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Original Research Article

FETAL SKELETAL GROWTH PATTERN ANALYSIS IN FETUSES WITH SKELETAL DYSPLASIA– AN ULTRASONOGRAPHY STUDY IN WESTERN INDIAN POPULATION

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ABSTRACT

Background: In developing countries like India, several genetic, environmental factors and diverse cultural practices affect the normal growth and development of human embryo resulting in congenital malformations namely skeletal dysplasia. This article aims at analyzing the fetal skeletal growth pattern followed by its correlation to understand prenatal diagnosis of skeletal dysplasia.

Materials and Methods: Two studies, conducted by the authors at different places in western India population have been correlated. Primary prospective study, was conducted among 500 antenatal women coming for routine antenatal check-up during from 1st July 2015 to 31st May 2018. Routine fetal parameters like biparietal diameter, gestational sac, HC, AC, TC, FL along with all the other fetal long bones were measured using 2D/3D ultrasonography. Second retrospective study, was conducted on new-borns with congenital malformations in the same population.

Result: Out of the total 10,114 deliveries conducted during the study period, 182 new-borns had congenital malformations, out of which 23 cases had skeletal dysplasia. Fetal skeletal growth pattern analysis done was observed to be comparable with other populations residing in different parts of India and also abroad. Thanatophoric dysplasia was the most common type of skeletal dysplasia. Prenatal diagnosis of skeletal dysplasia was done correctly in 75% of cases. Peak of diagnoses were between 15 and 19 weeks of gestational age.

Conclusions: Fetal growth pattern analysis observed in western Indian population is similar to that observed in other groups of India and abroad. In majority of cases of thanatophoric dysplasia, prenatal ultrasound examination was diagnostic, providing a correct diagnosis using ultrasonography as the sole modality.

KEYWORDS: Skeletal Dysplasia, Prenatal Diagnosis, Fetal Growth Analysis, Thanatophoric Dysplasia.

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INTRODUCTION

Normal growth and development of human embryo is imperative and factors influencing it need to be bounteously studied by medical and non-medical professionals especially in developing countries like India. Fetal skeletal dysplasia is an osteochondroblastic disease, affecting approximately 2.4–4.5 of 10,000 births [1,2]. Thoughrare, skeletal dysplasia are commonly inherited as autosomal dominant, autosomal recessive, or X-linked disorders, and some disorders also result from imprinting

errors, somatic mosaicism, and teratogen exposure [3]. Substantial progress in identification of the molecular defects responsible for the osteo-chondro-dysplasia's, and the genetic defects have led to availability of DNA diagnostics for both molecular confirmation of ultrasound and post-mortem findings, as well as invasive prenatal diagnosis for at-risk families [4].

Routine mid-trimester ultrasound evaluation conducted among antenatal women in India include evaluation of the fetal skeleton in second trimester for detection of congenital malformations by two-dimensional ultrasound by 14 weeks, and measurements of the fetal femora and humeri. At present, the prenatal diagnosis of fetal skeletal dysplasia mostly relies on three dimensional ultrasound, X-ray and magnetic resonance imaging [5].

Studies on fetal growth pattern analysis has been conducted by several researchers from Mall F.P.,1914toTorvid Ket.al., 2017 by measuring foetal parts and correlated them with gestational age. Foetal growth pattern analysis is done by assessment of symphyseal-fundal height (clinically) and para-clinically by ultrasound biometry [6,7].Studies analysing multiple foetal parameters like Biparietal diameter (BPD), Head circumference (HC), Abdominal circumference (AC), Femur length (FL), Thoracic circumference (TC)for prediction offoetal growth have been carried out by plethora of researchers whereasfew have taken into account the study of alllong bones.

In thepresent article, observations from two separate research studies conducted by the authors separatelyin western Indian population have been correlated. In the primary (prospective) study, foetal skeletal growth pattern was analysedby taking routine fetal measurements like BPD,HC, AC, TC along with all fetal long bones, and if required special investigations were conducted to monitor foetal skeletal growth and development. The second (retrospective) study dealt with fetuses with congenital malformations and retrospectively studied the fetal skeletal growth pattern by retrieving the ultrasonography details and other investigations conducted during the gestational period. Finally, a correlation of fetal growth pattern analysis of structurally normal and skel-etally malformed fetuses was done to ensure better insight of prenatal diagnosis of skeletal dysplasia's.

MATERIALS AND METHODS

Participants and settings: Two separate observational studies wereconducted by the authors separately in two different Government Medical and Research Institutes of western part of India.Onestudy was conducted between 1st July 2015 to 31st May 2018 (henceforth referred as primary study) and another study was conducted between1st July 2010 and 31st May 2013 (henceforth referred as secondary study). The primary (prospective) study was carried on 500 pregnant women, which were selected by applying inclusion and exclusion criteria from amongstthe antenatal carewomenroutinely attending antenatal clinics for ultrasonographic screening at the Government civil hospital and its tie up hospitals.The ultrasonographic analysis of allstudy participants wasdone at the routine stationinantenatal ward in the civil hospitals. Ethical committee approval for the research study was taken from department of obstetrics and gynaecology, department of radiology, department of paediatrics and department of anatomy before commencement of the study. Informed consent from all the participants was also taken for any special or additional investigation/s like sampling of fetal umbilical cord blood and amniotic fluid, karyotyping, whole genome sequencing, and targeted next-generation sequencing required to be done on the patient or her spouse or blood relatives if in case suspicion arises with regards to the fetal growth and development. All these additional expenses were borne by the study researchers. No intramural or extra-mural funding was received.

Inclusion and exclusion criteria: The study participants belonged to age interval of 18 to 34 years, andwere born and brought up in western India, and only those ANC patients who had an uncomplicated obstetric history and an accurate knowledge of the last menstrual period were includedin the study. All the study participants attending ultrasonographic screening at the Medical College and Hospital were screened under the guidance of only one experienced sonographer to avoid observer bias.

Data collection procedure: Following procedure

was adopted to collect data for the primary study.

1) General informationa bout the participants selected for the study with special reference to their menstrual history, socio-economic score, Hb%, past medical and surgical history, h/o consanguineous marriage, h/o previous child with congenital malformation, etc. was recorded in a proforma. For the same, a specially designed, self-administered questionnaire was constituted in Hindi and Marathi. After taking informed consent, the questionnaire was handed over to the participants and data collected. Discussion was not allowed among the participant during filling of the questionnaire, which was assured by ensuring presence of minimum one researcher supervising the process.

2) Ultrasound screening examination was performed on the participants with full urinary bladder. The participants were placed on the ultrasound scanning table in supine position with their abdomen exposed. Then a mineral oil jelly " SONOGEL" was applied all over the surface so as to ensure an airless contact between the tissue and the transducer probe.

3) The transducer probe was placed in a longitudinal direction and moved all over the surface of the abdomen. The foetal position was then assessed.

4) The measurements of all the parameters were made as per the recommendations of the American Institute of Ultrasound in Medicine [8], 2018.

a. The measurements of the gestational sac and the crown rump length were recorded only in the first trimester of pregnancy i.e. during the first 12 weeks of gestation as assessed from the menstrual dates.

i. The gestational sac parameter is a measureof the anechoic space containing the fluid, embryo and extra embryonic structures. To compensate for the error due to different shapes (e.g. ovoid) of the sac, the mean of long, antero-posterior and transverse axis measurements, one each, is recommended.

ii. The crown-rump length is a measurement of the embryo is a measurement along its longest axis.

b. The Biparietal diameter (BPD) is measured in the trans-axial plane at the widest portion of the skull, with the thalamus positioned in the midline. A leading edge to leading edge measurement is obtained from the first echo of the closer temporoparietal calvarial table to the first echo of the farther temporoparietal calvarial table. It is measured with a freeze framed electronic calliper technique.

c. The foetal head circumference was measured at the same level as that of the BPD.

d. The foetal abdomen was identified and with the help of the digitaliser, the perimeter of the body wall was traced and the foetal abdominal circumference was measured at the level where the portal vein is inside the liver, equidistant from the lateral body walls.

e. The transducer probe was moved along the foetal spine to identify the inferior extremity. Femur was identified and a straight measurement of it was taken from one end to the other, along the long axis of the diaphysis, the osseous portion of the shaft.

f. Similarly, humerus, tibia, fibula, radius and ulna were identified and straight line measurements of their ossified shafts were taken.

g. The information so collected was recorded in using a proforma.

Any fetus showing femora or humeri length measurements less than 5th centile or -2 SD from the mean inthe second trimester (<24 weeks) and fetuses with chest-to-abdominal circumference ratio of <0.6 or femur length to abdominal circumference ratio of 0.16 (suggesting lethality)were referred to a radiologyandgenetic lab for CT/MRI and genetic/ molecular evaluation respectively. All theroutine fetal measurements thus taken (like BPD, AC,HC, TC and length of all fetal longbones)along with findings of additional special investigation sconducted were analysedand fetalmaturity in terms of weeks was recorded.

Data analysis procedure: The data collected from 500 subjects was then subjected to statistical analysis. Following statistical formulae were used:

1) Average: This is the arithmetic mean which is the sum of measurements in a series divided by the number of measurements. It stands for the whole series, as typical of the values in the series.

$$X = \frac{\sum x}{n}$$

Where X is the average or mean

$\sum x$ is the sum total of values in the series.

n is the number of observations

2) Standard deviation: It is by far the most important and widely used measure of dispersion or variation in the observations and is calculated as

$$\sigma = \sqrt{\frac{\sum(x-\bar{x})^2}{N}}$$

Where, x= individual values, x = mean of all values, N= no. of observations.

3) Coefficient of variation: It is the ratio of the standard deviation to the mean, expressed as percentage. Low value of coefficient of variation implies almost uniformity in the values of the variation in the observations of a variable. High values of coefficient of variation signifies a larger variation in the observations of a variable.

$$C.V. = \frac{S.D.}{Mean}$$

All thestudy participantswere screened using ultrasonography every week starting from 7th week till 38th week of gestational period and all mentioned foetal parameters were measured for assessing the general and skeletal foetal growth pattern. All the participants were followed regularly tillterm and their off-springs were examined for presence of any congenital malformations by paediatrician.

The second (retrospective)study was conducted on 182 new-borns with congenitally malformations arising out of 10,114 deliveries which were conducted in the medical institute and tie up hospitals from 1st July2010 to 31st May 2013. The study was done in western Indian population at Government medical college and hospital, Miraj. The 182 cases were studied regarding the sex of proband, birth order, type and subtype of congenital malformation, h/o consanguinity, maternal and paternal education and occupation. Details of ultrasonography and other investigations done in the gestational period of their mothers was retrieved and analysis was done pertaining to the prenatal diagnosis done in these cases. The data arising from both the studies regarding ultrasonographic findings and fetal congenital malformations was tabulated, graphically represented, statistical analysed and discussed.

OBSERVATIONS

Table 1: The data regarding the measurements of fetal long bones.

Menstrual age in weeks	No. of observations	Average FL	Average TL	Average Fib. L	Average HL	Average RL	Average UL
13	6	1.35	1.15	1.05	1.25	0.97	1.07
14	17	1.29	1.1	1.05	1.23	0.99	1.08
15	17	1.58	1.32	1.42	1.46	1.26	1.35
16	19	1.96	1.77	1.67	1.78	1.62	1.71
17	16	2.5	2.17	2.13	2.27	2.02	2.19
18	32	2.75	2.46	2.45	2.56	2.31	2.41
19	17	2.9	2.65	2.65	2.7	2.41	2.57
20	26	3.24	2.94	2.91	2.99	2.69	2.89
21	21	3.28	2.98	2.99	3.12	2.78	2.98
22	50	3.62	3.3	3.34	3.31	3.03	3.19
23	21	3.81	3.59	3.53	3.6	3.25	3.42
24	19	4.38	3.93	3.96	3.98	3.66	3.8
25	27	4.52	4.14	4.17	4.21	3.87	4.04
26	25	4.78	4.3	4.3	4.36	4	4.17
27	17	4.65	4.22	4.3	4.29	3.87	4.09
28	19	4.96	4.55	4.67	4.51	4.11	4.32
29	11	5.55	5.17	5.27	5.12	4.72	4.89
30	22	5.6	5.16	5.23	5.13	4.75	4.98
31	11	5.68	5.23	5.09	5.11	4.63	4.85
32	12	6.1	5.68	5.75	5.57	5.04	5.33
33	9	5.93	5.68	5.5	5.52	5.05	5.31
34	7	6.2	5.5	5.65	5.5	5.16	5.34
35	6	6.38	5.77	5.87	5.73	5.28	5.42
36	2	6.6	6.05	6.2	6.1	5.5	5.75
38	2	7.05	6.2	6.15	6.15	5.5	5.8
TOTAL	**433**						

Table 2: The data regarding the measurements of fetal long bones.

Menstrual age in weeks	No. of observations	Standard deviation - FL	Standard deviation -TL	Standard deviation – Fib. L	Standard deviation - HL	Standard deviation - RL	Standard deviation - UL
13	6	0.05	0.05	0.05	0.05	0.05	0.05
14	17	0.24	0.23	0.26	0.25	0.24	0.23
15	17	0.28	0.23	0.27	0.3	0.25	0.24
16	19	0.29	0.3	0.3	0.3	0.29	0.32
17	16	0.41	0.47	0.42	0.42	0.41	0.4
18	32	0.4	0.39	0.38	0.4	0.38	0.39
19	17	0.24	0.27	0.3	0.23	0.19	0.22
20	26	0.2	0.26	0.18	0.25	0.21	0.27
21	21	0.19	0.2	0.23	0.23	0.16	0.19
22	50	1.18	0.26	0.28	0.24	0.21	0.21
23	21	0.19	0.21	0.24	0.17	0.26	0.23
24	19	0.28	0.31	0.29	0.28	0.3	0.28
25	27	0.31	0.34	0.34	0.25	0.31	0.25
26	25	0.19	0.28	0.36	0.23	0.33	0.25
27	17	0.29	0.34	0.42	0.26	0.27	0.25
28	19	0.4	0.4	0.4	0.34	0.36	0.29
29	11	0.34	0.28	0.24	0.31	0.42	0.38
30	22	0.34	0.37	0.35	0.42	0.44	0.4
31	11	0.52	0.64	0.69	0.58	0.49	0.54
32	12	0.48	0.41	0.33	0.28	0.36	0.3
33	9	0.47	0.42	0.35	0.39	0.63	0.54
34	7	0.2	0.18	0.19	0.25	0.21	0.21
35	6	0.13	0.29	0.27	0.18	0.27	0.19
36	2	0.1	0.05	0.1	0.1	0.1	0.15
38	2	0.15	0.2	0.15	0.15	0.3	0.1
TOTAL	**433**						

Primary study: All the study participants were screened using ultrasonography weekly and followed till the term. Foetal growth pattern analysis was conducted usingall said foetal parameters with focus onearly detection of congenital malformations in the foetuses. 500 antenatal women were studied after selection from among the antenatal women admitted in medical college and civil hospitals during the study period. 411 of 500 study participants had normal vaginal delivery after reaching full term, whereas 88 had to undergo LSCS and remaining 1 had miscarriage. Out of the 499 new-borns delivered, 9 neonates had congenital malformations (involving any system of the body) and remaining 490 were normal births.

The data regarding the measurements of fetal long bones was tabulated and shown in table 1 and 2.

Second study: Out of the total 10,114 deliveries conducted in the government medical college and its hospitals during the study period, 182 new-borns had congenital malformations (belonging to any system of body). Incidence of congenital malformations was calculated as 1.79%.

The total 182 off spring with congenital mal formations were classified depending on the system to which themal formation belonged. Cardiovascular cases (39) were the most common. Twenty-threecaseswere identified with Musculo-skeletal malformation. Mixed group (35) consists of those caseswho exhibited more than one anomaly and each belonging to different system. Refer table 3.

Table 3: Showing the no of cases and malformation.

Type of malformation	No. of cases out of 182	Percentage (%)
Cardiovascular	**39**	**21.4**
Respiratory	3	1.6
Cerebrovascular	14	7.7
Musculoskeletal	**23**	**12.6**
Renal	8	4.4
Urogenital	22	12.1
Anorectal	14	7.7
Gastrointestinal	24	13.2
Mixed (combination of anomalies)	35	19.2
Total	**182**	**100**

All the 182 cases were further classified based on the subtype of congenital malformation. Atrial septal defect (18 cases) was the most common subtype of cardiovascular variety. Twenty three cases were affected by musculo-skeletal type of congenital malformation. Out of 23, 8 cases had features of lethal skeletal dysplasia and were identified as a syndrome. Other 15 cases,exhibited non-lethal, isolated

individual variety of skeletal dysplasia's and couldn't be classified as any syndrome.For details refer table 4.

Table 4: Isolated individual variety of skeletal dysplasia's.

Sl. No.	Type of congenital malformation	No of cases (n)	Most common subtype (n)
1	Cardiovascular	**39**	Atrial septal defect (18)
2	Respiratory	3	-
3	Cerebrovascular	14	Hydrocephaly (6)
4	Musculoskeletal	**23**	**Skeletal dysplasia (8)**
5	Renal	8	Hydronephrosis + PUJ obstruction + vesicoureteral reflux (4)
6	Urogenital	22	Hypospadias (7)
7	Anorectal	14	Anal atresia (9)
8	Gastrointestinal	24	Cleft lip and palate (8)
9	Mixed (combination of anomalies)	35	**Skeletal malformations**
	Total	**182**	

On considering the total 182 fetuses, born with congenital malformations to total 10,114 deliveries conducted during the study period, 23 cases were identified with musculoskeletal malformations. 8 cases were confirmed as lethal skeletal dysplasia's. All the 8 cases displayed features of hypoplasia affecting upper limb or lower limb, depressed nasal bridge, dysmorphicfacial features, thoracic hypoplasia, deformed femur (reduced femur length), etc. and werediagnosed as thanatophoric dysplasia type 1. Remaining were identified as non-lethal, isolated, individual musculoskeletal malformations. 4 cases were identified with unilateral CTEV while 3 cases were of bilateral CTEV. 3 cases were identified with Kypho-scoliosis and another 3 cases were identified with Proximal femoral focal deficiency. One case with polydactyly and another with syn-polydactyly was also identified. Molecular testing was conducted only in 9 cases out of 23, because the dysplasia were of lethal variety. In the second study, the observations stated that only 6 cases out of total 8, were correctly diagnosed prenatally and identified as Thanatophoric dysplasia type 1. They were diagnosed between 18-25 weeks of gestational age. Two cases out of 8 cases of TD were incorrectly diagnosed prenatally as structurally normal IUGR fetuses. They were correctly diagnosed post-natally.The fetal measurements (FL, BPD, HC, long bones and ThC) data were available at thefirst diagnostic examination. The FL was short (by definition (<5th centile) as well as Z-scores <"2). The HC was increased in the case.

Lethality in skeletal dysplasia's is caused by impaired lung development, secondary to a narrow thorax, hence thoracic circumference was measured in suspected cases. Prenatal two dimensional sonographic imaging examination displayed short limbs and FL/AC ratio < 0.16. after genetic counselling, the pregnancy was terminated and a malformed fetus was delivered. Postnatal radiography findings were consistent with diagnosis of TD 1 with features of right thumb hypoplastic atrophy, femur – 'telephone receiver shape', depressed nasal bridge, dysmorphic facial features with macrocephaly, microtia, frontal bossing, short neck with hypoplastic lung with microcephaly, short ribs, long narrow trunk. Molecular genetic testing identified FGFR3 gene mutant.

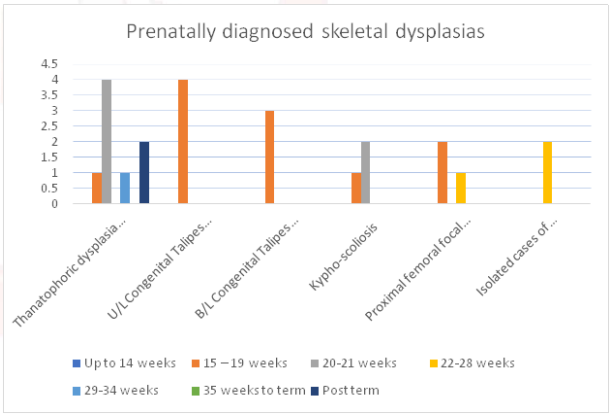

Fig. 1: Prenatally diagnosed skeletal dysplasias.

The case had to be differentially diagnosed from other conditions like asphyxiating thoracic dysplasia, homozygous achondroplasia, short rib polydactyly, osteogenesis imperfecta, campomelic syndrome.

Seven cases of congenital talipes equinovarus(4 unilateral, 3 bilateral) were identified. All of them were correctly diagnosed prenatally between 17-19 weeks. They were identified as foetuses showing positional abnormality of left foot with persistent adduction & inward rotation and normal liquor. Though the abnormality was suspected on 2D-mode, yet 3D-mode played a key role in confirming the diagnosis. No evidence ofany other sonographic fetal abnormality was noted. Based on the above findings, isolated, unilateral and bilateral club foot was diagnosed.

Table 5: Showing the Various types of skeletal dysplasia and number of cases.

Type of Skeletal dysplasia	No (out of total 182 cases)	Foetuses with PD Correctly diagnosed	GA at PD (completed weeks)	Pregnancy Outcome
Thanatophoric dysplasia (TD -1)	8	6/8	1-18 wks.	All pregnancies were terminated
			2-21 wks.	One - Beyond 35 weeks
			3-21wks.	Four - 22 -28 weeks
			4-29 wks.	Two - 20-21 weeks
			5-20 wks.	
			6-21 wks.	
			7-false negative	
Right thumb hypoplastic atrophy, femur – 'telephone receiver shape'			8-False negative	
Depressed nasal bridge				
Dysmorphic facial features with macrocephaly, microtia, frontal bossing				
Short neck with hypoplastic lung with microcephaly				
Short ribs, long narrow trunk				
U/L Congenital Talipes Equino Varus	4	4/4	17-18 wks.	Vaginally delivered full term babies in all the cases
B/L Congenital Talipes Equino Varus	3	3/3	18-19 wks.	
Kypho-scoliosis	2- scoliosis 1-kyphosis	3/3	1. 18 wks.	1,2 - Pregnancy terminated
			2. 21 wks.	3 – live birth at 39 wks.
			3. 21 wks.	
Proximal femoral focal deficiency (PFFD)	3	3/3	1. 18 wks.	Elective caesarean section
			2. 23 wks.	
			3. 18 wks.	
Isolated cases of polydactyly and poly-syndactyly	1-Polydactyly	2/2	1- 23 wks.	Both fetuses born alive - good perinatal outcome
	1-Poly-syndactyly		2- 24 wks.	
Total	**23**			

The ladies were counselled about the fetal anomaly with its inherent prognosis and was followed-up in postpartum period when clinical examination vaginally delivered; full-term baby confirmed the antenatal diagnosis.

Three cases of kypho-scoliotic disease were identified all correctly prenatally at 18, 21, 21weeks respectively. First case was of pure structural scoliosis—acondition that, even without other associated structural abnormalities carries a poor prognosis. Ultrasound scan revealed gross fetal spinal scoliosis, almost to an angle of 90 degrees, at the lower thoracic level. The spinal canal and overlying skin appeared intact, with no evidence of a classical neural tubedefect. The serum AFP estimation was 51 Ku/L. This normal serum AFP result was explained by the lack of obvious breach of the meninges. The parents opted forpregnancy termination. The fetus was found to have webbing of the neck and an imperforate anus in addition to vertebral defects.Second case had ultrasonography findings at 21 weeks of scoliosis with lower thoracic and lumbar hemivertebrae, fused ribs, possible sacral agenesis. Prenatal MRI at 22 weeks was non diagnostic. Pregnancy was terminated at 23 weeks. Third case was of kyphosis. USG scan at 21 weeks showed hemivertebrae at two levels. Additional investigations were declined. Pregnancy was continued resulting in live birth of 39 weeks, 2905 grams, female. Post-natal examination showed left sided u/l segmentation defect at T-10/11 and resultant right sided scoliosis. B/L segmentation defect at L4-5 with resultant block vertebra and narrowing of spinal canal was seen.

Three isolated cases of proximal femur focal deficiency were correctly identified pre-natally at 18, 21 and 18 weeks in 18, 33 and 32 years old pregnant women respectively. The family history was negative for congenital anomalies and no h/oconsanguineous marriage. The patients were negative for diabetes mellitus. Prenatal exams performed in the first trimester of pregnancy showed normal results. The right femurwas shorter than the left one in the second and third cases and the left femur were

shorter than the right one inthe second case. Computer Tomography (CT) wasperformed after 30 weeks of gestation and confirmed the images obtained by 2D-US and3D-US. The presumptive diagnosis was congenital isolated short femur. Elective caesarean sections were carried out at 38 weeks 'for the first and second fetuses and 39 weeks for the third fetus. The first andsecond infants were both 2 years old and showed a 5.2-and 6.0-cm difference between limb lengths, respectively. The third infant showed a 6.7-cm difference between limb lengths.

Two isolated cases, one of polydactyly and other syn-polydactyly were correctly identified at 23 and 24 weeks respectively. Both the fetuses were scanned and found no other congenital malformation. they born alive. So, it was concluded that isolated polydactyly identified by prenatal sonography is associated with good perinatal outcome. Refer to Table 5 and Figure 1 for details.

DISCUSSION

Fetal growth pattern analysis in normal offspring: In the primary study, fetal growth pattern analysis was done by measuring the gestational sac size and crown rump length during first trimester of pregnancy. In second and third trimester, measurementsof biparietal diameter, head circumference, abdominal circumference and fetal long bones were done. Observations regarding gestational sac size in the present study were similar to those studied by Hellman F. L et al [9]. 1969 in United States. Observations regarding crown rump length in the present study and those published by Robinson H. P et al [10]. In 1975 inUnited States were similar. Observations regarding measurements of bi-parietal diameter and head circumference in primary study matched with those of Rajan R et al [5]. 1990. The slight variation in observations of primary study and the above said studieswere negligible from statistical point of view.

Study observations specify that the gestational sac is identified as early as 5th week and the embryonic crown rump length is identified a week later, i.e. by about 6th week. This knowledge is important for pregnancy detection in early first trimester, if medical termination is to be done.The differentiation between hard and soft tissues of the embryo is possible after about 10th week of gestation when other parameters like bi-parietal diameter, head circumference, abdominal circumference, length of long bones can be measured and become more important than the crown rump length.

Comparison of study observations in normal fetuses: Several studies on the subject has been conducted on western population. Studies by Indian authors (Rajan R et al [5]. 1990; Mukherjee B et al [11]. 1986; Ghamande S. A et al [12]. 1989) were reflection of the fetal growth parameters in a particular region of India. India being a multiracial country, regional differences in the growth pattern of fetal parameters is expected. Table 4 depicts the comparison of (week wise averages of measurements of fetal limb bones) primary study observations with findings of previous studiesconducted among Indian and foreign population.Observations of primary study match with those of previous studies.

Fetal growth pattern analysis in cases with skeletal dysplasia (second study): While skeletal dysplasia whichpresent individually are quite rare. A recent Indian study reports that incidence of SDs in Indiais 19.6 per 10,000 births and 5.2 per 10,000 births for lethal dysplasia's [14].

Classification and frequencies of skeletal dysplasia's: Currently, the prenatal diagnosis of fetal skeletal dysplasia mostly relies on ultrasound, X-ray and magnetic resonance imaging. In 40–49% of cases with fetal skeletal dysplasia, ultrasound cannot differentiate among the different types of skeletal dysplasia. Hence, this has been merely used to identify severe lethal skeletal dysplasia [15,16]. In the 2010 revision of the Nosology and Classification of Genetic Skeletal Disorders, 456 conditions were classified into 40 groups defined by molecular, biochemical and radiographic criteria [17]. Among these conditions, 316 conditions were associated with mutations in one or more of 226 different genes, providing a basis for the molecular genetic diagnosis of fetal skeletal dysplasia. In the present study, 8 cases could be identified as thanatophoric dysplasia and rest were isolated

cases of non-lethal unclassified congenital malformations which required further investigations. Our casescame from various districts of state of Maharashtra, Telangana and Karnataka and not from a particular region only. We therefore could not calculate the incidence of individual skeletal dysplasia's. However, thecommon types of skeletal dysplasia in observed in our fetal population and their frequencies were similar to those found by researchers in previous studies namely Sharony et al [18]., 1993, Goncalves and Jeanty [19], 1994, Gaffney et al [20]., 1998, Doray et al [21]., 2000.

Diagnostic accuracy of prenatal diagnosis: The probability of achieving the correct specific diagnosis by prenatal ultrasound depends on the type of skeletal dysplasia. Themost common type of skeletal dysplasia inour study was than atophoric dysplasia, which was diagnosed correctly prenatally in 75% of cases between 18 to 25 weeks. Two cases out of 8 cases of TD were incorrectly diagnosed prenatally as structurally normal IUGR fetuses. Tretter et al [22]. and Parilla et al [23]. report that they had no false negatives.

Lethality: Lethality in skeletal dysplasia's is caused mainly by pulmonary hypoplasia, secondary to thoracichypoplasia. The discrimination between lethal and non-lethal formsof skeletal dysplasia's is, of utmost clinical significance. The findings should be conveyed to the physicians caring for the patient and to thepatient. In our study, except three cases, all fetuses with lethal disease(TD) were correctly classified (88%). These findings match with Schramm et al [24](113/114, 99%), Gaffney et al [20]. (100%), Doray et al. [21] (17⁄21) and Parilla et al [23]. (100%).

Gestational ages at diagnosis: In India, legal gestational age of termination of pregnancy is 20 weeks and hence earlydetection iscrucial. In our study, almost all cases of TD except one were diagnosedbefore 21weeks. Non-lethal skeletal dysplasia was identified relatively earlier than TD.

Biometry: In ultrasound screening, the FL is the best parameter for the detection of skeletal dysplasia's. However,not all cases of skeletal dysplasia have shortening below -2 SD. Previous studies commented that theFL is the best parameter to distinguish at least among the most common typeof skeletal dysplasia's. Our findings match with this, the measurements of the other long bones and the head and thorax circumferences contributing mainly to the differential diagnoses.

Genetic analysis: Two- or three-dimensional ultrasonography assisted with computerised tomography appropriately whenever required can give a specific diagnosis in the majority of cases.

Various chromosomal abnormalities complicate the wide, diverse variety of skeletal abnormalities. Fetuses with trisomy 13, trisomy 18, or even trisomy 21 (Down's syndrome) may present abnormal skeletal development. Molecular genetic testing can contribute essential diagnostic information and complement prenatal sonographic assessment in inconclusive cases. In inconclusive cases ultrasound assisted by CT canbeused to make probable diagnosis initially and appropriate molecular tests, including molecular genetic analysis could be selected to confirm the diagnoses later. Inpresent study, all cases identified as thanatophoricdysplasia– 1, were testedsubsequently formolecular analysis which involved either full sequence analysis of all coding exons of the FGFR3 gene, or more targeted initial analysis of exons 7, 10, 15, and 19, which are the exons containing themutations responsible for 99% of cases of TDI reported to date.For TDI a series of missense mutationshave been identified: R248C*, Y373C*, S249C, G370C, S371C (Rousseau et al [25].1996; Passos-Buenoet al [26].1999). The most common mutants(*) account for 60- 80% of TDI (of note all mutations create new, unpaired cysteine residues in theFGFR3). Further more, stop codon mutations (X807L, X807G,X807R, X807C, X807W) have been identified (Rousseau et al [27]. 1995; Rousseau et al [25]. 1996). Even patients with the same FGFR3 mutation may have phenotypic differences. The TD variants occasionally lack typical characteristics, such as facial deformation or different degrees of femur bowing [26]. Wilcox et al. [28]and Castori et al. [29] suggested that the phenotypic variability of TD may be due to nonallelic genetic variability, epigenetic/environmental, and stochastic factors.

Table 6: Gestational age related changes Primary study findings in agreement with literature.

Gestational age	Primary study						E. Merz et al [13], 1987					Rajan et al [5], 1990
	FL	HL	TL	FiL	RL	UL	HL	TL	FiL	RL	UL	FL
13	1.35	1.25	1.15	1.05	0.97	1.07	1	1	0.8	0.6	0.8	1.1
14	1.29	1.23	1.1	1.05	0.99	1.08	1.2	1.3	0.9	0.8	1	1.4
15	1.58	1.46	1.32	1.42	1.26	1.35	1.4	1.6	1.2	1.1	1.2	1.7
16	1.96	1.78	1.77	1.67	1.62	1.71	1.7	1.9	1.5	1.4	1.6	1.9
17	2.5	2.27	2.17	2.13	2.02	2.19	2	2.2	1.7	1.5	1.7	2.3
18	2.75	2.56	2.46	2.45	2.31	2.41	2.3	2.5	2.1	1.9	2.2	2.6
19	2.9	2.7	2.65	2.65	2.41	2.57	2.6	2.7	2.3	2.1	2.4	2.9
20	3.24	2.99	2.94	2.91	2.69	2.89	2.9	3	2.6	2.4	2.7	3.1
21	3.28	3.12	2.98	2.99	2.78	2.98	3.2	3.2	2.9	2.7	3	3.3
22	3.62	3.31	3.3	3.34	3.03	3.19	3.3	3.5	3.1	2.8	3.1	3.6
23	3.81	3.6	3.59	3.53	3.25	3.42	3.7	3.7	3.4	3.1	3.5	3.9
24	4.38	3.98	3.93	3.96	3.66	3.8	3.8	3.9	3.6	3.3	3.6	4.2
25	4.52	4.21	4.14	4.17	3.87	4.04	4.2	4.2	3.9	3.5	3.9	4.6
26	4.78	4.36	4.3	4.3	4	4.17	4.3	4.4	4	3.6	4	4.8
27	4.65	4.29	4.22	4.3	3.87	4.09	4.5	4.6	4.2	3.7	4.1	4.9
28	4.96	4.51	4.55	4.67	4.11	4.32	4.7	4.8	4.4	3.9	4.4	5.3
29	5.55	5.12	5.17	5.27	4.72	4.89	4.8	5	4.5	4	4.5	5.3
30	5.6	5.13	5.16	5.23	4.75	4.98	5	5.1	4.7	4.1	4.7	5.6
31	5.68	5.11	5.23	5.09	4.63	4.85	5.3	5.3	4.9	4.2	4.9	6
32	6.1	5.57	5.68	5.75	5.04	5.33	5.4	5.5	5.1	4.4	5	6.1
33	5.93	5.52	5.68	5.5	5.05	5.31	5.6	5.6	5.3	4.5	5.2	6.4
34	6.2	5.5	5.5	5.65	5.16	5.34	5.8	5.8	5.5	4.7	5.4	6.6
35	6.38	5.73	5.77	5.87	5.28	5.42	5.9	5.9	5.6	4.8	5.4	6.7
36	6.6	6.1	6.05	6.2	5.5	5.75	6.4	6.1	5.6	4.9	5.5	6.9
38	7.05	6.15	6.2	6.15	5.5	5.8	6.6	6.3	6	5.1	5.8	7
40	-	-	-	-	-	-	6.6	6.5	6.2	5.3	6	7.1

CONCLUSION

Fetal growth pattern analysis observed in western Indian population is similar to that observed amongother population groups of India. In majority of cases of thanatophoric dysplasia, prenatal ultrasound examination was diagnostic, providing a correct diagnosis using ultrasonography as the sole modality. The spectrum of skeletal dysplasia in western India, is similar to that of other parts of the World, but recessive entities may be more frequent because of widespread consanguinity in India. Presently, India provides molecular genetic testing facilities at extremely limited medical centres, hence precise clinical-radiographic phenotyping remains the mainstay of diagnosis and counselling and of gatekeeping to efficient laboratory testing.

In cases where ultrasound findings suggest a lethal prognosis, then computer tomography/ magnetic resonance imaging should be used to confirm the diagnosis. This should be followed by counselling based on clinical and radiological findings. Molecular testing should be considered to confirm the clinical findings.

Fetuses with probable prenatal diagnosis of skeletal dysplasia, and those who are expected to be delivered at a viable gestational age, insuch cases, a proper management delivery plan should be constituted after consultations between the obstetrical, neonatal, anaesthesia, and geneticists.

In cases where fetuses are delivered with skeletal dysplasia, the diagnosis should be confirmed by post-delivery clinical and radiologic evaluation. In case of fetal death or aborted fetuses, molecular testing should also be done along with clinical and radiological evaluation.

Suggestions: High risk pregnancies for homozygosity orcompound heterozygosity for skeletal dysplasia's should be offered molecular testing. Individuals with skeletal dysplasia's should be encouraged to obtain molecular analysis before pregnancy.Research studies focusing on targeted gene sequencing technologywhich can significantly improve the prenatal diagnosis of systemicskeletal abnormalities, thereby allowing for a more comprehensiveand useful prenatal genetic counselling guidance for parents, should be encouraged by government and private sector in India.

Conflicts of Interests: **None**

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