the terrace of the Anatomy Department. The side walls of the maceration room were made by metallic sieves so that sufficient air and sun light would be available. The indication of the completion of the maceration was that no live maggots found in the jars and only few dead maggots were found floating on the surface of the water. The harvested bones of the fetus

were cleaned with water. The bones were filtered by metal sieve with minute holes to avoid losing even small bones. Although full sets of fetal bones of human fetuses were prepared, only ulna bones have been selected for the present study. As the epiphyses of the ulna bones not formed during intrauterine life, only the diaphyses of ulna bones were observed and measured (Fig.1). The prepared fetal bone sets were kept in closed plastic boxes and stored at the Department of Anatomy, BJMC, Pune India.

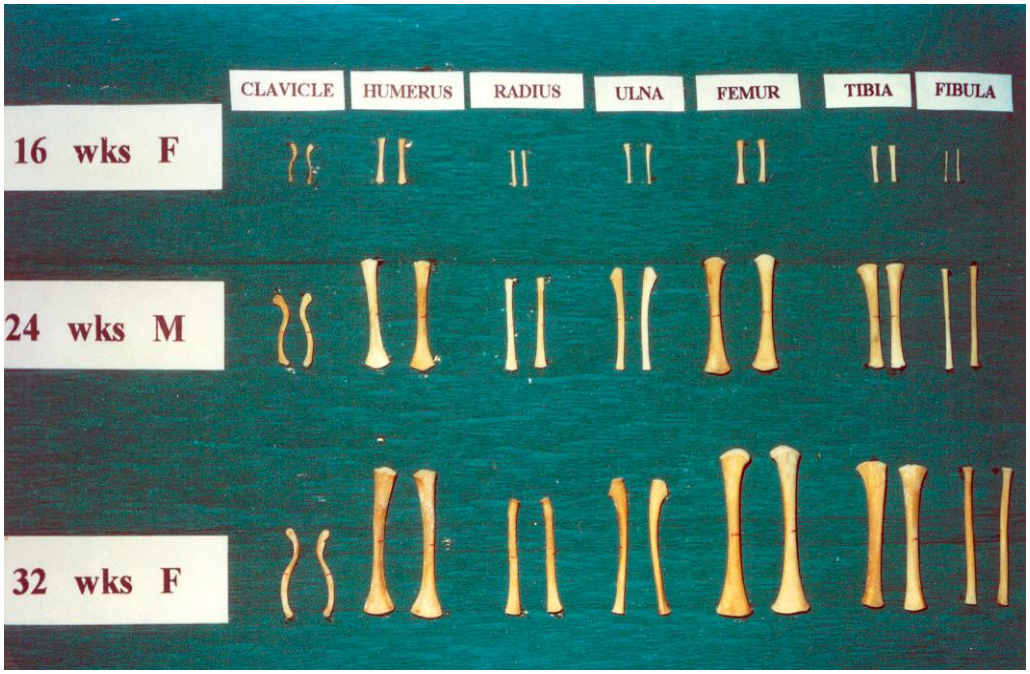

Fig. 1. Shafts of Fetal Long Bones (16 to 32 weeks)

Osteometry

In addition to the two somatometric measurements, there were seven osteometric measurements taken on ulna bones for the present study (Table 3). All the seven osteometric measurements were measured on both right and left side bones. Except the maximum length of ulna all the remaining six osteometric measurements taken on fetal ulna were newly introduced ones for the first time.

Table 3. Osteometric Measurements on Fetal Ulna

S.No.	Osteometric Measurement	Abbreviation
1	Ulna-Maximum Length	u-ml
2	Ulna-Proximal Antero-Posterior Diameter	u-pap
3	Ulna-Proximal Medio-Lateral Diameter	u-pml
4	Ulna-Distal Antero-Posterior Diameter	u-dap
5	Ulna-Distal Medio-Lateral Diameter	u-dml
6	Ulna-Middle Antero-Posterior Diameter	u-map
7	Ulna-Middle Medio-Lateral Diameter	u-mml

The maximum length of ulna is the maximum straight line distance between the highest point on the proximal end and the lowest point on the distal end of ulna. The remaining measurements were taken at the proximal/distal/middle part of ulna from the maximum straight line distance from anterior/posterior/medial/lateral most points as per the name of the measurement.

Number of Measurements

The somatometric and the osteometric studies undertaken for the present work, involved a very large number of measurements taken on all the 456 fetuses. In all, there were 'in order' 7296 somatometric and osteometric measurements recorded on 456 fetuses (Table 4).

Table 4. Details of Measurements on Human Fetuses and Fetal Ulna Bones

Measurement			Each fetus		Total on Somatometry / Osteometry on all fetuses
Name	Sample	Type	Number of Measurements	Total Number Of	
Somatometry	456	Single	2	(2 × 1) 2	(2 × 456) 912

Osteometry	456	Bilateral	7	(7 x 2) 14	(14 × 456) 6384
Grand Total					**7296**

Inter-age Groups

As there were four age groups viz. I, II, III, IV, the absolute growth rate was calculated between these four groups. Thus, there were three inter-age groups formed I-II, II-III, and III-IV from the four basic groups. Each inter-age group had the total number of fetuses from both the groups concerned. The absolute growth rate was calculated only for the Maximum Length of Ulna for the present study.

Statistical Considerations

Means and Standard Deviations and "t" values for bilateral and bisexual differences were calculated. Regression values for Growth Rate for fetal ulna with CRL and CHL were calculated. Regression values for estimating CRL and CHL from fetal ulna were also calculated (Singh and Bhasin 1989). Calculation of Absolute Growth Rate per cent per month for three inter-age groups was done by following the formula from Biswas and Bhattacharya (1966).

Results and Discussion

Bilateral Differences

Postnatal growth in human populations is characterized by bilateral asymmetry. Thus, dextral (Right) and sinistrel (Left) dominance in the use of hand and feet, hand clasping, arm folding, eyedness, etc. reveal at times significant variations across populations (Malhotra 1968). It is thus natural to pose the same question with reference to fetus. Unfortunately, we do not have any previous data to provide any insight into such characters and their status at fetal context. In the present study the u-ml shows significant right side dominance at 1% level from the group III in the males. In the females,

from group II, significant right side dominance is observed in the u-ml at 1% level and the u-dap at 5% level. And also in the females, the u-dml and the u-map show left side dominance at 5% level from the group II. It is observed that only 4.46% show significant bilateral differences in the osteometric measurements. Therefore, in the present study, all the analyses including the mean and standard deviations, correlation coefficients, regression analyses, analyses on growth and age estimation were calculated on the basis of the mean values of both the left and right sides merging together.

Bisexual Differences

Significant differences are found in between males and females in the u-ml, u-dml, u-mml at 5% level and in the u-pap, u-dap at 1% level from group II. In all these measurements females show higher values. On the basis of the analyses, it is observed that only 8.93% show significant bisexual differences. In spite of the very low percentage of bisexual differences in the present study, all the obtained results on correlation coefficients, regression constants for growth rate and also scattergrams along with regression fit lines and bar diagram are presented sex wise separately for evolving a broad comparative perspective on both the sexes.

Growth Rate

In the present study all the osteometric measurements, show increasing trend of growth rate for every 1 mm increase in CRL and CHL. The b1 values (Tables 5 and 6) show increase in the osteometric measurements for every one mm increase in CRL/CHL. Among all the measurements, the u-ml shows faster rate of growth in both males and females. The fastest rate of growth is observed in female ulna from the group I (11 to 16 weeks). For every 1 mm in CRL, the Maximum Length of Ulna (u-ml) is increased by .306 mm. In the group I (11 to 16 weeks) mostly females show slightly higher values in most of the osteometric measurements than males. The

group II (17 to 24 weeks) shows slightly higher rate of growth when compared to group III (25 to 32 weeks) in most of the osteometric measurements in both males and females. The growth rate of u-ml is higher in the group I compared to the group IV in both males and females.

Table 5. Growth Rate of Ulna with CRL in four age groups in males and females. b1 showing increase in the osteometric measurements for every one mm increase in CRL

ULNA	**Group – I**	**Group - II**	**Group III**	**Group IV**
Measurements				
Males	**b1**	**b1**	**b1**	**b1**
u-ml	.210	.212	.128	.178
u-pap	.029	.034	.028	.045
u-pml	.017	.020	.012	.024
u-dap	.012	.022	.017	.026
u-dml	.010	.015	.017	.018
u-map	.008	.014	.009	.015
u-mml	.011	007	.010	.017
Females	**b1**	**b1**	**b1**	**b1**
u-ml	.306	.211	.170	.192
u-pap	.094	.036	.037	.047
u-pml	.027	.019	.019	.032
u-dap	.010	.021	.019	.033
u-dml	.006	.016	.014	.027
u-map	.048	.014	.008	.019
u-mml	.083	.008	.009	.012

Table 6. Growth Rate of Ulna with CHL in four age groups in males and females. b1 showing increase in the osteometric measurements for every one mm increase in CHL

ULNA	**Group –I**	**Group -II**	**Group – III**	**Group –IV**
Measurements				
Males	**b1**	**b1**	**b1**	**b1**
u-ml	.133	.140	.104	.128
u-pap	.018	.022	.022	.031
u-pml	.011	.013	.011	.017
u-dap	.008	.014	.013	.018
u-dml	.006	.010	.013	.012
u-map	.005	.009	.007	.011
u-mml	.007	.004	.008	.011
Females	b1	b1	b1	b1
u-ml	.201	.138	.129	.141
u-pap	.058	.023	.028	.029
u-pml	.016	.013	.014	.018
u-dap	.007	.014	.015	.020
u-dml	-.001	.010	.011	.017
u-map	.041	.009	.006	.013
u-mml	.053	.005	.007	.008

Out of the seven osteometric measurements of the present study as the remaining six measurements are new ones there were no other studies available for comparison with the new measurements. Studies on bone growth, based on quantitative analyses, help better understanding the growth pattern. On the basis of the analyses on osteometry, the present study reveals that there is a positive growth trend, which is observed in all the

measurements taken on the fetal ulna from all the four age groups considered. Saettle (1951) plotted growth curves of shafts against fetal height on the basis of the growth curve which was more accurate. Moss et al. (1955) also stated that the several combinations of osseous shaft lengths revealed a constant ratio between the specific growth rates of all the bones.

The present study could not make proper comparison with the above studies, as the methodology adopted in the earlier studies to prepare the fetal bones are variable with the present study. Moss et al. (1955) measured 106 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 are 333 fetuses. The present osteometric study is carried out on 912 dried bones from 456 fetuses. Variations observed with the earlier results (Table 7) might be attributed to the less sample size of the earlier studies and the mode of preparation of the bone material for the osteometric study. Moss et al. (1955) noted a characteristic interphase in the growth of the body shaft in the CRL interval of 80 - 89 mm. Before that interval, the body shafts of all the long bones grew relatively faster than the CRL and after that the growth rate was not as fast as it was before.

Table 7. Mean of Maximum Length of Ulna: Comparison

	Group I		**Group II**	
Bones	Moss et al. (1955)	Present study	Moss et al. (1955)	Present study
Ulna	8.46	12.22	24.14	26.87

Vare and Bansal (1977) observed a linear correlation between the diaphyseal length of upper and lower limbs and the CRL from 185 fetuses of 116 males and 69 females 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, the periosteum was left intact. Vare and Bansal (1977) reported about all the long bones except clavicle. In these studies, the calculation of growth rate was found not on the basis of age groups. Whereas in the present study the growth rate is calculated using regression equations, in all the four age groups under male and female categories separately, for all the osteometic measurements. Thus in the present study a broad understanding is evolved on the growth rate of fetal ulna, age wise and sex wise. Vare and Bansal (1977) reported that for every 1 mm increase in CRL the length of ulna increased by 0.17 mm. Whereas the present study reports that the groups I, II, III and IV show 0.210 mm, 0.212 mm, 0.128 mm and 0.178 mm respectively in males and 0.306 mm, 0.211 mm, 0.170 mm and 0.192 mm respectively in females.

Absolute Growth Rate

The u-ml of males from the inter-age group I-II (11 weeks to 24 weeks) shows the highest absolute rate of fetal growth as 74.12% per month. The lowest absolute growth rate is found in the u-ml of females from the inter-age group III-IV (25 weeks to 40 weeks) as15.36% per month (Table 8). Absolute growth rate for the Maximum Length of Ulna is higher in the inter-age group I-II (11 weeks to 24 weeks) and gradually declining through the proceeding two inter-age groups II-III (17 weeks to 32 weeks) and III-IV (25 weeks to 40 weeks). The bar diagram (Fig. 2) reveals the absolute growth rate for the Maximum Length of Ulna in both males and females between the three inter-age groups. Thus the prepared bar diagram not only helps to understand the trend of the absolute growth rate but also shows male-female differences.

Table 8. Absolute Growth Rate of Maximum Length of Ulna Percent Per month

Sex	Inter-age group I	Inter-age group II	Inter-age group III
Males	74.12	25.28	18.39
Females	49.14	23.75	15.36

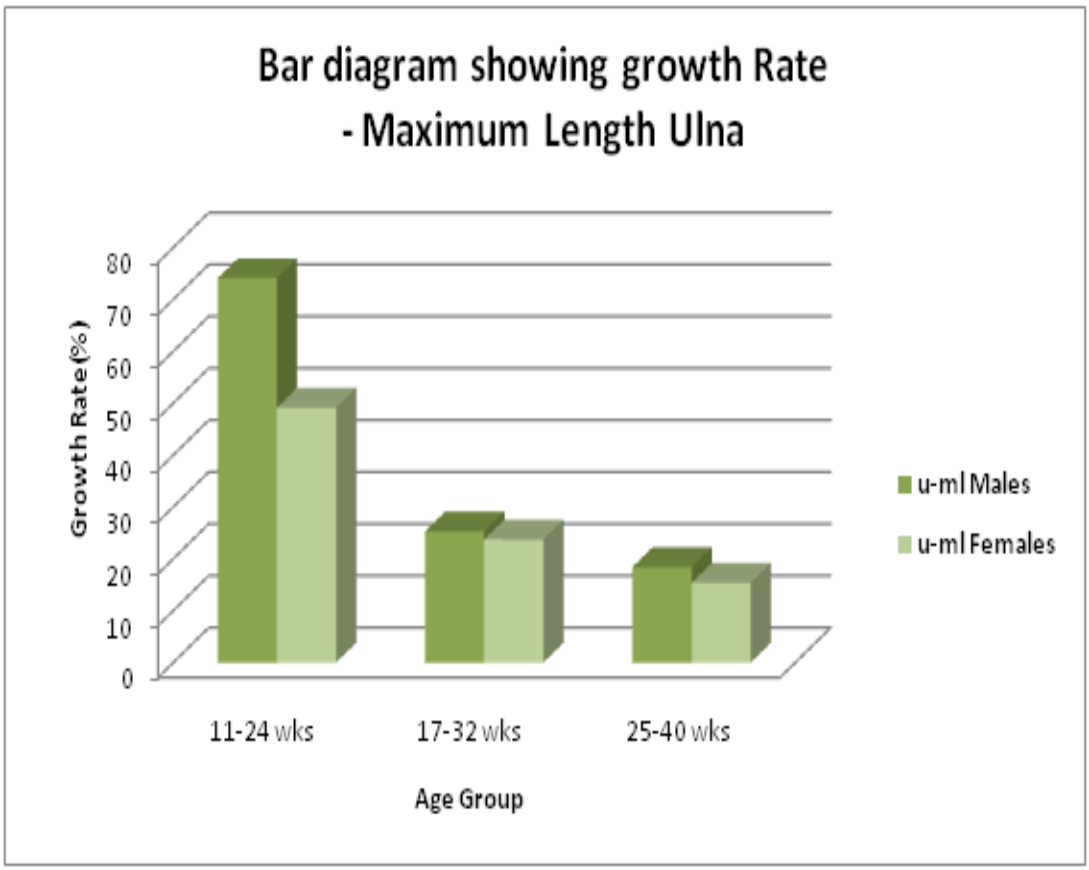

Fig. 2. Absolute Growth Rate of Maximum Length of Ulna

Bivariate distribution

In the case of osteometry, variations between CRL with the u-ml were computed in males and females separately. Because of the smaller sample size in the groups I and IV, scatter diagrams were prepared only for the groups II and III. The scattergrams (Figs. 3 and 4) show that there are very

close relationships found between the CRL with u-ml. The obtained scatter diagrams show a good fit between the variables correlated.

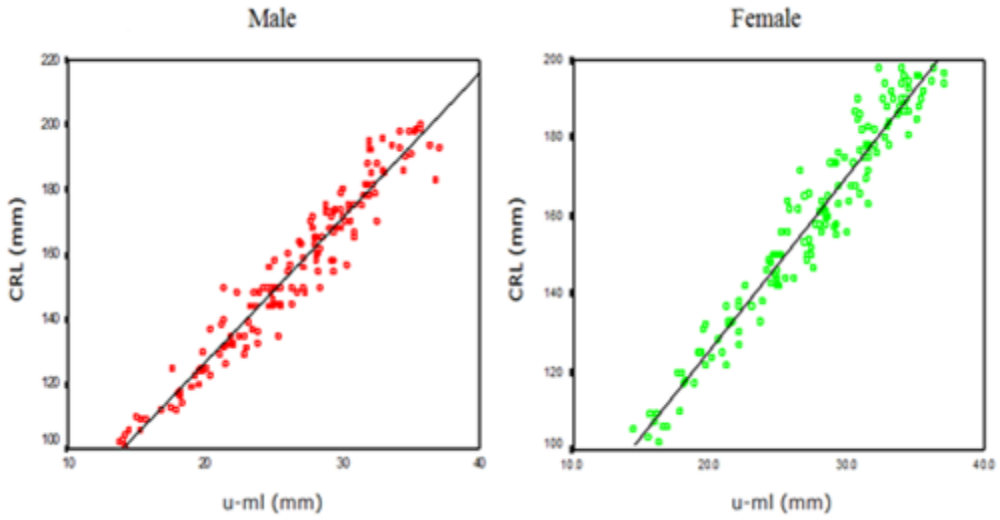

Fig. 3. Scatter Diagrams and Regression Fit Lines for Group II for u-ml with CRL

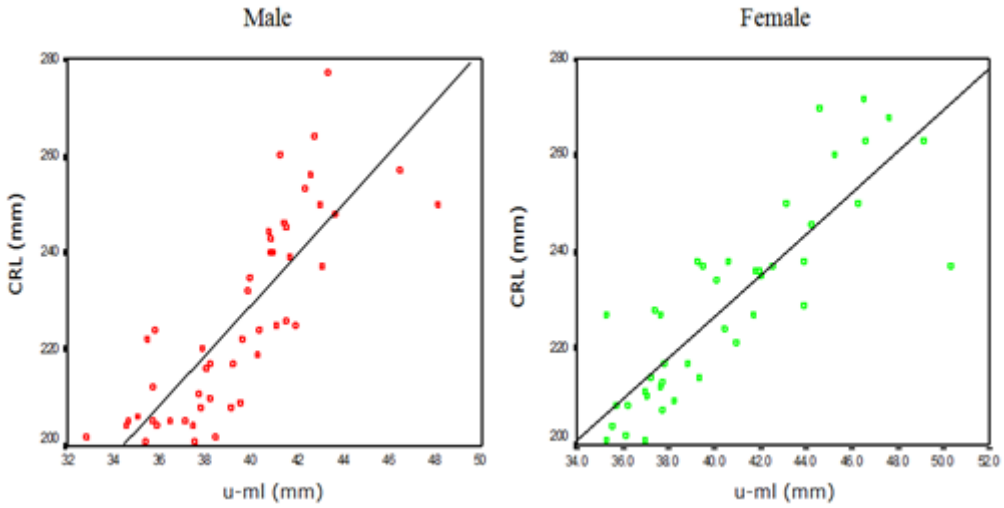

Fig. 4. Scatter Diagrams and Regression Fit Lines for Group III for u-ml with CRL

Correlations

In the present study, there are highly significant correlations obtained between the CRL/CHL with all the other measurements. In the males, the highest value of correlation coefficient .991 at 1% level is found between the

CRL and u-ml in group I. In the females, the highest value .981 at 1% level is found in between the CHL and u-ml in group II.

Age Estimation of Human Fetus

As there are highly significant correlations obtained between the CRL/CHL with all the osteometric measurements, necessary regression equations (b1, b1) were calculated for the fetal age estimation. As there are already established scales available to estimate fetal age from CRL and CHL, it is restricted to calculate only CRL and CHL from all the osteometric measurements. Once the CRL/CHL is calculated, age can be estimated from the already established age estimation scale. With the help of the tables 9 and 10, CRL and CHL can be calculated using the two values b0 and b1 and given measurements.

The formula to calculate CRL/CHL is as follows:

CRL/CHL = (b1 x measurement) + b0

Table 9. Regression Values (b0, b1) for Estimating CRL, from Osteometry on Ulna

Osteometric Measurements on Ulna	**Group –All**	
	b0	**b1**
u-ml	21.472	5.118
u-pap	43.540	26.984
u-pml	26.872	50.046
u-dap	48.861	44.309
u-dml	45.180	57.181
u-map	41.825	74.052
u-mml	35.785	100.974

Table 10. Regression Values (b0, b1) for Estimating CHL, from Osteometry on Ulna

Osteometric Measurements on Ulna	**Group –All**	
	b0	**b1**
u-ml	31.291	7.651
u-pap	65.341	40.127
u-pml	40.217	74.534
u-dap	73.279	65.882
u-dml	67.864	84.995
u-map	62.086	110.502
u-mml	54.009	150.012

As in the present study, the CRL and CHL are found closely correlated with all the osteometric measurements, which clearly reveal the dynamic relationships between the CRL/CHL with all the osteometric measurements. Mehta and Singh 1972; Vare and Bansal 1977; Kosa 1997 attempted to estimate fetal age from chemically preserved fetal long bones. Vidhu et al. (2014) dissected fetal femora from fetuses and measured. Simon et al. (1992); Simon and Baig (2015)[a]; Simon and Baig (2015)[b] estimated CRL and CHL from fetal clavicle, fetal humerus and fetal femur respectively from naturally macerated fetal bones without adding any preservatives. The analysis in the present study to estimate fetal age from the measurements on human fetal ulna bones would definitely help in solving problems facing estimation of fetal age, a crucial factor in medico-legal cases.

Conclusion

The present study is a departure from the earlier attempts in terms of large sample size with almost equal sex ratio, osteometry taken directly on naturally prepared ulna bones belong to full fetal period. Six new osteometic

measurements are newly introduced. Increasing growth trend is found on the basis all the measurements taken on fetal ulna. Age estimation formula is found which would be solving problems in forensics. The significance in the clinical aspects would enable to advance a standard which would help to comprehend the differential growth pattern between normal and abnormal fetuses. The anatomical aspects of this study on growth pattern in relation to population base, age, sex variations would open new vistas of researches in the fetal growth.

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